POLYNUCLEOTIDES ENCODING NOVEL HUMAN PHOSPHATASES

This application claims benefit to provisional application U.S. Serial No. 60/256,868, filed December 20, 2000; to provisional application U.S. Serial No. 60/280,186, filed March 30, 2001; to provisional application U.S. Serial No. 60/287,735, filed May 01, 2001, to provisional application U.S. Serial No. 60/295,848, filed June 05, 2001, and to provisional application U.S. Serial No. 60/300,465, filed June 25, 2001.

FIELD OF THE INVENTION

The present invention provides novel polynucleotides encoding human phosphatase polypeptides, fragments and homologues thereof. Also provided are vectors, host cells, antibodies, and recombinant and synthetic methods for producing said polypeptides. The invention further relates to diagnostic and therapeutic methods for applying these novel human phosphatase polypeptides to the diagnosis, treatment, and/or prevention of various diseases and/or disorders related to these polypeptides, particularly cardiovascular diseases and/or disorders. The invention further relates to screening methods for identifying agonists and antagonists of the polynucleotides and polypeptides of the present invention.

BACKGROUND OF THE INVENTION

Phosphorylation of proteins is a fundamental mechanism for regulating diverse cellular processes. While the majority of protein phosphorylation occurs at serine and threonine residues, phosphorylation at tyrosine residues is attracting a great deal of interest since the discovery that many oncogene products and growth factor receptors possess intrinsic protein tyrosine kinase activity. The importance of protein tyrosine phosphorylation in growth factor signal transduction, cell cycle progression and neoplastic transformation is now well established (Hunter et al., Ann. Rev. Biochem. 54:987-930 (1985), Ullrich et al., Cell 61:203-212 (1990), Nurse, Nature 344:503-508 (1990), Cantley et al, Cell 64:281-302 (1991)).

Biochemical studies have shown that phosphorylation on tyrosine residues of a variety of cellular proteins is a dynamic process involving competing phosphorylation

and dephosphorylation reactions. The regulation of protein tyrosine phosphorylation is mediated by the reciprocal actions of protein tyrosine kinases (PTKases) and protein tyrosine phosphatases (PTPases). The tyrosine phosphorylation reactions are catalyzed by PTKases. Tyrosine phosphorylated proteins can be specifically dephosphorylated through the action of PTPases. The level of protein tyrosine phosphorylation of intracellular substances is determined by the balance of PTKase and PTPase activities. (Hunter, T., Cell 58:1013-1016 (1989)).

The protein tyrosine kinases (PTKases) are a large family of proteins that includes many growth factor receptors and potential oncogenes. (Hanks et al., Science 241:42-52 (1988)). Many PTKases have been linked to initial signals required for induction of the cell cycle (Weaver et al., Mol. Cell. Biol. 11, 9:4415-4422 (1991)). PTKases comprise a discrete family of enzymes having common ancestry with, but major differences from, serine/threonine-specific protein kinases (Hanks et al., supra). The mechanisms leading to changes in activity of PTKases are best understood in the case of receptor-type PTKases having a transmembrane topology (Ullrich et al. (1990) supra). The binding of specific ligands to the extracellular domain of members of receptor-type PTKases is thought to induce their oligomerization leading to an increase in tyrosine kinase activity and activation of the signal transduction pathways (Ullrich et al., (1990) supra). Deregulation of kinase activity through mutation or overexpression is a well established mechanism for cell transformation (Hunter et al., (1985) supra; Ullrich et al., (1990) supra).

The protein phosphatases are composed of at least two separate and distinct families (Hunter, T. (1989) supra) the protein serine/threonine phosphatases and the protein tyrosine phosphatases (PTPases).

The protein tyrosine phosphatases (PTPases) are a family of proteins that have been classified into two subgroups. The first subgroup is made up of the low molecular weight, intracellular enzymes that contain a single conserved catalytic phosphatase domain. All known intracellular type PTPases contain a single conserved catalytic phosphatase domain. Examples of the first group of PTPases include (1) placental PTPase 1B (Charbonneau et al., Proc. Natl. Acad. Sci. USA 86:5252-5256 (1989); Chernoff et al., Proc. Natl. Acad. Sci. USA 87:2735-2789 (1989)), (2) T-cell PTPase (Cool et al., Proc. Natl. Acad. Sci. USA 86:5257-5261 (1989)), (3) rat brain

PTPase (Guan et al., Proc. Natl. Acad. Sci. USA 87:1501-1502 (1990)), (4) neuronal phosphatase (STEP) (Lombroso et al., Proc. Natl. Acad. Sci. USA 88:7242-7246 (1991)), and (5) cytoplasmic phosphatases that contain a region of homology to cytoskeletal proteins (Gu et al., Proc. Natl. Acad. Sci. USA 88:5867-57871 (1991); Yang et al., Proc. Natl. Acad. Sci. USA 88:5949-5953 (1991)).

Enzymes of this class are characterized by an active site motif of CX_5R. Within ths motif the Cysteine sulfur acts as a nucleophile which cleaves the P-O bond and releases the phosphate; the Arginine interacts with the phosphate and facilitates nucleophic attack. In many cases the Cysteine is preceded by a Histidine and the Arginine is followed by a Serine or Threonine. In addition, an Aspartate residue located 20 or more amino acids N terminal to the Cysteine acts as a general acid during cleavage [Fauman, 1996].

The second subgroup of protein tyrosine phosphatases is made up of the high molecular weight, receptor-linked PTPases, termed R-PTPases. R-PTPases consist of a) an intracellular catalytic region, b) a single transmembrane segment, and c) a putative ligand-binding extracellular domain (Gebbink et al., supra).

The structures and sizes of the c) putative ligand-binding extracellular "receptor" domains of R-PTPases are quite divergent. In contrast, the a) intracellular catalytic regions of R-PTPases are highly homologous. All RPTPases have two tandemly duplicated catalytic phosphatase homology domains, with the prominent exception of an R-PTPase termed HPTP.beta., which has "only one catalytic phosphatase domain. (Tsai et al., J. Biol. Chem... 266(16):10534-10543 (1991)).

One example of R-PTPases are the leukocyte common antigens (LCA) (Ralph, S. J., EMBO J. 6:1251-1257 (1987)). LCA is a family of high molecular weight glycoproteins expressed on the surface of all leukocytes and their hemopoietic progenitors (Thomas, Ann. Rev. Immunol. 7:339-369 (1989)). A remarkable degree of similarity is detected with the sequence of LCA from several species (Charbonneau et al., Proc. Natl. Acad. Sci. USA 85:7182-7186 (1988)). LCA is referred to in the literature by different names, including T200 (Trowbridge et al., Eur. J. Immunol. 6:557-562 (1962)), B220 for the B cell form (Coffman et al., Nature 289:681-683 (1981)), the mouse allotypic marker Ly-5 (Komuro et al., Immunogenetics 1:452-456

(1975)), and more recently CD45 (Cobbold et al., Leucocyte Typing III, ed. A. J. McMichael et al., pp. 788-803 (1987)).

Several studies suggest that CD45 plays a critical role in T cell activation. These studies are reviewed in Weiss A., Ann. Rev. Genet. 25:487-510 (1991). In one study, T-cell clones that were mutagenized by NSG and selected for their failure to express CD45 had impaired responses to T-cell receptor stimuli (Weaver et al., (1991) supra). These T-cell clones were functionally defective in their responses to signals transmitted through the T cell antigen receptor, including cytolysis of appropriate targets, proliferation, and lymphokine production (Weaver et al., (1991) supra).

Other studies indicate that the PTPase activity of CD45 plays a role in the activation of pp56.sup.lck, a lymphocyte-specific PTKase (Mustelin et al., Proc. Natl. Acad. Sci. USA 86:6302-6306 (1989); Ostergaard et al., Proc. Natl. Acad. Sci. USA 86:8959-8963 (1989)). These authors hypothesized that the phosphatase activity of CD45 activates pp56.sup.lck by dephosphorylation of a C-terminal tyrosine residue, which may, in turn, be related to T-cell activation.

Another example of R-PTPases is the leukocyte common antigen related molecule (LAR) (Streuli et al., J. Exp. Med. 168:1523-1530 (1988)). LAR was initially identified as a homologue of LCA (Streuli et al., supra). Although the a) intracellular catalytic region of the LAR molecule contains two catalytic phosphatase homology domains (domain I and domain II), mutational analyses suggest that only domain I has catalytic phosphatase activity, whereas domain II is enzymatically inactive (Streuli et al., EMBO J. 9(8):2399-2407 (1990)). Chemically induced LAR mutants having tyrosine at amino acid position 1379 changed to a phenylalanine are temperature-sensitive (Tsai et al., J. Biol. Chem... 266(16):10534-10543 (1991)).

A new mouse R-PTP, designated mRPTP.mu., has been cloned which has a) an extracellular domain that shares some structural motifs with LAR. (Gebbink et al., (1991) supra). In addition, these authors have cloned the human homologue of RPTP.mu. and localized the gene on human chromosome 18.

Two Drosophila PTPases, termed DLAR and DPTP, have been predicted based on the sequences of cDNA clones (Streuli et al., Proc. Natl. Acad. Sci. USA 86:8698-8702 (1989)). cDNAs coding for another Drosophila R-PTPase, termed

DPTP 99A, have been cloned and characterized (Hariharan et al., Proc. Natl. Acad. Sci. USA 88:11266-11270 (1991)).

Other examples of R-PTPases include R-PTPase-.alpha., .beta., .gamma., and .zeta. (Krueger et al., EMBO J. 9:3241-3252 (1990), Sap et al., Proc. Natl. Acad. Sci. USA 87:6112-6116 (1990), Kaplan et al., Proc. Natl. Acad. Sci. USA 87:7000-7004 (1990), Jirik et al., FEBS Lett. 273:239-242 (1990); Mathews et al., Proc. Natl. Acad. Sci. USA 87:4444-4448 (1990), Ohagi et al., Nucl. Acids Res. 18:7159 (1990)). Published application W092/01050 discloses human R-PTPase-.alpha., .beta. and .gamma., and reports on the nature of the structural homologies found among the conserved domains of these three R-PTPases and other members of this protein family. The murine R-PTPase-.alpha. has 794 amino acids, whereas the human R-PTPase-.alpha. has 802 amino acids. R-PTPase-.alpha. has an intracellular domain homologous to the catalytic domains of other tyrosine phosphatases. The 142 amino acid extracellular domain (including signal peptide of RPTPase-.alpha.) has a high serine and threonine content (32%) and 8 potential N-glycosylation sites. cDNA clones have been produced that code for the R-PTPase-.alpha., and R-PTPase-.alpha. has been expressed from eukaryotic hosts. Northern analysis has been used to identify the natural expression of R-PTPase-.alpha. in various cells and tissues. A polyclonal antibody to R-PTPase-.alpha. has been produced by immunization with a synthetic peptide of R-PTPase-.alpha., which identifies a 130 kDa protein in cells transfected with a cDNA clone encoding a portion of R-PTPase-.alpha..

Another example of R-PTPases is HePTP. (Jirik et al, FASEB J. 4:82082 (1990) Abstract 2253). Jirik et al. screened a cDNA library derived from a hepatoblastoma cell line, HepG2, with a probe encoding the two PTPase domains of LCA, and discovered a cDNA clone encoding a new RPTPase, named HePTP. The HePTP gene appeared to be expressed in a variety of human and murine cell lines and tissues.

Since the initial purification, sequencing, and cloning of a PTPase, additional potential PTPases have been identified at a rapid pace. The number of different PTPases that have been identified is increasing steadily, leading to speculations that this family may be as large as the PTKase family (Hunter (1989) supra).

Conserved amino acid sequences in the catalytic domains of known PTPases have been identified and defined (Krueger et al., EMBO J. 9:3241-3252 (1990) and Yi et al., Mol. Cell. Biol. 12:836-846 (1992), which are incorporated herein by reference.) These amino acid sequences are designated "consensus sequences" herein.

Yi et al. aligned the catalytic phosphatase domain sequences of the following PTPases: LCA, PTP1B, TCPTP, LAR, DLAR, and HPTP.alpha., HPTP.beta., and HPTP.gamma.. This alignment includes the following "consensus sequences" (Yi et al., supra, FIG. 2(A), lines 1 and 2): DYINAS/N (SEQ ID NO:77), CXXYWP (SEQ ID NO:78), and I/VVMXXXXE (SEQ ID NO:79).

Krueger et al., aligned the catalytic phosphatase domain sequences of PTP1B, TCPTP, LAR, LCA, HPTP.alpha., .beta., .gamma., .GAMMA., .delta., .epsilon. and .zeta. and DLAR and DPTP. This alignment includes the following "consensus sequences: (Krueger et al., supra, FIG. 7, lines 1 and 2): D/NYINAS/N (SEQ ID NO:80), CXXYWP (SEQ ID NO:81), and I/VVMXXXXE (SEQ ID NO:82).

It is becoming clear that dephosphorylation of tyrosine residues can by itself function as an important regulatory mechanism. Dephosphorylation of a C-terminal tyrosine residue has been shown to activate tyrosine kinase activity in the case of the src family of tyrosine kinases (Hunter, T. Cell 49:1-4 (1987)). Tyrosine dephosphorylation has been suggested to be an obligatory step in the mitotic activation of the maturation-promoting factor (MPF) kinase (Morla et al., Cell 58:193-203 (1989)). These observations point out the need in the art for understanding the mechanisms that regulate tyrosine phosphatase activity.

Modulators (inhibitors or activators) of human phosphatase expression or activity could be used to treat a subject with a disorder characterized by aberrant phosphatase expression or activity or by decreased phosphorylation of a phosphatase substrate protein. Examples of such disorders include but are not limited to: an immune, anti-proliferative, proliferative (e.g. cancer), metabolic (e.g. diabetes or obesity), bone (e.g., osteoporosis), neural, and/or cardiovascular diseases and/or disorders, in addition to, viral pathogenesis.

It is clear that further analysis of structure-function relationships among PTPases are needed to gain important understanding of the mechanisms of signal transduction, cell cycle progression and cell growth, and neoplastic transformation.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells, in addition to their use in the production of human phosphatase polypeptides or peptides using recombinant techniques. Synthetic methods for producing the polypeptides and polynucleotides of the present invention are provided. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the human phosphatase polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

BRIEF SUMMARY OF THE INVENTION

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the human BMY_HPP1 phosphatase protein having the amino acid sequence shown as SEQ ID NO:150, or the amino acid sequence encoded by the cDNA clone, BMY_HPP1, deposited as ATCC Deposit Number XXXXXX on XXXXXX.

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the human BMY_HPP2 phosphatase protein having the amino acid sequence shown as SEQ ID NO:152, or the amino acid sequence encoded by the cDNA clone, BMY_HPP2, deposited as ATCC Deposit Number XXXXXX on XXXXXX.

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the human BMY_HPP5 phosphatase protein having the amino acid sequence shown as SEQ ID NO:42, or the amino acid sequence encoded by the cDNA clone, BMY_HPP5 (also referred to as 7IC-5-E2), deposited as ATCC Deposit Number PTA-2966 on January 24th, 2001.

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the human RET31 phosphatase protein having the amino acid sequence shown as SEQ ID NO:109, or the amino acid sequence encoded by the cDNA clone, RET31 (also referred to as 1hrTNF031, and/or Clone 31), deposited as ATCC Deposit Number PTA-3434 on June 7, 2001.

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the mouse RET31 phosphatase protein having the amino acid sequence shown as SEQ ID NO:114, or the amino acid sequence encoded by the cDNA clone, mRET31, deposited as ATCC Deposit Number XXXXXX on XXXXXX.

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the human BMY_HPP1 phosphatase protein having the amino acid sequence shown as SEQ ID NO:150, or the amino acid sequence encoded by the cDNA clone, BMY_HPP1, deposited as ATCC Deposit Number XXXXX on XXXXX.

The present invention provides isolated nucleic acid molecules, that comprise, or alternatively consist of, a polynucleotide encoding the BMY_HPP2 phosphatase protein having the amino acid sequence shown as SEQ ID NO:152, or the amino acid sequence encoded by the cDNA clone, BMY_HPP2, deposited as ATCC Deposit Number XXXXX on XXXXX.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells, in addition to their use in the production of human phosphatase polypeptides or peptides using recombinant techniques. Synthetic methods for producing the polypeptides and polynucleotides of the present invention are provided. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the human phosphatase polypeptides and polynucleotides, and therapeutic methods for treating such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of the polypeptides.

The invention further provides an isolated BMY_HPP1 human phosphatase polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The invention further provides an isolated BMY_HPP2 human phosphatase polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The invention further provides an isolated BMY_HPP5 human phosphatase polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The invention further provides an isolated RET31 human phosphatase polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The invention further provides an isolated RET31 mouse phosphatase polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The invention further relates to a polynucleotide encoding a polypeptide fragment of SEQ ID NO:150, 152, 8, 10, 42, or 109, or a polypeptide fragment encoded by the cDNA sequence included in the deposited clone, which is hybridizable to SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to a polynucleotide encoding a polypeptide domain of SEQ ID NO:150, 152, 8, 10, 42, or 109 or a polypeptide domain encoded by the cDNA sequence included in the deposited clone, which is hybridizable to SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to a polynucleotide encoding a polypeptide epitope of SEQ ID NO:150, 152, 8, 10, 42, or 109 or a polypeptide epitope encoded by the cDNA sequence included in the deposited clone, which is hybridizable to SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to a polynucleotide encoding a polypeptide of SEQ ID NO:150, 152, 8, 10, 42, or 109 or the cDNA sequence included in the deposited clone, which is hybridizable to SEQ ID NO:149, 151, 7, 9, 41, or 108, having biological activity.

The invention further relates to a polynucleotide which is a variant of SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to a polynucleotide which is an allelic variant of SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to a polynucleotide which encodes a species homologue of the SEQ ID NO:150, 152, 8, 10, 42, or 109.

The invention further relates to a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified herein, wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

The invention further relates to an isolated nucleic acid molecule of SEQ ID NO:150, 152, 8, 10, 42, or 109, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a human phosphatase protein.

The invention further relates to an isolated nucleic acid molecule of SEQ ID NO: 149, 151, 7, 9, 41, or 108 wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:150, 152, 8, 10, 42, or 109 or the polypeptide encoded by the cDNA sequence included in the deposited clone, which is hybridizable to SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to an isolated nucleic acid molecule of of SEQ ID NO: 149, 151, 7, 9, 41, or 108, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:149, 151, 7, 9, 41, or 108 or the cDNA sequence included in the deposited clone, which is hybridizable to SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to an isolated nucleic acid molecule of SEQ ID NO:1, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

The invention further relates to an isolated polypeptide comprising an amino acid sequence that comprises a polypeptide fragment of SEQ ID NO:150, 152, 8, 10, 42, or 109 or the encoded sequence included in the deposited clone.

The invention further relates to a polypeptide fragment of SEQ ID NO:150, 152, 8, 10, 42, or 109 or the encoded sequence included in the deposited clone, having biological activity.

The invention further relates to a polypeptide domain of SEQ ID NO:150, 152, 8, 10, 42, or 109 or the encoded sequence included in the deposited clone.

The invention further relates to a polypeptide epitope of SEQ ID NO:150, 152, 8, 10, 42, or 109 or the encoded sequence included in the deposited clone.

The invention further relates to a full length protein of SEQ ID NO:150, 152, 8, 10, 42, or 109 or the encoded sequence included in the deposited clone.

The invention further relates to a variant of SEQ ID NO:150, 152, 8, 10, 42, or 109.

The invention further relates to an allelic variant of SEQ ID NO:150, 152, 8, 10, 42, or 109. The invention further relates to a species homologue of SEQ ID NO:150, 152, 8, 10, 42, or 109.

The invention further relates to the isolated polypeptide of of SEQ ID NO:150, 152, 8, 10, 42, or 109, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

The invention further relates to an isolated antibody that binds specifically to the isolated polypeptide of SEQ ID NO:150, 152, 8, 10, 42, or 109.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of SEQ ID NO:150, 152, 8, 10, 42, or 109 or the polynucleotide of SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to a method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising the steps of (a) determining the presence or absence of a mutation in the polynucleotide of SEQ ID NO:149, 151, 7, 9, 41, or 108; and (b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

The invention further relates to a method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising the steps of (a) determining the presence or amount of expression of the polypeptide of of SEQ ID NO:150, 152, 8, 10, 42, or 109 in a biological sample; and diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

The invention further relates to a method for identifying a binding partner to the polypeptide of SEQ ID NO:150, 152, 8, 10, 42, or 109 comprising the steps of (a) contacting the polypeptide of SEQ ID NO:150, 152, 8, 10, 42, or 109 with a binding

partner; and (b) determining whether the binding partner effects an activity of the polypeptide.

The invention further relates to a gene corresponding to the cDNA sequence of SEQ ID NO:149, 151, 7, 9, 41, or 108.

The invention further relates to a method of identifying an activity in a biological assay, wherein the method comprises the steps of expressing SEQ ID NO:149, 151, 7, 9, 41, or 108 in a cell, (b) isolating the supernatant; (c) detecting an activity in a biological assay; and (d) identifying the protein in the supernatant having the activity.

The invention further relates to a process for making polynucleotide sequences encoding gene products having altered activity selected from the group consisting of SEQ ID NO:150, 152, 8, 10, 42, or 109 activity comprising the steps of (a) shuffling a nucleotide sequence of SEQ ID NO:149, 151, 7, 9, 41, or 108, (b) expressing the resulting shuffled nucleotide sequences and, (c) selecting for altered activity selected from the group consisting of SEQ ID NO:150, 152, 8, 10, 42, or 109 activity as compared to the activity selected from the group consisting of SEQ ID NO:150, 152, 8, 10, 42, or 109 activity of the gene product of said unmodified nucleotide sequence.

The invention further relates to a shuffled polynucleotide sequence produced by a shuffling process, wherein said shuffled DNA molecule encodes a gene product having enhanced tolerance to an inhibitor of any one of the activities selected from the group consisting of SEQ ID NO:150, 152, 8, 10, 42, or 109 activity.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition with the polypeptide provided as SEQ ID NO:150, 152, 8, 10, 42, or 109, in addition to, its encoding nucleic acid, wherein the medical condition is a condition related to aberrant phosphatase activity.

The invention further relates to a method of identifying a compound that modulates the biological activity of a phosphatase, comprising the steps of, (a) combining a candidate modulator compound with a phosphatase having the sequence set forth in one or more of SEQ ID NO:150, 152, 8, 10, 42, or 109; and measuring an effect of the candidate modulator compound on the activity of a phosphatase.

The invention further relates to a method of identifying a compound that modulates the biological activity of a phosphatase, comprising the steps of, (a)

combining a candidate modulator compound with a host cell expressing a phosphatase having the sequence as set forth in SEQ ID NO:150, 152, 8, 10, 42, or 109; and , (b) measuring an effect of the candidate modulator compound on the activity of the expressed a phosphatase.

The invention further relates to a method of identifying a compound that modulates the biological activity of a phosphatase, comprising the steps of, (a) combining a candidate modulator compound with a host cell containing a vector described herein, wherein a phosphatase is expressed by the cell; and, (b) measuring an effect of the candidate modulator compound on the activity of the expressed a phosphatase.

The invention further relates to a method of screening for a compound that is capable of modulating the biological activity of a phosphatase, comprising the steps of: (a) providing a host cell described herein; (b) determining the biological activity of a phosphatase in the absence of a modulator compound; (c) contacting the cell with the modulator compound; and (d) determining the biological activity of a phosphatase in the presence of the modulator compound; wherein a difference between the activity of a phosphatase in the presence of the modulator compound and in the absence of the modulator compound indicates a modulating effect of the compound.

The invention further relates to a compound that modulates the biological activity of human a phosphatase as identified by the methods described herein.

The invention also relates to *in silico* screening methods including *in silico* docking and methods of structure based drug design which utilize the three dimensional coordinates of BMY_HPP1 (Figure 28, Table VIII). Also provided are methods of identifying modulators of BMY_HPP1 that include modulator building or searching utilizing computer programs and algorithms. In an embodiment of the invention a method is provided for designing potential modulators of BMY_HPP1 comprising any combination of steps which utilize said three dimensional structure to design or select potential modulators.

The present invention also provides structure coordinates of the homology model of BMY_HPP1. The complete coordinates are listed in Table VIII and visualized in Figure 28.. The model present in this invention further provides a basis

for designing stimulators and inhibitors or antagonists of one or more of the biological functions of BMY_HPP1, or of mutants with altered specificity.

The invention also relates to *in silico* screening methods including *in silico* docking and methods of structure based drug design which utilize the three dimensional coordinates of BMY_HPP2 (Figure 32, Table IX). Also provided are methods of identifying modulators of BMY_HPP2 that include modulator building or searching utilizing computer programs and algorithms. In an embodiment of the invention a method is provided for designing potential modulators of BMY_HPP2 comprising any combination of steps which utilize said three dimensional structure to design or select potential modulators.

The present invention also provides structure coordinates of the homology model of BMY_HPP2. The complete coordinates are listed in Table IX and visualized in Figure 32. The model present in this invention further provides a basis for designing stimulators and inhibitors or antagonists of one or more of the biological functions of BMY_HPP2, or of mutants with altered specificity.

The invention also relates to *in silico* screening methods including *in silico* docking and methods of structure based drug design which utilize the three dimensional coordinates of BMY_HPP5 (Figure 38, Table X). Also provided are methods of identifying modulators of BMY_HPP5 that include modulator building or searching utilizing computer programs and algorithms. In an embodiment of the invention a method is provided for designing potential modulators of BMY_HPP5 comprising any combination of steps which utilize said three dimensional structure to design or select potential modulators.

The present invention also provides structure coordinates of the homology model of BMY_HPP5. The complete coordinates are listed in Table X and visualized in Figure 38. The model present in this invention further provides a basis for designing stimulators and inhibitors or antagonists of one or more of the biological functions of BMY_HPP5, or of mutants with altered specificity.

The invention also provides a machine readable storage medium which comprises the structure coordinates of BMY_HPP1, including all or any parts conserved active site regions. Such storage medium encoded with these data are capable of displaying on a computer screen or similar viewing device, a three-

dimensional graphical representation of a molecule or molecular complex which comprises said regions or similarly shaped homologous regions.

The invention also provides methods for designing, evaluating and identifying compounds which bind to all or parts of the aforementioned regions. The methods include three dimensional model building (homology modeling) and methods of computer assisted-drug design which can be used to identify compounds which bind or modulate the forementioned regions of the BMY_HPP1 polypeptide. Such compounds are potential inhibitors of BMY_HPP1 or its homologues.

The invention also provides a machine readable storage medium which comprises the structure coordinates of BMY_HPP2, including all or any parts conserved active site regions. Such storage medium encoded with these data are capable of displaying on a computer screen or similar viewing device, a three-dimensional graphical representation of a molecule or molecular complex which comprises said regions or similarly shaped homologous regions.

The invention also provides methods for designing, evaluating and identifying compounds which bind to all or parts of the aforementioned regions. The methods include three dimensional model building (homology modeling) and methods of computer assisted-drug design which can be used to identify compounds which bind or modulate the forementioned regions of the BMY_HPP2 polypeptide. Such compounds are potential inhibitors of BMY_HPP2 or its homologues.

The invention also provides a machine readable storage medium which comprises the structure coordinates of BMY_HPP5, including all or any parts conserved active site regions. Such storage medium encoded with these data are capable of displaying on a computer screen or similar viewing device, a three-dimensional graphical representation of a molecule or molecular complex which comprises said regions or similarly shaped homologous regions.

The invention also provides methods for designing, evaluating and identifying compounds which bind to all or parts of the aforementioned regions. The methods include three dimensional model building (homology modeling) and methods of computer assisted-drug design which can be used to identify compounds which bind or modulate the forementioned regions of the BMY_HPP5 polypeptide. Such compounds are potential inhibitors of BMY_HPP5 or its homologues.

The invention also provides a computer for producing a three-dimensional representation of a molecule or molecular complex, wherein said molecule or molecular complex comprises the structural coordinates of the model BMY_HPP1 in accordance with Table VIII, or a three-dimensional representation of a homologue of said molecule or molecular complex, wherein said homologue comprises backbone atoms that have a root mean square deviation from the backbone atoms of not more than 3.5 angstroms. wherein said computer comprises:

The invention also provides a machine-readable data storage medium, comprising a data storage material encoded with machine readable data, wherein the data is defined by the set of structure coordinates of the model BMY_HPP1 according to Table VIII, or a homologue of said model, wherein said homologue comprises backbone atoms that have a root mean square deviation from the backbone atoms of not more than 3.5Å; a working memory for storing instructions for processing said machine-readable data; a central-processing unit coupled to said working memory and to said machine-readable data storage medium for processing said machine readable data into said three-dimensional representation; and a display coupled to said central-processing unit for displaying said three-dimensional representation. The invention also provides said computer wherein the machine-readable data storage medium is defined by the set of structure coordinates of the model for BMY_HPP1 according to Table VIII, or a homologue of said molecule, said homologue having a root mean square deviation from the backbone atoms of not more than 3.0 Å.

The invention also provides a model comprising all or any part of the model defined by structure coordinates of BMY_HPP1 according to Table VIII, or a mutant or homologue of said molecule or molecular complex.

The invention also provides a method for identifying a mutant of BMY_HPP1 with altered biological properties, function, or reactivity, the method comprising the step selected from the group consisting of: Using the BMY_HPP1 model or a homologue of said model according to Table VIII, for the design of protein mutants with altered biological function or properties.

The invention also provides a method for identifying structural and chemical features of BMY_HPP1 using the structural coordinates set forth in Table VIII, comprising any steps or combination of steps consisting of: employing identified

structural or chemical features to design or select compounds as potential BMY_HPP1 modulators; employing the three-dimensional structural model to design or select compounds as potential BMY_HPP1 modulators; synthesizing the potential BMY_HPP1 modulators; and screening the potential BMY_HPP1 modulators in an assay characterized by binding of a protein to the BMY_HPP1. The invention further provides said method wherein the potential BMY_HPP1 modulator is selected from a database. The invention further provides said method wherein the potential BMY_HPP1 modulator is designed de novo. The invention further provides said method wherein the potential BMY_HPP1 modulator is designed from a known modulator of activity.

The invention also provides a method for identifying a compound that modulates BMY_HPP1 activity, the method comprising any combination of steps of: Modeling test compounds that fit spatially into or near the active site region defined by residues D161-Y162 and H189-C190-G193-R196 of BMY_HPP1 as defined by structure coordinates according to Table VIII, or modeling test compounds that fit spatially into a three-dimensional structural model of the catalytic domain of BMY_HPP1, mutant homologue or portion thereof; using said structure coordinates or said active site region as set forth in prior claims to identify structural and chemical features; employing identified structural or chemical features to design or select compounds as potential BMY_HPP1 modulators including substrates, antagonists and agonists; employing the three-dimensional structural model or the catalytic domain of BMY_HPP1 to design or select compounds as potential BMY_HPP1 inhibitors; screening the potential BMY_HPP1 inhibitors in an assay characterized by binding of a test compound to BMY_HPP1; and/or modifying or replacing one or more amino acids from BMY_HPP1 including but not limited to the residues corresponding to the active site region as set forth in prior claims of BMY_HPP1 according to Table VIII.

The invention also provides a computer for producing a three-dimensional representation of a molecule or molecular complex, wherein said molecule or molecular complex comprises the structural coordinates of the model BMY_HPP2 in accordance with Table IX, or a three-dimensional representation of a homologue of said molecule or molecular complex, wherein said homologue comprises backbone

atoms that have a root mean square deviation from the backbone atoms of not more than 3.5 angstroms. wherein said computer comprises:

The invention also provides a machine-readable data storage medium, comprising a data storage material encoded with machine readable data, wherein the data is defined by the set of structure coordinates of the model BMY_HPP2 according to Table IX, or a homologue of said model, wherein said homologue comprises backbone atoms that have a root mean square deviation from the backbone atoms of not more than 3.5Å; a working memory for storing instructions for processing said machine-readable data; a central-processing unit coupled to said working memory and to said machine-readable data storage medium for processing said machine readable data into said three-dimensional representation; and a display coupled to said central-processing unit for displaying said three-dimensional representation. The invention also provides said computer wherein the machine-readable data storage medium is defined by the set of structure coordinates of the model for BMY_HPP2 according to Table IX, or a homologue of said molecule, said homologue having a root mean square deviation from the backbone atoms of not more than 3.0 Å.

The invention also provides a model comprising all or any part of the model defined by structure coordinates of BMY_HPP2 according to Table IX, or a mutant or homologue of said molecule or molecular complex.

The invention also provides a method for identifying a mutant of BMY_HPP2 with altered biological properties, function, or reactivity, the method comprising the step selected from the group consisting of: Using the BMY_HPP2 model or a homologue of said model according to Table IX, for the design of protein mutants with altered biological function or properties.

The invention also provides a method for identifying structural and chemical features of BMY_HPP2 using the structural coordinates set forth in Table IX, comprising any steps or combination of steps consisting of: employing identified structural or chemical features to design or select compounds as potential BMY_HPP2 modulators; employing the three-dimensional structural model to design or select compounds as potential BMY_HPP2 modulators; synthesizing the potential BMY_HPP2 modulators; and screening the potential BMY_HPP2 modulators in an assay characterized by binding of a protein to the BMY_HPP2. The invention further

provides said method wherein the potential BMY_HPP2 modulator is selected from a database. The invention further provides said method wherein the potential BMY_HPP2 modulator is designed de novo. The invention further provides said method wherein the potential BMY_HPP2 modulator is designed from a known modulator of activity.

The invention also provides a method for identifying a compound that modulates BMY_HPP2 activity, the method comprising any combination of steps of: Modeling test compounds that fit spatially into or near the active site region defined by residues residues D65, H94-C95, G98, and R101 of BMY_HPP2 as defined by structure coordinates according to Table IX, or modeling test compounds that fit spatially into a three-dimensional structural model of the catalytic domain of BMY_HPP2, mutant homologue or portion thereof; using said structure coordinates or said active site region as set forth in prior claims to identify structural and chemical features; employing identified structural or chemical features to design or select compounds as potential BMY_HPP2 modulators including substrates, antagonists and agonists; employing the three-dimensional structural model or the catalytic domain of BMY_HPP2 to design or select compounds as potential BMY_HPP2 inhibitors; screening the potential BMY_HPP2 inhibitors in an assay characterized by binding of a test compound to BMY_HPP2; and/or modifying or replacing one or more amino acids from BMY_HPP2 including but not limited to the residues corresponding to the active site region as set forth in prior claims of BMY_HPP2 according to Table IX.

The invention also provides a computer for producing a three-dimensional representation of a molecule or molecular complex, wherein said molecule or molecular complex comprises the structural coordinates of the model BMY_HPP5 in accordance with Table X, or a three-dimensional representation of a homologue of said molecule or molecular complex, wherein said homologue comprises backbone atoms that have a root mean square deviation from the backbone atoms of not more than 3.5 angstroms. wherein said computer comprises:

The invention also provides a machine-readable data storage medium, comprising a data storage material encoded with machine readable data, wherein the data is defined by the set of structure coordinates of the model BMY_HPP5 according to Table X, or a homologue of said model, wherein said homologue comprises

backbone atoms that have a root mean square deviation from the backbone atoms of not more than 3.5Å; a working memory for storing instructions for processing said machine-readable data; a central-processing unit coupled to said working memory and to said machine-readable data storage medium for processing said machine readable data into said three-dimensional representation; and a display coupled to said central-processing unit for displaying said three-dimensional representation. The invention also provides said computer wherein the machine-readable data storage medium is defined by the set of structure coordinates of the model for BMY_HPP5 according to Table X, or a homologue of said molecule, said homologue having a root mean square deviation from the backbone atoms of not more than 3.0 Å.

The invention also provides a model comprising all or any part of the model defined by structure coordinates of BMY_HPP5 according to Table X, or a mutant or homologue of said molecule or molecular complex.

The invention also provides a method for identifying a mutant of BMY_HPP5 with altered biological properties, function, or reactivity, the method comprising the step selected from the group consisting of: Using the BMY_HPP5 model or a homologue of said model according to Table X, for the design of protein mutants with altered biological function or properties.

The invention also provides a method for identifying structural and chemical features of BMY_HPP5 using the structural coordinates set forth in Table X, comprising any steps or combination of steps consisting of: employing identified structural or chemical features to design or select compounds as potential BMY_HPP5 modulators; employing the three-dimensional structural model to design or select compounds as potential BMY_HPP5 modulators; synthesizing the potential BMY_HPP5 modulators; and screening the potential BMY_HPP5 modulators in an assay characterized by binding of a protein to the BMY_HPP5. The invention further provides said method wherein the potential BMY_HPP5 modulator is selected from a database. The invention further provides said method wherein the potential BMY_HPP5 modulator is designed de novo. The invention further provides said method wherein the potential BMY_HPP5 modulator is designed from a known modulator of activity.

The invention also provides a method for identifying a compound that modulates BMY_HPP5 activity, the method comprising any combination of steps of: Modeling test compounds that fit spatially into or near the active site region defined by residues residues D213, H243, C244, and R250 of BMY_HPP5 as defined by structure coordinates according to Table X, or modeling test compounds that fit spatially into a three-dimensional structural model of the catalytic domain of BMY_HPP5, mutant homologue or portion thereof; using said structure coordinates or said active site region as set forth in prior claims to identify structural and chemical features; employing identified structural or chemical features to design or select compounds as potential BMY_HPP5 modulators including substrates, antagonists and agonists; employing the three-dimensional structural model or the catalytic domain of BMY_HPP5 to design or select compounds as potential BMY_HPP5 inhibitors; screening the potential BMY_HPP5 inhibitors in an assay characterized by binding of a test compound to BMY_HPP5; and/or modifying or replacing one or more amino acids from BMY_HPP5 including but not limited to the residues corresponding to the active site region as set forth in prior claims of BMY_HPP5 according to Table X.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a renal condition.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is an inflammatory disease.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is an inflammatory disease where dual-specificity phosphatases, either directly or indirectly, are involved in disease progression.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a cancer.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a neural disorder.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a reproductive disorder.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is an immunological disorder.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a musculo-degenerative disorder.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a muscle disorder.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a hepatic disorder.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is an endocrine disorder.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a pulmonary disorder.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a disorder associated, either directly or indirectly, with TNF-alpha.

The invention further relates to a method for preventing, treating, or ameliorating a medical condition, wherein the medical condition is a disorder associated, either directly or indirectly, with IL-1.

BRIEF DESCRIPTION OF THE FIGURES/DRAWINGS

Figure 1 shows the polynucleotide sequences (SEQ ID NO: 1 and 3) and deduced amino acid sequence (SEQ ID NO:2 and 4) of gene fragments A and B, respectfully, of the novel human phosphatase, BMY_HPP1, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of fragment A contains a sequence of 144 nucleotides (SEQ ID NO:1), encoding a polypeptide of 48 amino acids (SEQ ID NO:2), while the polynucleotide sequence of fragment B contains a sequence of 33 nucleotides (SEQ ID NO:3), encoding a polypeptide of 11 amino acids (SEQ ID NO:4).

Figure 2 shows the polynucleotide sequence (SEQ ID NO: 5) and deduced amino acid sequence (SEQ ID NO:6) of a gene fragment of the novel human phosphatase, BMY_HPP2, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of this fragment contains a sequence of 746 nucleotides (SEQ ID NO:5), encoding 248 amino acids (SEQ ID NO:6) of the full-length BMY_HPP2 polypeptide, and/or translated portions of the 5' and/or 3' UTR of clone BMY_HPP2. The asterisks ("*") may represent any amino acid.

Figure 3 shows the polynucleotide sequence (SEQ ID NO: 7) and deduced amino acid sequence (SEQ ID NO:8) of a gene fragment of the novel human phosphatase, BMY_HPP3, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of this fragment contains a sequence of 511 nucleotides (SEQ ID NO:5), encoding 170 amino acids (SEQ ID NO:8) of the full-length BMY_HPP3 polypeptide, and/or translated portions of the 5' and/or 3' UTR of clone BMY_HPP3. The asterisks ("*") may represent any amino acid.

Figures 4A-B show the polynucleotide sequence (SEQ ID NO: 9) and deduced amino acid sequence (SEQ ID NO:10) of a gene fragment of the novel human phosphatase, BMY_HPP4, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of this fragment contains a sequence of 1710 nucleotides (SEQ ID NO:9), encoding 570 amino acids (SEQ ID NO:10) of the full-length BMY_HPP3 polypeptide, and/or translated portions of the 5' and/or 3' UTR of clone BMY_HPP4. The asterisks ("*") may represent any amino acid.

Figures 5A-E show the polynucleotide sequence (SEQ ID NO: 41) and deduced amino acid sequence (SEQ ID NO:42) of the novel full-length human phosphatase, BMY_HPP5, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of this protein contains a sequence of 5111 nucleotides (SEQ ID NO:41), encoding 665 amino acids (SEQ ID NO:42) of the full-length BMY_HPP5 polypeptide.

Figures 6A-D show the regions of identity between the encoded full-length human phosphatase protein BMY_HPP1 (BMY_HPP1_FL; SEQ ID NO:150), and fragments A and B of BMY_HPP1 (BMY_HPP1_A and BMY_HPP1_B; SEQ ID NO:2 and 4, respectfully), to other phosphatase proteins, specifically, the Schizosacchromyces Pombe protein tyrosine phosphatase PYP3 protein (PYP3_SP; Genbank Accession No:gi| P32587; SEQ ID NO:Y7); the mouse protein tyrosine phosphatase, receptor type, O, protein (MM_RPTPO; Genbank Accession No:gi| NP_035346; SEQ ID NO:Y8); and the human protein tyrosine phosphatase, receptor type, O, protein (HS_RPTPO; Genbank Accession No:gi| NP_002839; SEQ ID NO:Y9). The alignment was performed using the CLUSTALW algorithm. The darkly shaded amino acids represent regions of matching identity. The lightly shaded amino acids represent regions of matching similarity. Dots ("•") between residues indicate gapped regions of non-identity for the aligned polypeptides. Catalytic residues are indicated in bold.

Figures 7A-B show the regions of identity between the encoded full-length human phosphatase protein BMY_HPP2 (BMY_HPP2.FL; SEQ ID NO:152), and the fragment of BMY_HPP2 (BMY_HPP2.partial; SEQ ID NO:6) to other phosphatase proteins, specifically, the human CDC14 (also known as the cell division cycle 14, S. cerevisiae Gene A protein) homologue A (HS_CDC14A; Genbank Accession No:gi| NP_003663; SEQ ID NO:30); the human S. cerevisiae CDC14 homolog, gene B (HS_CDC14B; Genbank Accession No:gi| NP_003662; SEQ ID NO:31); and the yeast soluble tyrosine-specific protein phosphatase Cdc14p protein (SC_CDC14; Genbank Accession No:gi| NP_002839; SEQ ID NO:32). The alignment was performed using the CLUSTALW algorithm. The darkly shaded amino acids represent regions of matching identity. The lightly shaded amino acids represent regions of matching similarity. Dots ("•") between residues indicate gapped regions of non-identity for the aligned polypeptides. Catalytic residues are indicated in bold.

Figure 8 shows the regions of identity between the encoded human phosphatase protein fragment of BMY_HPP3 (SEQ ID NO:8) to other phosphatase proteins, specifically, the human protein tyrosine phosphatase PTPCAAX1 PROTEIN (HS_PTPCAAX1; Genbank Accession No:gi| AAB40597; SEQ ID NO:33); the human protein tyrosine phosphatase PTPCAAX2 (HS_PTPCAAX2; Genbank Accession No:gi| AAB40598; SEQ ID NO:34); the mouse prenylated protein tyrosine phosphatase (MM_PTPCAAX; Genbank Accession No:gi| JC5981; SEQ ID NO:35); and the Drosophila PRL-1 protein (DM_PRL1; Genbank Accession No:gi| AAF53506; SEQ ID NO:36). The alignment was performed using the CLUSTALW algorithm. The darkly shaded amino acids represent regions of matching identity. The lightly shaded amino acids represent regions of matching similarity. Dots ("•") between residues indicate gapped regions of non-identity for the aligned polypeptides. Catalytic residues are indicated in bold.

Figures 9A-B show the regions of identity between the encoded human phosphatase protein fragment of BMY_HPP4 (SEQ ID NO:10) to other phosphatase proteins, specifically, the mouse osteotesticular protein tyrosine phosphatase (MM_OST-PTP; Genbank Accession No:gi| AAG28768; SEQ ID NO:37); and the rat protein-tyrosine-

phosphatase (RN_PTP-OST; Genbank Accession No:gi| A55148; SEQ ID NO:38). The alignment was performed using the CLUSTALW algorithm. The darkly shaded amino acids represent regions of matching identity. The lightly shaded amino acids represent regions of matching similarity. Dots ("•") between residues indicate gapped regions of non-identity for the aligned polypeptides. Catalytic residues are indicated in bold.

Figures 10A-B shows the regions of identity between the encoded human phosphatase protein fragment of BMY_HPP5 (SEQ ID NO:42) to other phosphatase proteins, specifically, the human dual specificity phosphatase 8 (hs_dspp8; Genbank Accession No:gi| NP_004411; SEQ ID NO:39); and the mouse neuronal tyrosine/threonine phosphatase 1 (r mm_npp1; Genbank Accession No:gi| NP_032774; SEQ ID NO:40). The alignment was performed using the CLUSTALW algorithm. The darkly shaded amino acids represent regions of matching identity. The lightly shaded amino acids represent regions of matching similarity. Dots ("•") between residues indicate gapped regions of non-identity for the aligned polypeptides. Catalytic residues are indicated in bold.

Figure 11 shows an expression profile of the novel human phosphatase protein BMY_HPP5. The figure illustrates the relative expression level of BMY_HPP5 amongst various mRNA tissue sources. As shown, the BMY_HPP5 polypeptide was expressed to a significant extent, in the testis and spinal cord, and to a lesser extent, in bone marrow, brain, liver, and thymus. Expression data was obtained by measuring the steady state BMY_HPP5 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NO:67 and 68 as described herein.

Figure 12 shows a table illustrating the percent identity and percent similarity between the BMY_HPP5 (SEQ ID NO:42), the human RET31 (SEQ ID NO:109), and the mouse RET31 (SEQ ID NO:114) polypeptides of the present invention with other phosphatase proteins. The percent identity and percent similarity values were determined based upon the GAP algorithm (GCG suite of programs; and Henikoff, S.

and Henikoff, J. G., Proc. Natl. Acad. Sci. USA 89: 10915-10919(1992)) using the following parameters: gap weight = 8, and length weight = 2.

Figures 13A-F show the polynucleotide sequence (SEQ ID NO: 108) and deduced amino acid sequence (SEQ ID NO:109) of the novel full-length human phosphatase, RET31, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of this protein contains a sequence of 5450 nucleotides (SEQ ID NO:108), encoding 665 amino acids (SEQ ID NO:109) of the full-length RET31 polypeptide. An analysis of the RET31 polypeptide determined that it comprised the following features: a dual specificity phosphatase catalytic domain located from about amino acid 158 to about amino acid 297 (SEQ ID NO:134) of SEQ ID NO:109 represented by double underlining; and a catalytic cysteine amino acid residue located at amino acid 244 of SEQ ID NO:109 represented by shading.

Figures 14A-C show the regions of identity between the encoded human phosphatase protein of RET31 (SEQ ID NO:109) to other phosphatase proteins, specifically, the human protein-tyrosine phosphatase DUS8 protein, also referred to as hVH-5 (DUS8; Genbank Accession No:gi|U27193; SEQ ID NO:110); the human dual specificity MAP kinase DUSP6 protein (DUSP6; Genbank Accession No:gi|AB013382; SEQ ID NO:111); the human map kinase phosphatase MKP-5 protein (MKP-5; Genbank Accession No:gi|AB026436; SEQ ID NO:112); and the mouse RET31 protein of the present invention (mRET31; SEQ ID NO:114). The alignment was performed using the CLUSTALW algorithm. The darkly shaded amino acids represent regions of matching identity. The lightly shaded amino acids represent regions of matching similarity. Dots ("•") between residues indicate gapped regions of non-identity for the aligned polypeptides.

Figure 15 shows the results of a northern hybridization illustrating the expression profile of the novel human phosphatase protein RET31. The figure illustrates the relative expression level of RET31 amongst various mRNA tissue sources. As shown, the RET31 polypeptide was expressed predominately in adrenal gland, testis, and

skeletal muscle; to a significant extent, in the liver, prostate ovary, and to a lesser extent, in placenta, pancreas, thymus, small intestine, thyroid, heart, kidney and liver. Expression data was obtained by the hybridization of a 408bp P^{32}-labeled RET31 polynucleotide fragment correponding to SEQ ID NO:108 (specifically the RsaI fragment of SEQ ID NO:115) to several multiple tissue northern mRNA blots as described herein.

Figures 16A-C show the polynucleotide sequence (SEQ ID NO: 113) and deduced amino acid sequence (SEQ ID NO:114) of the novel full-length mouse phosphatase, mRET31, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of this protein contains a sequence of 2756 nucleotides (SEQ ID NO:113), encoding 660 amino acids (SEQ ID NO:114) of the full-length mRET31 polypeptide. An analysis of the mRET31 polypeptide determined that it comprised the following features: a dual specificity phosphatase catalytic domain located from about amino acid 158 to about amino acid 297 (SEQ ID NO:135) of SEQ ID NO:114 represented by double underlining.

Figure 17 shows the regions of identity between the dual specificity phosphatase catalytic (DSPc) domain of the encoded human phosphatase protein of RET31 (SEQ ID NO:109) to the dual specificity phosphatase catalytic (DSPc) domain of other phosphatase proteins, specifically, the DSPc domain of the human protein-tyrosine phosphatase DUS8 protein, also referred to as hVH-5 (DUS8_DSPc; Genbank Accession No:gi|U27193; SEQ ID NO:110); the DSPc domain of the human dual specificity MAP kinase DUSP6 protein (DUSP6_DSPc; Genbank Accession No:gi|AB013382; SEQ ID NO:111); and the DSPc domain of the human map kinase phosphatase MKP-5 protein (MKP-5_DSPc; Genbank Accession No:gi|AB026436; SEQ ID NO:112. Red boxes indicate conservation among all four DSPc domains, blue boxes indicate conservation among three DSPc domains, and green boxes indicate conservation between RET31 and one of the other protein domains. Dots ("•") between residues indicate gapped regions of non-identity for the aligned polypeptides.

Figure 18 shows the results of a northern hybridization illustrating the expression profile of the novel human phosphatase protein RET31 in human lung microvascular endothelial cells (HMCEC) after the administration of TNF-alpha for 0, 1, 6, and 24 hours. As shown, the RET31 polypeptide is up-regulated by TNF-α, reaching a peak of expression of about 6 hours. Expression data was obtained by the hybridization of a 408bp P^{32}-labeled RET31 polynucleotide fragment correponding to SEQ ID NO:108 (specifically the RsaI fragment of SEQ ID NO:115) to northern blots containing the isolated HMVEC mRNA for each indicated sample as described herein.

Figures 19A-F show the predicted polynucleotide sequence (SEQ ID NO: 147) and deduced amino acid sequence (SEQ ID NO:148) of the novel full-length human phosphatase, RET31, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of this protein contains a sequence of 5450 nucleotides (SEQ ID NO:147), encoding 665 amino acids (SEQ ID NO:148) of the full-length RET31 polypeptide. A portion of the sequence was determined based upon the sequence provided from the Incyte gene cluster 1026659.7 using bioinformatic methods.

Figures 20A-D show the predicted polynucleotide sequence (SEQ ID NO:149) and deduced amino acid sequence (SEQ ID NO:150) of the novel full-length human phosphatase, BMY_HPP1, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of this protein contains a sequence of 4393 nucleotides (SEQ ID NO:149), encoding 607 amino acids (SEQ ID NO:150) of the full-length BMY_HPP1 polypeptide. An analysis of the BMY_HPP1 polypeptide determined that it comprised the following features: a predicted dual specificity phosphatase catalytic domain located from about amino acid 41 to about amino acid 49 of SEQ ID NO:150 represented by shading; and conserved phophatase catalytic residues at amino acid 14, at amino acid 42, and at amino acid 48 of SEQ ID NO:150 (Figures 20A-D).

Figure 21 shows the polynucleotide sequence (SEQ ID NO:151) and deduced amino acid sequence (SEQ ID NO:152) of the novel full-length human phosphatase, BMY_HPP2, of the present invention. The standard one-letter abbreviation for amino acids is used to illustrate the deduced amino acid sequence. The polynucleotide sequence of this protein contains a sequence of 878 nucleotides (SEQ ID NO:151), encoding 150 amino acids (SEQ ID NO:152) of the full-length BMY_HPP2 polypeptide. An analysis of the BMY_HPP2 polypeptide determined that it comprised the following features: a predicted dual specificity phosphatase catalytic domain located from about amino acid 93 and 94, and from about amino acid 100 and 101 of SEQ ID NO:152 represented by shading; and conserved phosphatase catalytic residues located at amino acid 65, 94, and 100 of SEQ ID NO: 152 represented in bold.

Figure 22 shows an expression profile of the novel full-length human phosphatase protein BMY_HPP1. The figure illustrates the relative expression level of BMY_HPP1 amongst various mRNA tissue sources. As shown, the BMY_HPP1 polypeptide was expressed predominately in testis; to a significant extent, in the spinal cord, and to a lesser extent, in pancreas, brain, pituitary, heart, and lung. Expression data was obtained by measuring the steady state BMY_HPP1 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NO:154 and 155 as described herein.

Figure 23 shows an expression profile of the novel full-length human phosphatase protein BMY_HPP2. The figure illustrates the relative expression level of BMY_HPP2 amongst various mRNA tissue sources. As shown, the BMY_HPP2 polypeptide was expressed predominately in liver and kidney; to a significant extent, in the spleen, and to a lesser extent, in lung, testis, heart, intestine, pancreas, lymph node, spinal cord, and prostate. Expression data was obtained by measuring the steady state BMY_HPP2 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NO:156 and 157 as described herein.

Figure 24 shows a table illustrating the percent identity and percent similarity between the full-length BMY_HPP1 polypeptide (SEQ ID NO:150), and the full-

length BMY_HPP2 polypeptide (SEQ ID NO:152) of the present invention with other phosphatase proteins. The percent identity and percent similarity values were determined based upon the GAP algorithm (GCG suite of programs; and Henikoff, and Henikoff, J. G., Proc. Natl. Acad. Sci. USA 89: 10915-10919(1992)) using the following parameters: gap weight = 8, and length weight = 2.

Figure 25 shows a table illustrating the percent identity and percent similarity between the full-length RET31 polypeptide (SEQ ID NO:109) of the present invention with other phosphatase proteins. The percent identity and percent similarity values were determined based upon the GAP algorithm (GCG suite of programs; and Henikoff, and Henikoff, J. G., Proc. Natl. Acad. Sci. USA 89: 10915-10919(1992)) using the following parameters: gap weight = 8, and length weight = 2.

Figure 26 shows an expanded expression profile of the novel full-length human phosphatase protein BMY_HPP1. The figure illustrates the relative expression level of BMY_HPP1 amongst various mRNA tissue sources. As shown, the BMY_HPP1 polypeptide was expressed predominately in brain subregions and other central nervous system tissues, in particular the caudate, hippocampus and nucleus accumbens of the brain. Significant expression was observed in the in the adrenal, pineal and pituitary glands, the atrium of the heart, in the testis, and to a lesser extent in a number of other tissues as shown. Expression data was obtained by measuring the steady state BMY_HPP1 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NO:194 and 195, and Taqman probe (SEQ ID NO:196) as described in Example 59 herein.

Figure 27 shows the regions of identity between amino acid residues M1 to E301 of the BMY_HPP1 polypeptide (amino acids M1 to E301 of SEQ ID NO:150) to amino acid residues D11 to N321 of the human tyrosine specific phosphatase 1aax (Protein Data Bank, PDB entry 1aax chain A; Genbank Accession No. gi|2981942; SEQ ID NO:206) which was used as the basis for building the BMY_HPP1 homology model as represented in Table VIII and visualized in Figure 28. Amino acids defining active

site residues are highlighted with asterisks ("*"). The alignment was created using the FASTA algorithm (Pearson, *et. al.* 1990).

Figure 28 shows a three-dimensional homology model of amino acid residues M1 to E301 of the BMY_HPP1 polypeptide based upon the homologous structure of amino acid residues D11 to N321 of the human tyrosine specific phosphatase 1aax (Protein Data Bank, PDB entry 1aax chain A; Genbank Accession No. gi|2981942; SEQ ID NO:206). The structural coordinates of the BMY_HPP1 polypeptide are provided in Table VIII herein. The homology model of BMY_HPP1 was derived from generating a sequence alignment with the the human tyrosine specific phosphatase 1aax (Protein Data Bank, PDB entry 1aax chain A; Genbank Accession No. gi|2981942; SEQ ID NO:206) using the INSIGHTII (Accelrys Inc., San Diego, CA) version 2000 as described herein.

Figure 29 shows an energy graph for the BMY_HPP1 model of the present invention (dotted line) and the tyrosine specific phosphatase 1aax template (solid line) from which the model was generated. The energy distribution for each protein fold is displayed on the y-axis, while the amino acid residue position of the protein fold is displayed on the x-axis. As shown, the BMY_HPP1 model has slightly higher energies in the C-terminal region while the N-terminal region of the structural model appears to represent a "native-like" conformation of the BMY_HPP1 polypeptide. This graph supports the motif and sequence alignments in confirming that the three dimensional structure coordinates of BMY_HPP1 are an accurate and useful representation of the structure of the BMY_HPP1 polypeptide.

Figure 30 shows an expanded expression profile of the novel full-length human phosphatase protein BMY_HPP2. The figure illustrates the relative expression level of BMY_HPP2 amongst various mRNA tissue sources. As shown, the BMY_HPP2 polypeptide was expressed predominately in adrenal gland; significantly in the pineal and pituitary gland, lung parenchyma, bronchi, kidney, liver, blood vessels from the choroid plexus, coronary artery, pulmonary artery, the nucleus accumbens of the brain, and to a lesser extent in the trachea, breast and uterus and in other tissues as

shown. Expression data was obtained by measuring the steady state BMY_HPP2 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NO:197 and 198, and Taqman probe (SEQ ID NO:199) as described in Example 59 herein.

Figure 31 shows the regions of identity between amino acid residues M1 to K150 of the BMY_HPP2 polypeptide (amino acids M1 to K150 of SEQ ID NO:152) to amino acid residues N31 to K179 of the N-terminus of the human dual specificity phosphatase, 1vhr (vaccinia H1-related phosphatase VN1) (residues N31-K179; Protein Data Bank, PDB entry 1vhr chain A; Genbank Accession No. gi|1633321; SEQ ID NO:207) which was used as the basis for building the BMY_HPP2 homology model as represented in Table IX and visualized in Figure 32. Amino acids defining active site residues are highlighted in bold. The alignment was created using the FASTA algorithm (Pearson, *et. al.* 1990).

Figure 32 shows a three-dimensional homology model of amino acid residues M1 to K150 of the BMY_HPP2 polypeptide based upon the homologous structure of amino acid residues N31 to K179 of the N-terminus of the human dual specificity phosphatase, 1vhr (vaccinia H1-related phosphatase VN1) (residues N31-K179; Protein Data Bank, PDB entry 1vhr chain A; Genbank Accession No. gi|1633321; SEQ ID NO:207). The structural coordinates of the BMY_HPP2 polypeptide are provided in Table IX herein. The homology model of BMY_HPP2 was derived from generating a sequence alignment with the human dual specificity phosphatase, 1vhr (vaccinia H1-related phosphatase VN1) (residues N31-K179; Protein Data Bank, PDB entry 1vhr chain A; Genbank Accession No. gi|1633321; SEQ ID NO:207) using the INSIGHTII (Accelrys Inc., San Diego, CA) version 2000 as described herein.

Figure 33 shows an energy graph for the BMY_HPP2 model of the present invention (dotted line) and the phosphatase VHR template (PDB code 1vhr) (solid line) from which the model was generated. The energy distribution for each protein fold is displayed on the y-axis, while the amino acid residue position of the protein fold is displayed on the x-axis. As shown, the BMY_HPP2 model and 1vhr template have

similar energies over the aligned region, suggesting that the structural model of BMY_HPP2 represents a "native-like" conformation of the BMY_HPP2 polypeptide. This graph supports the motif and sequence alignments in confirming that the three dimensional structure coordinates of BMY_HPP2 are an accurate and useful representation of the structure of the BMY_HPP1 polypeptide.

Figure 34 shows an expanded expression profile of the novel full-length human phosphatase protein BMY_HPP4. The figure illustrates the relative expression level of BMY_HPP4 amongst various mRNA tissue sources. As shown, the BMY_HPP4 polypeptide was expressed predominately in cerebellum; significantly in other subregions of the brain, and in the pineal and pituitary glands. Expression data was obtained by measuring the steady state BMY_HPP4 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NO:200 and 201, and Taqman probe (SEQ ID NO:202) as described in Example 59 herein.

Figure 35 shows an expanded expression profile of the novel full-length human phosphatase protein BMY_HPP5. The figure illustrates the relative expression level of BMY_HPP5 amongst various mRNA tissue sources. As shown, the BMY_HPP5 polypeptide was expressed predominately in the adrenal, pineal and pituitary glands; significantly in the cerebellum, prostate, testis, and to a lesser extent in other tissues as shown. Expression data was obtained by measuring the steady state BMY_HPP5 mRNA levels by quantitative PCR using the PCR primer pair provided as SEQ ID NO:203 and 204, and Taqman probe (SEQ ID NO:205) as described in Example 59 herein.

Figure 36 shows the results of para-nitrophenylphosphate (pNPP) phosphatase activity assays of the purified RET31-GST full length (FL), and M1 to T302 RET31 C-terminal deletion mutant (trunc) fusion proteins, as compared to purified GST alone. The bars represent the average of triplicate determinations, and the standard deviations are as shown. Each protein preparation was assayed in the absence and presence of 2 mM orthovanadate ("-van"). As shown, both the full-length RET31 and M1 to T302 RET31 C-terminal deletion mutant demonstrated phosphatase activity via

cleavage of the NPP substrate which was blocked by the phosphatase-specific inhibitor, vanadate. Of particular significance is the unexpected five fold increase in phosphatase activity of the M1 to T302 RET31 C-terminal deletion mutant relative to the full-length RET31 polypeptide. The phosphatase assays were performed as described in Example 57 herein. The full length and truncated versions clearly demonstrated phosphatase activity compared to the GST protein.

Figure 37 shows the regions of identity between amino acid residues N157 to I300 of the BMY_HPP5 polypeptide (amino acids N157 to I300 of SEQ ID NO:42) to amino acid residues A204 to L347 of the human dual specificity phosphatase MAP Kinase phosphatase 3, also called PYST1, 1mkp (residues A204-L347; Protein Data Bank, PDB entry 1mkp chain A; Genbank Accession No. gi|5822131; SEQ ID NO:208) which was used as the basis for building the BMY_HPP5 homology model as represented in Table X and visualized in Figure 38. Amino acids defining active site residues are highlighted in bold. The alignment was created using the FASTA algorithm (Pearson, *et. al.* 1990).

Figure 38 shows a three-dimensional homology model of amino acid residues N157 to I300 of the BMY_HPP5 polypeptide based upon the homologous structure of amino acid residues A204 to L347 of the human dual specificity phosphatase MAP Kinase phosphatase 3, also called PYST1, 1mkp (residues A204-L347; Protein Data Bank, PDB entry 1mkp chain A; Genbank Accession No. gi|5822131; SEQ ID NO:208). The structural coordinates of the BMY_HPP2 polypeptide are provided in Table IX herein. The homology model of BMY_HPP2 was derived from generating a sequence alignment with the human dual specificity phosphatase MAP Kinase phosphatase 3, also called PYST1, 1mkp (residues A204-L347; Protein Data Bank, PDB entry 1mkp chain A; Genbank Accession No. gi|5822131; SEQ ID NO:208) using the INSIGHTII (Accelrys Inc., San Diego, CA) version 2000 as described herein.

Figure 39 shows an energy graph for the BMY_HPP5 model of the present invention (dotted line) and the phosphatase VHR template (PDB code 1vhr) (solid line) from

which the model was generated. The energy distribution for each protein fold is displayed on the y-axis, while the amino acid residue position of the protein fold is displayed on the x-axis. As shown, the BMY_HPP5 model and 1vhr template have similar energies over the aligned region, suggesting that the structural model of BMY_HPP5 represents a "native-like" conformation of the BMY_HPP5 polypeptide. This graph supports the motif and sequence alignments in confirming that the three dimensional structure coordinates of BMY_HPP5 are an accurate and useful representation of the structure of the BMY_HPP5 polypeptide.

Table I provides a summary of the novel polypeptides and their encoding polynucleotides of the present invention.

Table II illustrates the preferred hybridization conditions for the polynucleotides of the present invention. Other hybridization conditions may be known in the art or are described elsewhere herein.

Table III provides the amino acid sequences of known phosphatases that were used to identify the novel human phosphatases of the present invention using the BLAST algorithm as described herein.

Table IV provides the PFAM motifs that were used in Hidden Markov Model (HMM) searches to identify the novel human phosphtases of the present invention as described herein.

Table V provides the predicted exon structure of the BMY_HPP4 gene. The 'Start' and 'End' designations refer to the respective nucleotide positions of the BMY_HPP4 as they appear for the corresponding genomic sequence in BAC AL 354751. The numbering begins at the start of BAC AL354751; nucleotide 71352 in the BAC is equivalent to nucleotide 1 of the BMY_HPP4 transcript (SEQ ID NO:9; Figure 4).

Table VI provides representative primers for sequencing and/or cloning any one of the human phosphatases of the present invention in conjunction with the teachings

described herein. 'Left Cloning Primer', and 'Right Cloning Primer' represent the forward and reverse sequencing primers, while the 'Internal RevComp Cloning Primer' and/or 'Internal Cloning Primer' represent antisense cloning primers as described in the Examples herein.

Table VII provides a summary of various conservative substitutions encompassed by the present invention.

Table VIII provides the structural coordinates of the homology model of the BMY_HPP1 polypeptide provided in Figure 28. A description of the headings are as follows: "Atom No" refers to the atom number within the BMY_HPP1 homology model; "Atom name" refers to the element whose coordinates are measured, the first letter in the column defines the element; "Residue" refers to the amino acid of the BMY_HPP1 polypeptide within which the atom resides; "Residue No" refers to the amino acid position in which the atom resides, "X Coord", "Y Coord", and "Z Coord" structurally define the atomic position of the element measured in three dimensions.

Table IX provides the structural coordinates of the homology model of the BMY_HPP2 polypeptide provided in Figure 32. A description of the headings are as follows: "Atom No" refers to the atom number within the BMY_HPP2 homology model; "Atom name" refers to the element whose coordinates are measured, the first letter in the column defines the element; "Residue" refers to the amino acid of the BMY_HPP2 polypeptide within which the atom resides; "Residue No" refers to the amino acid position in which the atom resides, "X Coord", "Y Coord", and "Z Coord" structurally define the atomic position of the element measured in three dimensions.

Table X provides the structural coordinates of the homology model of the BMY_HPP5 polypeptide provided in Figure 38. A description of the headings are as follows: "Atom No" refers to the atom number within the BMY_HPP5 homology model; "Atom name" refers to the element whose coordinates are measured, the first letter in the column defines the element; "Residue" refers to the amino acid of the BMY_HPP5 polypeptide within which the atom resides; "Residue No" refers to the

amino acid position in which the atom resides, "X Coord", "Y Coord", and "Z Coord" structurally define the atomic position of the element measured in three dimensions.

DETAILED DESCRIPTION OF THE INVENTION

The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the Examples included herein. All references to "phosphatase" and/or "human phosphatases" shall be construed to apply to BMY_HPP1, BMY_HPP2, BMY_HPP3, BMY_HPP4, BMY_HPP5, RET31, mouse RET31, and/or fragments thereof unless otherwise specified herein. Moreover, since BMY_HPP5 is believed to represent a splice variant of the RET31 polypeptide, all references to "BMY_HPP5" shall be construed to apply to RET31, and all references to "RET31" shall be construed to apply to "BMY_HPP5".

The invention provides human polynucleotide sequences encoding novel human phosphatases with substantial homology to the class of phosphatases known as phosphotyrosine or dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases. Members of this class of phosphatases have been implicated in a number of diseases and/or disorders, which include, but are not limited to, bone disorders, (Yoon, HK., Baylink, DJ., Lau, KH, Am. J. Nephrol., 20(2):153-62, (2000)), disease resistance to pathogens, reproductive disorders (Gloria, Bottini, F., Nicotra, M., Lucarini, N., Borgiani, P., La, Torre, M., Amante, A., Gimelfarb, A., Bottini, E, Dis. Markers., 12(4):261-9, (1996)), neural disorders (Shimohama, S., Fujimoto, S., Taniguchi, T., Kameyama, M., Kimura, J. Ann, Neurol., 33(6):616-21, (1993)), prostate cancer (Nguyen, L., Chapdelaine, A., and Chevalier, S., Clin. Chem. 36(8 Pt 1): 1450-5 (1990)), immune disorders, particularly those relating to haematopoietic cell development, apoptosis, activation, and nonresponsiveness (Frearson, JA., Alexander, DR, Bioessays., 19(5): 417-27 (1997)), etc.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of

matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5 kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:7, 9, 41, 108, 149, 151 or the cDNA contained within the clone deposited with the ATCC. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, with or without a signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In the present invention, the full length sequence identified as SEQ ID NO: 7, 9, 41, 108, 149, 151 was often generated by overlapping sequences contained in one or more clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X was deposited with the American Type Culture Collection ("ATCC"). As shown in Table I, each clone is identified by a cDNA Clone ID (Identifier) and the ATCC Deposit Number. The ATCC is located at 10801

University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure. The deposited clone is inserted in the pSport plasmid (Life Technologies) using SalI and NotI restriction sites as described herein.

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucleotide sequence provided as SEQ ID NO: 7, 9, 41, 108, 149, 151, a nucleic acid molecule of the present invention encoding a human phosphatase polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, the complement thereof, or the cDNA within the clone deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC

(750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH2PO4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-

stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing,

phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:X" refers to a polynucleotide sequence while "SEQ ID NO:Y" refers to a polypeptide sequence, both sequences are identified by an integer specified in Table I.

"A polypeptide having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention.)

The term "organism" as referred to herein is meant to encompass any organism referenced herein, though preferably to eukaryotic organsisms, more preferably to mammals, and most preferably to humans.

The present invention encompasses the identification of proteins, nucleic acids, or other molecules, that bind to polypeptides and polynucleotides of the present invention (for example, in a receptor-ligand interaction). The polynucleotides of the present invention can also be used in interaction trap assays (such as, for example, that described by Ozenberger and Young (Mol Endocrinol., 9(10):1321-9, (1995); and Ann. N. Y. Acad. Sci., 7;766:279-81, (1995)).

The polynucleotide and polypeptides of the present invention are useful as probes for the identification and isolation of full-length cDNAs and/or genomic DNA which correspond to the polynucleotides of the present invention, as probes to

hybridize and discover novel, related DNA sequences, as probes for positional cloning of this or a related sequence, as probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides, as probes to quantify gene expression, and as probes for microarrays.

In addition, polynucleotides and polypeptides of the present invention may comprise one, two, three, four, five, six, seven, eight, or more membrane domains.

Also, in preferred embodiments the present invention provides methods for further refining the biological function of the polynucleotides and/or polypeptides of the present invention.

Specifically, the invention provides methods for using the polynucleotides and polypeptides of the invention to identify orthologs, homologs, paralogs, variants, and/or allelic variants of the invention. Also provided are methods of using the polynucleotides and polypeptides of the invention to identify the entire coding region of the invention, non-coding regions of the invention, regulatory sequences of the invention, and secreted, mature, pro-, prepro-, forms of the invention (as applicable).

In preferred embodiments, the invention provides methods for identifying the glycosylation sites inherent in the polynucleotides and polypeptides of the invention, and the subsequent alteration, deletion, and/or addition of said sites for a number of desirable characteristics which include, but are not limited to, augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

In further preferred embodiments, methods are provided for evolving the polynucleotides and polypeptides of the present invention using molecular evolution techniques in an effort to create and identify novel variants with desired structural, functional, and/or physical characteristics.

The present invention further provides for other experimental methods and procedures currently available to derive functional assignments. These procedures include but are not limited to spotting of clones on arrays, micro-array technology, PCR based methods (e.g., quantitative PCR), anti-sense methodology, gene knockout experiments, and other procedures that could use sequence information from clones to build a primer or a hybrid partner.

As used herein the terms "modulate or modulates" refer to an increase or decrease in the amount, quality or effect of a particular activity, DNA, RNA, or protein.

Polynucleotides and Polypeptides of the Invention

Features of the Polypeptide Encoded by Gene No:1

Polypeptide fragments A and B corresponding to this gene provided as SEQ ID NO:2 and 4 (Figure 1), encoded by the polynucleotide sequence according to SEQ ID NO:1 and 3 (Figure 1), the predicted full-length polypeptide sequence corresponding to this gene provided as SEQ ID NO:150 (Figures 20A-D), encoded by the full-length polynucleotide sequence according to SEQ ID NO:149 (Figures 20A-D), and/or encoded by the polynucleotide contained within the deposited clone, BMY_HPP1, has significant homology at the nucleotide and amino acid level to a number of phosphatases, which include, for example, the Schizosacchromyces Pombe protein tyrosine phosphatase PYP3 protein (PYP3_SP; Genbank Accession No:gi| P32587; SEQ ID NO:Y7); the mouse protein tyrosine phosphatase, receptor type, O, protein (MM_RPTPO; Genbank Accession No:gi| NP_035346; SEQ ID NO:Y8); and the human protein tyrosine phosphatase, receptor type, O, protein (HS_RPTPO; Genbank Accession No:gi| NP_002839; SEQ ID NO:Y9); as determined by BLASTP. An alignment of the human phosphatase polypeptide with these proteins is provided in Figures 6A-D. The conserved catalytic residues are noted.

BMY_HPP1 is a novel phosphoprotein phosphatase encoded by a human genomic BAC clone, Genbank accession AL360020. Aside from the predicted full-length BMY_HPP1 polypeptide sequence, two separate homologous regions in BAC AL360020 have been identified. Fragment A of BMY_HPP1 includes key conserved phosphatase catalytic residues: an Aspartate ("D") at amino acid 11 of SEQ ID NO:2 (Figure 1), a Cysteine ("C") at amino acid 40 of SEQ ID NO:2 (Figure 1), and an Arginine ("R") at amino acid 46 of SEQ ID NO:2 (Figure 1). Fragment B of BMY_HPP1 represents a more N-terminal fragment and is not predicted to include any catalytic residues. The predicted conserved phosphatase catalytic residues for the predicted full-length BMY_HPP1 polypeptide are as follows: conserved phophatase catalytic residues: an Aspartate ("D") at amino acid 14 of SEQ ID NO:150 (Figures 20A-D), a Cysteine ("C") at amino acid 42 of SEQ ID NO:150 (Figures 20A-D), and an Arginine ("R") at amino acid 48 of SEQ ID NO:150 (Figures 20A-D).

An alignment of the BMY_HPP1 polypeptide fragments and predicted full-length polypeptide with other phosphatase proteins (Figures 6A-D) illustrates the conserved phosphatase catalytic residues.

Based upon the strong homology to members of the phosphatase proteins, the polypeptide encoded by the human BMY_HPP1 phosphatase of the present invention is expected to share at least some biological activity with phosphatase proteins, preferably with members of the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases, particularly the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases referenced herein.

The present invention encompasses the use of BMY_HPP1 inhibitors and/or activators of BMY_HPP1 activity for the treatment, detection, amelioaration, or prevention of phosphatase associated disorders, including but not limited to metabolic diseases such as diabetes, in addition to neural and/or cardiovascular diseases and disorders. The present invention also encompasses the use of BMY_HPP1 inhibitors and/or activators of BMY_HPP1 activity as immunosuppressive agents, anti-inflammatory agents, and/or anti-tumor agents

The present invention encompasses the use of BMY_HPP1 phosphatase inhibitors, including, antagonists such as antisense nucleic acids, in addition to other antagonists, as described herein, in a therapeutic regimen to diagnose, prognose, treat, ameliorate, and/or prevent diseases where a kinase activity is insufficient. One, non-limiting example of a disease which may occur due to insufficient kinase activity are certain types of diabetes, where one or more kinases involved in the insulin receptor signal pathway may have insufficient activity or insufficient expression, for example.

Moreover, the present invention encompasses the use of BMY_HPP1 phosphatase activators, and/or the use of the BMY_HPP1 phosphatase gene or protein in a gene therapy regimen, as described herein, for the diagnoses, prognoses, treatment, amelioration, and/or prevention of diseases and/or disorders where a kinase activity is overly high, such as a cancer where a kinase oncogene product has excessive activity or excessive expression.

The present invention also encompasses the use of catalytically inactive variants of BMY_HPP1 proteins, including fragments thereof, such as a protein therapeutic, or the use of the encoding polynucleotide sequence or as gene therapy,

for example, in the diagnoses, prognosis, treatment, amelioration, and/or prevention of diseases or disorders where phosphatase activity is overly high.

The present invention encompasses the use of antibodies directed against the BMY_HPP1 polypeptides, including fragment and/or variants thereof, of the present invention in diagnostics, as a biomarkers, and/or as a therapeutic agents.

The present invention encompasses the use of an inactive, non-catalytic, mutant of the BMY_HPP1 phosphatase as a substrate trapping mutant to bind cellular phosphoproteins or a library of phosphopeptides to identify substrates of the BMY_HPP1 polypeptides.

The present invention encompasses the use of the BMY_HPP1 polypeptides, to identify inhibitors or activators of the BMY_HPP1 phosphatase activity using either in vitro or 'virtual' (in silico) screening methods.

One embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of the BMY_HPP1 phosphatase comprising the steps of: i.) contacting a BMY_HPP1 phosphatase inhibitor or activator labeled with an analytically detectable reagent with the BMY_HPP1 phosphatase under conditions sufficient to form a complex with the inhibitor or activator; ii.) contacting said complex with a sample containing a compound to be identified; iii) and identifying the compound as an inhibitor or activator by detecting the ability of the test compound to alter the amount of labeled known BMY_HPP1 phosphatase inhibitor or activator in the complex.

Another embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of a BMY_HPP1 phosphatase comprising the steps of: i.) contacting the BMY_HPP1 phosphatase with a compound to be identified; and ii.) and measuring the ability of the BMY_HPP1 phosphatase to remove phosphate from a substrate.

The present invention also encomposses a method for identifying a ligand for the BMY_HPP1 phosphatase comprising the steps of: i.) contacting the BMY_HPP1 phosphatase with a series of compounds under conditions to permit binding; and ii.) detecting the presence of any ligand-bound protein.

Preferably, the above referenced methods comprise the BMY_HPP1 phosphatase in a form selected from the group consisting of whole cells, cytosolic cell

fractions, membrane cell fractions, purified or partially purified forms. The invention also relates to recombinantly expressed BMY_HPP1 phosphatase in a purified, substantially purified, or unpurified state. The invention further relates to BMY_HPP1 phosphatase fused or conjugated to a protein, peptide, or other molecule or compound known in the art, or referenced herein.

The present invention also encompasses pharmaceutical composition of the BMY_HPP1 phosphatase polypeptide comprising a compound identified by above referenced methods and a pharmaceutically acceptable carrier.

Expression profiling designed to measure the steady state mRNA levels encoding the BMY_HPP1 polypeptide showed predominately high expression levels in testis; to a significant extent, in the spinal cord, and to a lesser extent, in pancreas, brain, pituitary, heart, and lung (as shown in Figure 22).

Moreover, additional expression profiling of the BMY_HPP1 polypeptide in normal tissues showed strong expression in a number of brain subregions and other central nervous system tissues, in particular the caudate, hippocampus and nucleus accumbens of the brain (as shown in Figure 26). These regions are known to be involved in a number of neurological disorders such as depression, bipolar disorder, schizophrenia, dementia, cognitive disorders and obesity. This data suggests a role for modulators of BMY_HPP1 activity in the treatment of neural disorders. In addition, BMY_HPP1 is strongly expressed in the adrenal, pineal and pituitary glands, suggesting a role for modulators of BMY_HPP1 activity in the treatment of endocrine disorders; in the atrium of the heart, suggesting a role for modulators of BMY_HPP1 activity in the treatment of cardiac failure or other diseases of the heart; and in the testis, suggesting a role for modulators of BMY_HPP1 activity in the treatment of male infertility caused by defective or insufficient spermatogenesis, as a contraceptive agent, or in the treatment of testicular cancer. In addition, BMY_HPP1 was expressed at lower levels across a number of tissues as well.

The strong homology to dual specificity phosphatases, combined with the predominate localized expression in testis tissue emphasizes the potential utility for BMY_HPP1 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing testicular, in addition to reproductive disorders.

In preferred embodiments, BMY_HPP1 polynucleotides and polypeptides including agonists and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing the following, non-limiting, diseases or disorders of the testis: spermatogenesis, infertility, Klinefelter's syndrome, XX male, epididymitis, genital warts, germinal cell aplasia, cryptorchidism, varicocele, immotile cilia syndrome, and viral orchitis. The BMY_HPP1 polynucleotides and polypeptides including agonists and fragments thereof, may also have uses related to modulating testicular development, embryogenesis, reproduction, and in ameliorating, treating, and/or preventing testicular proliferative disorders (e.g., cancers, which include, for example, choriocarcinoma, Nonseminoma, seminona, and testicular germ cell tumors).

Likewise, the predominate localized expression in testis tissue also emphasizes the potential utility for BMY_HPP1 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing metabolic diseases and disorders which include the following, not limiting examples: premature puberty, incomplete puberty, Kallman syndrome, Cushing's syndrome, hyperprolactinemia, hemochromatosis, congenital adrenal hyperplasia, FSH deficiency, and granulomatous disease, for example.

This gene product may also be useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. The testes are also a site of active gene expression of transcripts that is expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications.

The strong homology to dual specificity phosphatase proteins, combined with the localized expression in spinal cord, brain subregions, and other central nervous system tissues, suggests the BMY_HPP1 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in the Examples, and elsewhere herein. Briefly, the uses include, but are not

limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

The BMY_HPP1 polypeptide has been shown to comprise one glycosylation sites according to the Motif algorithm (Genetics Computer Group, Inc.). As discussed more specifically herein, protein glycosylation is thought to serve a variety of functions including: augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

Asparagine glycosylation sites have the following consensus pattern, N-{P}-[ST]-{P}, wherein N represents the glycosylation site. However, it is well known that that potential N-glycosylation sites are specific to the consensus sequence Asn-Xaa-Ser/Thr. However, the presence of the consensus tripeptide is not sufficient to conclude that an asparagine residue is glycosylated, due to the fact that the folding of the protein plays an important role in the regulation of N-glycosylation. It has been shown that the presence of proline between Asn and Ser/Thr will inhibit N-glycosylation; this has been confirmed by a recent statistical analysis of glycosylation sites, which also shows that about 50% of the sites that have a proline C-terminal to

Ser/Thr are not glycosylated. Additional information relating to asparagine glycosylation may be found in reference to the following publications, which are hereby incorporated by reference herein: Marshall R.D., Annu. Rev. Biochem. 41:673-702(1972); Pless D.D., Lennarz W.J., Proc. Natl. Acad. Sci. U.S.A. 74:134-138(1977); Bause E., Biochem. J. 209:331-336(1983); Gavel Y., von Heijne G., Protein Eng. 3:433-442(1990); and Miletich J.P., Broze G.J. Jr., J. Biol. Chem... 265:11397-11404(1990).

In preferred embodiments, the following asparagine glycosylation site polypeptide is encompassed by the present invention: LTPLRNISCCDPKA (SEQ ID NO:158). Polynucleotides encoding this polypeptide are also provided. The present invention also encompasses the use of this BMY_HPP1 asparagine glycosylation site polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

The BMY_HPP1 polypeptides of the present invention were determined to comprise several phosphorylation sites based upon the Motif algorithm (Genetics Computer Group, Inc.). The phosphorylation of such sites may regulate some biological activity of the BMY_HPP1 polypeptide. For example, phosphorylation at specific sites may be involved in regulating the proteins ability to associate or bind to other molecules (e.g., proteins, ligands, substrates, DNA, etc.). In the present case, phosphorylation may modulate the ability of the BMY_HPP1 polypeptide to associate with other potassium channel alpha subunits, beta subunits, or its ability to modulate potassium channel function.

The BMY_HPP1 polypeptide was predicted to comprise four PKC phosphorylation sites using the Motif algorithm (Genetics Computer Group, Inc.). In vivo, protein kinase C exhibits a preference for the phosphorylation of serine or threonine residues. The PKC phosphorylation sites have the following consensus pattern: [ST]-x-[RK], where S or T represents the site of phosphorylation and 'x' an intervening amino acid residue. Additional information regarding PKC phosphorylation sites can be found in Woodget J.R., Gould K.L., Hunter T., Eur. J. Biochem. 161:177-184(1986), and Kishimoto A., Nishiyama K., Nakanishi H., Uratsuji Y., Nomura H., Takeyama Y., Nishizuka Y., J. Biol. Chem... 260:12492-12499(1985); which are hereby incorporated by reference herein.

In preferred embodiments, the following PKC phosphorylation site polypeptides are encompassed by the present invention: TLSFWSQKFGGLE (SEQ ID NO:159), VQNSRTPRSPLDC (SEQ ID NO:160), PLDCGSSKAQFLV (SEQ ID NO:161), and/or PTVYNTKKIFKHT (SEQ ID NO:162). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these BMY_HPP1 PKC phosphorylation site polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In further confirmation of the human BMY_HPP1 polypeptide representing a novel human phosphatase polypeptide, the BMY_HPP1 polypeptide has been shown to comprise a tyrosine specific protein phosphatase active site domain according to the Motif algorithm (Genetics Computer Group, Inc.).

Tyrosine specific protein phosphatases (EC 3.1.3.48) (PTPase) are enzymes that catalyze the removal of a phosphate group attached to a tyrosine residue. These enzymes are very important in the control of cell growth, proliferation, differentiation and transformation. Multiple forms of PTPase have been characterized and can be classified into two categories: soluble PTPases and transmembrane receptor proteins that contain PTPase domain(s).

The currently known PTPases are listed below: Soluble PTPases, PTPN1 (PTP-1B), PTPN2 (T-cell PTPase; TC-PTP), PTPN3 (H1) and PTPN4 (MEG), enzymes that contain an N-terminal band 4.1-like domain and could act at junctions between the membrane and cytoskeleton, PTPN5 (STEP), PTPN6 (PTP-1C; HCP; SHP) and PTPN11 (PTP-2C; SH-PTP3; Syp), enzymes which contain two copies of the SH2 domain at its N-terminal extremity (e.g., the Drosophila protein corkscrew (gene csw) also belongs to this subgroup), PTPN7 (LC-PTP; Hematopoietic protein-tyrosine phosphatase; HePTP), PTPN8 (70Z-PEP), PTPN9 (MEG2), PTPN12 (PTP-G1; PTP-P19), Yeast PTP1, Yeast PTP2 which may be involved in the ubiquitin-mediated protein degradation pathway, Fission yeast pyp1 and pyp2 which play a role in inhibiting the onset of mitosis, Fission yeast pyp3 which contributes to the dephosphorylation of cdc2, Yeast CDC14 which may be involved in chromosome segregation, Yersinia virulence plasmid PTPAses (gene yopH), Autographa californica nuclear polyhedrosis virus 19 Kd PTPase, Dual specificity PTPases, DUSP1 (PTPN10; MAP kinase phosphatase-1; MKP-1); which dephosphorylates

MAP kinase on both Thr-183 and Tyr-185, DUSP2 (PAC-1), a nuclear enzyme that dephosphorylates MAP kinases ERK1 and ERK2 on both Thr and Tyr residues, DUSP3 (VHR), DUSP4 (HVH2), DUSP5 (HVH3), DUSP6 (Pyst1; MKP-3), DUSP7 (Pyst2; MKP-X), Yeast MSG5, a PTPase that dephosphorylates MAP kinase FUS3, Yeast YVH1, Vaccinia virus H1 PTPase - a dual specificity phosphatase,

Structurally, all known receptor PTPases, are made up of a variable length extracellular domain, followed by a transmembrane region and a C-terminal catalytic cytoplasmic domain. Some of the receptor PTPases contain fibronectin type III (FN-III) repeats, immunoglobulin-like domains, MAM domains or carbonic anhydrase-like domains in their extracellular region. The cytoplasmic region generally contains two copies of the PTPAse domain. The first seems to have enzymatic activity, while the second is inactive but seems to affect substrate specificity of the first. In these domains, the catalytic cysteine is generally conserved but some other, presumably important, residues are not.

PTPase domains consist of about 300 amino acids. There are two conserved cysteines, the second one has been shown to be absolutely required for activity. Furthermore, a number of conserved residues in its immediate vicinity have also been shown to be important.

A consensus sequence for tyrosine specific protein phophatases is provided as follows:

[LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAGP]-x-[LIVMFY], wherein C is the active site residue and "X" represents any amino acid.

Additional information related to tyrosine specific protein phosphatase domains and proteins may be found in reference to the following publications Fischer E.H., Charbonneau H., Tonks N.K., Science 253:401-406(1991); Charbonneau H., Tonks N.K., Annu. Rev. Cell Biol. 8:463-493(1992); Trowbridge I.S., J. Biol. Chem... 266:23517-23520(1991); Tonks N.K., Charbonneau H., Trends Biochem. Sci. 14:497-500(1989); and Hunter T., Cell 58:1013-1016(1989); which are hereby incorporated herein by reference in their entirety.

In preferred embodiments, the following tyrosine specific protein phosphatase active site domain polypeptide is encompassed by the present invention: QEGKVIHCHAGLGRTGVLIAYLV (SEQ ID NO:163). Polynucleotides encoding these

polypeptides are also provided. The present invention also encompasses the use of this tyrosine specific protein phosphatase active site domain polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

In preferred embodiments, the following N-terminal BMY_HPP1 deletion polypeptides are encompassed by the present invention: M1-L607, E2-L607, A3-L607, G4-L607, I5-L607, Y6-L607, F7-L607, Y8-L607, N9-L607, F10-L607, G11-L607, W12-L607, K13-L607, D14-L607, Y15-L607, G16-L607, V17-L607, A18-L607, S19-L607, L20-L607, T21-L607, T22-L607, I23-L607, L24-L607, D25-L607, M26-L607, V27-L607, K28-L607, V29-L607, M30-L607, T31-L607, F32-L607, A33-L607, L34-L607, Q35-L607, E36-L607, G37-L607, K38-L607, V39-L607, A40-L607, I41-L607, H42-L607, C43-L607, H44-L607, A45-L607, G46-L607, L47-L607, G48-L607, R49-L607, T50-L607, G51-L607, V52-L607, L53-L607, I54-L607, A55-L607, C56-L607, Y57-L607, L58-L607, V59-L607, F60-L607, A61-L607, T62-L607, R63-L607, M64-L607, T65-L607, A66-L607, D67-L607, Q68-L607, A69-L607, I70-L607, I71-L607, F72-L607, V73-L607, R74-L607, A75-L607, K76-L607, R77-L607, P78-L607, N79-L607, S80-L607, I81-L607, Q82-L607, T83-L607, R84-L607, G85-L607, Q86-L607, L87-L607, L88-L607, C89-L607, V90-L607, R91-L607, E92-L607, F93-L607, T94-L607, Q95-L607, F96-L607, L97-L607, T98-L607, P99-L607, L100-L607, R101-L607, N102-L607, I103-L607, F104-L607, S105-L607, C106-L607, C107-L607, D108-L607, P109-L607, K110-L607, A111-L607, H112-L607, A113-L607, V114-L607, T115-L607, L116-L607, P117-L607, Q118-L607, Y119-L607, L120-L607, I121-L607, R122-L607, Q123-L607, R124-L607, H125-L607, L126-L607, L127-L607, H128-L607, G129-L607, Y130-L607, E131-L607, A132-L607, R133-L607, L134-L607, L135-L607, K136-L607, H137-L607, V138-L607, P139-L607, K140-L607, I141-L607, I142-L607, H143-L607, L144-L607, V145-L607, C146-L607, K147-L607, L148-L607, L149-L607, L150-L607, D151-L607, L152-L607, A153-L607, E154-L607, N155-L607, R156-L607, P157-L607, V158-L607, M159-L607, M160-L607, K161-L607, D162-L607, V163-L607, S164-L607, E165-L607, G166-L607, P167-L607, G168-L607, L169-L607, S170-L607, A171-L607, E172-L607, I173-L607, E174-L607, K175-L607, T176-L607, M177-L607, S178-L607, E179-L607, M180-L607, V181-L607, T182-L607, M183-L607, Q184-L607, L185-L607, D186-L607, K187-L607, E188-L607, L189-L607, L190-L607, R191-

L607, H192-L607, D193-L607, S194-L607, D195-L607, V196-L607, S197-L607, N198-L607, P199-L607, P200-L607, N201-L607, P202-L607, T203-L607, A204-L607, V205-L607, A206-L607, A207-L607, D208-L607, F209-L607, D210-L607, N211-L607, R212-L607, G213-L607, M214-L607, I215-L607, F216-L607, S217-L607, N218-L607, E219-L607, Q220-L607, Q221-L607, F222-L607, D223-L607, P224-L607, L225-L607, W226-L607, K227-L607, R228-L607, R229-L607, N230-L607, V231-L607, E232-L607, C233-L607, L234-L607, Q235-L607, P236-L607, L237-L607, T238-L607, H239-L607, L240-L607, K241-L607, R242-L607, R243-L607, L244-L607, S245-L607, Y246-L607, S247-L607, D248-L607, S249-L607, D250-L607, L251-L607, K252-L607, R253-L607, A254-L607, E255-L607, N256-L607, L257-L607, L258-L607, E259-L607, Q260-L607, G261-L607, E262-L607, T263-L607, P264-L607, Q265-L607, T266-L607, V267-L607, P268-L607, A269-L607, Q270-L607, I271-L607, L272-L607, V273-L607, G274-L607, H275-L607, K276-L607, P277-L607, R278-L607, Q279-L607, Q280-L607, K281-L607, L282-L607, I283-L607, S284-L607, H285-L607, C286-L607, Y287-L607, I288-L607, P289-L607, Q290-L607, S291-L607, P292-L607, E293-L607, P294-L607, D295-L607, L296-L607, H297-L607, K298-L607, E299-L607, A300-L607, L301-L607, V302-L607, R303-L607, S304-L607, T305-L607, L306-L607, S307-L607, F308-L607, W309-L607, S310-L607, Q311-L607, S312-L607, K313-L607, F314-L607, G315-L607, G316-L607, L317-L607, E318-L607, G319-L607, L320-L607, K321-L607, D322-L607, N323-L607, G324-L607, S325-L607, P326-L607, I327-L607, F328-L607, H329-L607, G330-L607, R331-L607, I332-L607, I333-L607, P334-L607, K335-L607, E336-L607, A337-L607, Q338-L607, Q339-L607, S340-L607, G341-L607, A342-L607, F343-L607, S344-L607, A345-L607, D346-L607, V347-L607, S348-L607, G349-L607, S350-L607, H351-L607, S352-L607, P353-L607, G354-L607, E355-L607, P356-L607, V357-L607, S358-L607, P359-L607, S360-L607, F361-L607, A362-L607, N363-L607, V364-L607, H365-L607, K366-L607, D367-L607, P368-L607, N369-L607, P370-L607, A371-L607, H372-L607, Q373-L607, Q374-L607, V375-L607, S376-L607, H377-L607, C378-L607, Q379-L607, C380-L607, K381-L607, T382-L607, H383-L607, G384-L607, V385-L607, G386-L607, S387-L607, P388-L607, G389-L607, S390-L607, V391-L607, R392-L607, Q393-L607, N394-L607, S395-L607, R396-L607, T397-L607, P398-L607, R399-

L607, S400-L607, P401-L607, L402-L607, D403-L607, C404-L607, G405-L607, S406-L607, S407-L607, P408-L607, K409-L607, A410-L607, Q411-L607, F412-L607, L413-L607, V414-L607, E415-L607, H416-L607, E417-L607, T418-L607, Q419-L607, D420-L607, S421-L607, K422-L607, D423-L607, L424-L607, S425-L607, E426-L607, A427-L607, A428-L607, S429-L607, H430-L607, S431-L607, A432-L607, L433-L607, Q434-L607, S435-L607, E436-L607, L437-L607, S438-L607, A439-L607, E440-L607, A441-L607, R442-L607, R443-L607, I444-L607, L445-L607, A446-L607, A447-L607, K448-L607, A449-L607, L450-L607, A451-L607, N452-L607, L453-L607, N454-L607, E455-L607, S456-L607, V457-L607, E458-L607, K459-L607, E460-L607, E461-L607, L462-L607, K463-L607, R464-L607, K465-L607, V466-L607, E467-L607, M468-L607, W469-L607, Q470-L607, K471-L607, E472-L607, L473-L607, N474-L607, S475-L607, R476-L607, D477-L607, G478-L607, A479-L607, W480-L607, E481-L607, R482-L607, I483-L607, C484-L607, G485-L607, E486-L607, R487-L607, D488-L607, P489-L607, F490-L607, I491-L607, L492-L607, C493-L607, S494-L607, L495-L607, M496-L607, W497-L607, S498-L607, W499-L607, V500-L607, E501-L607, Q502-L607, L503-L607, K504-L607, E505-L607, P506-L607, V507-L607, I508-L607, T509-L607, K510-L607, E511-L607, D512-L607, V513-L607, D514-L607, M515-L607, L516-L607, V517-L607, D518-L607, R519-L607, R520-L607, A521-L607, D522-L607, A523-L607, A524-L607, E525-L607, A526-L607, L527-L607, F528-L607, L529-L607, L530-L607, E531-L607, K532-L607, G533-L607, Q534-L607, H535-L607, Q536-L607, T537-L607, I538-L607, L539-L607, C540-L607, V541-L607, L542-L607, H543-L607, C544-L607, I545-L607, V546-L607, N547-L607, L548-L607, Q549-L607, T550-L607, I551-L607, P552-L607, V553-L607, D554-L607, V555-L607, E556-L607, E557-L607, A558-L607, F559-L607, L560-L607, A561-L607, H562-L607, A563-L607, I564-L607, K565-L607, A566-L607, F567-L607, T568-L607, K569-L607, V570-L607, N571-L607, F572-L607, D573-L607, S574-L607, E575-L607, N576-L607, G577-L607, P578-L607, T579-L607, V580-L607, Y581-L607, N582-L607, T583-L607, L584-L607, K585-L607, K586-L607, I587-L607, F588-L607, K589-L607, H590-L607, T591-L607, L592-L607, E593-L607, E594-L607, K595-L607, R596-L607, K597-L607, M598-L607, T599-L607, K600-L607, and/or D601-L607 of SEQ ID NO:150. Polynucleotide sequences encoding these

polypeptides are also provided. The present invention also encompasses the use of these N-terminal BMY_HPP1 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal BMY_HPP1 deletion polypeptides are encompassed by the present invention: M1-L607, M1-G606, M1-P605, M1-K604, M1-P603, M1-G602, M1-D601, M1-K600, M1-T599, M1-M598, M1-K597, M1-R596, M1-K595, M1-E594, M1-E593, M1-L592, M1-T591, M1-H590, M1-K589, M1-F588, M1-I587, M1-K586, M1-K585, M1-L584, M1-T583, M1-N582, M1-Y581, M1-V580, M1-T579, M1-P578, M1-G577, M1-N576, M1-E575, M1-S574, M1-D573, M1-F572, M1-N571, M1-V570, M1-K569, M1-T568, M1-F567, M1-A566, M1-K565, M1-I564, M1-A563, M1-H562, M1-A561, M1-L560, M1-F559, M1-A558, M1-E557, M1-E556, M1-V555, M1-D554, M1-V553, M1-P552, M1-I551, M1-T550, M1-Q549, M1-L548, M1-N547, M1-V546, M1-I545, M1-C544, M1-H543, M1-L542, M1-V541, M1-C540, M1-L539, M1-I538, M1-T537, M1-Q536, M1-H535, M1-Q534, M1-G533, M1-K532, M1-E531, M1-L530, M1-L529, M1-F528, M1-L527, M1-A526, M1-E525, M1-A524, M1-A523, M1-D522, M1-A521, M1-R520, M1-R519, M1-D518, M1-V517, M1-L516, M1-M515, M1-D514, M1-V513, M1-D512, M1-E511, M1-K510, M1-T509, M1-I508, M1-V507, M1-P506, M1-E505, M1-K504, M1-L503, M1-Q502, M1-E501, M1-V500, M1-W499, M1-S498, M1-W497, M1-M496, M1-L495, M1-S494, M1-C493, M1-L492, M1-I491, M1-F490, M1-P489, M1-D488, M1-R487, M1-E486, M1-G485, M1-C484, M1-I483, M1-R482, M1-E481, M1-W480, M1-A479, M1-G478, M1-D477, M1-R476, M1-S475, M1-N474, M1-L473, M1-E472, M1-K471, M1-Q470, M1-W469, M1-M468, M1-E467, M1-V466, M1-K465, M1-R464, M1-K463, M1-L462, M1-E461, M1-E460, M1-K459, M1-E458, M1-V457, M1-S456, M1-E455, M1-N454, M1-L453, M1-N452, M1-A451, M1-L450, M1-A449, M1-K448, M1-A447, M1-A446, M1-L445, M1-I444, M1-R443, M1-R442, M1-A441, M1-E440, M1-A439, M1-S438, M1-L437, M1-E436, M1-S435, M1-Q434, M1-L433, M1-A432, M1-S431, M1-H430, M1-S429, M1-A428, M1-A427, M1-E426, M1-S425, M1-L424, M1-D423, M1-K422, M1-S421, M1-D420, M1-Q419, M1-T418, M1-E417, M1-H416, M1-E415, M1-V414, M1-L413, M1-F412, M1-Q411, M1-A410, M1-K409, M1-P408, M1-S407, M1-S406, M1-G405, M1-C404, M1-D403, M1-L402, M1-P401,

M1-S400, M1-R399, M1-P398, M1-T397, M1-R396, M1-S395, M1-N394, M1-Q393, M1-R392, M1-V391, M1-S390, M1-G389, M1-P388, M1-S387, M1-G386, M1-V385, M1-G384, M1-H383, M1-T382, M1-K381, M1-C380, M1-Q379, M1-C378, M1-H377, M1-S376, M1-V375, M1-Q374, M1-Q373, M1-H372, M1-A371, M1-P370, M1-N369, M1-P368, M1-D367, M1-K366, M1-H365, M1-V364, M1-N363, M1-A362, M1-F361, M1-S360, M1-P359, M1-S358, M1-V357, M1-P356, M1-E355, M1-G354, M1-P353, M1-S352, M1-H351, M1-S350, M1-G349, M1-S348, M1-V347, M1-D346, M1-A345, M1-S344, M1-F343, M1-A342, M1-G341, M1-S340, M1-Q339, M1-Q338, M1-A337, M1-E336, M1-K335, M1-P334, M1-I333, M1-I332, M1-R331, M1-G330, M1-H329, M1-F328, M1-I327, M1-P326, M1-S325, M1-G324, M1-N323, M1-D322, M1-K321, M1-L320, M1-G319, M1-E318, M1-L317, M1-G316, M1-G315, M1-F314, M1-K313, M1-S312, M1-Q311, M1-S310, M1-W309, M1-F308, M1-S307, M1-L306, M1-T305, M1-S304, M1-R303, M1-V302, M1-L301, M1-A300, M1-E299, M1-K298, M1-H297, M1-L296, M1-D295, M1-P294, M1-E293, M1-P292, M1-S291, M1-Q290, M1-P289, M1-I288, M1-Y287, M1-C286, M1-H285, M1-S284, M1-I283, M1-L282, M1-K281, M1-Q280, M1-Q279, M1-R278, M1-P277, M1-K276, M1-H275, M1-G274, M1-V273, M1-L272, M1-I271, M1-Q270, M1-A269, M1-P268, M1-V267, M1-T266, M1-Q265, M1-P264, M1-T263, M1-E262, M1-G261, M1-Q260, M1-E259, M1-L258, M1-L257, M1-N256, M1-E255, M1-A254, M1-R253, M1-K252, M1-L251, M1-D250, M1-S249, M1-D248, M1-S247, M1-Y246, M1-S245, M1-L244, M1-R243, M1-R242, M1-K241, M1-L240, M1-H239, M1-T238, M1-L237, M1-P236, M1-Q235, M1-L234, M1-C233, M1-E232, M1-V231, M1-N230, M1-R229, M1-R228, M1-K227, M1-W226, M1-L225, M1-P224, M1-D223, M1-F222, M1-Q221, M1-Q220, M1-E219, M1-N218, M1-S217, M1-F216, M1-I215, M1-M214, M1-G213, M1-R212, M1-N211, M1-D210, M1-F209, M1-D208, M1-A207, M1-A206, M1-V205, M1-A204, M1-T203, M1-P202, M1-N201, M1-P200, M1-P199, M1-N198, M1-S197, M1-V196, M1-D195, M1-S194, M1-D193, M1-H192, M1-R191, M1-L190, M1-L189, M1-E188, M1-K187, M1-D186, M1-L185, M1-Q184, M1-M183, M1-T182, M1-V181, M1-M180, M1-E179, M1-S178, M1-M177, M1-T176, M1-K175, M1-E174, M1-I173, M1-E172, M1-A171, M1-S170, M1-L169, M1-G168, M1-P167, M1-G166, M1-E165, M1-S164, M1-V163, M1-D162, M1-K161, M1-M160, M1-M159, M1-V158,

M1-P157, M1-R156, M1-N155, M1-E154, M1-A153, M1-L152, M1-D151, M1-L150, M1-L149, M1-L148, M1-K147, M1-C146, M1-V145, M1-L144, M1-H143, M1-I142, M1-I141, M1-K140, M1-P139, M1-V138, M1-H137, M1-K136, M1-L135, M1-L134, M1-R133, M1-A132, M1-E131, M1-Y130, M1-G129, M1-H128, M1-L127, M1-L126, M1-H125, M1-R124, M1-Q123, M1-R122, M1-I121, M1-L120, M1-Y119, M1-Q118, M1-P117, M1-L116, M1-T115, M1-V114, M1-A113, M1-H112, M1-A111, M1-K110, M1-P109, M1-D108, M1-C107, M1-C106, M1-S105, M1-F104, M1-I103, M1-N102, M1-R101, M1-L100, M1-P99, M1-T98, M1-L97, M1-F96, M1-Q95, M1-T94, M1-F93, M1-E92, M1-R91, M1-V90, M1-C89, M1-L88, M1-L87, M1-Q86, M1-G85, M1-R84, M1-T83, M1-Q82, M1-I81, M1-S80, M1-N79, M1-P78, M1-R77, M1-K76, M1-A75, M1-R74, M1-V73, M1-F72, M1-I71, M1-I70, M1-A69, M1-Q68, M1-D67, M1-A66, M1-T65, M1-M64, M1-R63, M1-T62, M1-A61, M1-F60, M1-V59, M1-L58, M1-Y57, M1-C56, M1-A55, M1-I54, M1-L53, M1-V52, M1-G51, M1-T50, M1-R49, M1-G48, M1-L47, M1-G46, M1-A45, M1-H44, M1-C43, M1-H42, M1-I41, M1-A40, M1-V39, M1-K38, M1-G37, M1-E36, M1-Q35, M1-L34, M1-A33, M1-F32, M1-T31, M1-M30, M1-V29, M1-K28, M1-V27, M1-M26, M1-D25, M1-L24, M1-I23, M1-T22, M1-T21, M1-L20, M1-S19, M1-A18, M1-V17, M1-G16, M1-Y15, M1-D14, M1-K13, M1-W12, M1-G11, M1-F10, M1-N9, M1-Y8, and/or M1-F7 of SEQ ID NO:150. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these C-terminal BMY_HPP1 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following polypeptide is encompassed by the present invention:

MEAGIYFNFGWKDYGVASLTTIDMVKVMTFALQEGKVIHCHAGLGRTGVLI
AYLVFATRMTADQAIIVRAKRPNSIQTRGQLCVREFTQFLTPLRNISCCDPKA
HAVTLPQYIRQRHLLHGYEARLLHVPKIIHLVCKLLLDAENRPVMMKDVSEG
PLSAEIEKTMSEMVTMLDKELLRHDSDVSNPNPTAVAADFDNRGMISNEQQF
DPLWKRRNVCLQPLTHLKRRLSYSSDLKRAENLLEQGETQTVPAQILVGHKP
RQKLISHCYIPQSPEPDHKEALVRSTLSFWSQKFGGLEGLKDNGSPIHGRIIPKE
AQQSGAFADVSGSHSPGEPVSPFANVHKDPNPAHQQVHCQCKTHGVGSPGS
VQNSRTPRSPLDCGSSKAQFLVEHETQDSKDSEAASHSALQSELSAARRILAA

KALANLNEVEKEELKRKVEMWQKLNSRDGAWERICGERPFILCSLMWSWVE (SEQ ID NO:153). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of this polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

In preferred embodiments, the following BMY_HPP1 phosphatase active site domain amino acid substitutions are encompassed by the present invention: wherein M1 is substituted with either an A, C, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y; wherein E2 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A3 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein G4 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein I5 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Y6 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein F7 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein N8 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein F9 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein G10 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein W11 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or Y; wherein K12 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein D13 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Y14 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein G15 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V16 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein A17 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S18 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein L19 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein T20 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein T21 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein I22 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein D23 is substituted with either an

A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein M24 is substituted with either an A, C, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y; wherein V25 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein K26 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V27 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein M28 is substituted with either an A, C, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y; wherein T29 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein F30 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A31 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L32 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein Q33 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; wherein E34 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein G35 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein K36 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V37 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein I38 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein H39 is substituted with either an A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein C40 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein H41 is substituted with either an A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A42 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein G43 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L44 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein G45 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein R46 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein T47 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein G48 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V49 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein L50 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S,

T, V, W, or Y; wherein I51 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A52 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Y53 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein L54 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein V55 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein F56 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; and/or wherein A57 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y of SEQ ID NO:150, in addition to any combination thereof. The present invention also encompasses the use of these BMY_HPP1 phosphatase active site domain amino acid substituted polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following BMY_HPP1 phosphatase active site domain conservative amino acid substitutions are encompassed by the present invention: wherein M1 is substituted with either an A, G, S, or T; wherein E2 is substituted with a D; wherein A3 is substituted with either a G, I, L, M, S, T, or V; wherein G4 is substituted with either an A, M, S, or T; wherein I5 is substituted with either an A, V, or L; wherein Y6 is either an F, or W; wherein F7 is substituted with either a W, or Y; wherein N8 is substituted with a Q; wherein F9 is substituted with either a W, or Y; wherein G10 is substituted with either an A, M, S, or T; wherein W11 is either an F, or Y; wherein K12 is substituted with either a R, or H; wherein D13 is substituted with an E; wherein Y14 is either an F, or W; wherein G15 is substituted with either an A, M, S, or T; wherein V16 is substituted with either an A, I, or L; wherein A17 is substituted with either a G, I, L, M, S, T, or V; wherein S18 is substituted with either an A, G, M, or T; wherein L19 is substituted with either an A, I, or V; wherein T20 is substituted with either an A, G, M, or S; wherein T21 is substituted with either an A, G, M, or S; wherein I22 is substituted with either an A, V, or L; wherein D23 is substituted with an E; wherein M24 is substituted with either an A, G, S, or T; wherein V25 is substituted with either an A, I, or L; wherein K26 is substituted with either a R, or H; wherein V27 is substituted with either an A, I, or L; wherein M28 is substituted with either an A, G, S, or T; wherein T29 is substituted with either an A, G, M, or S; wherein F30 is substituted with either a W, or Y;

wherein A31 is substituted with either a G, I, L, M, S, T, or V; wherein L32 is substituted with either an A, I, or V; wherein Q33 is substituted with a N; wherein E34 is substituted with a D; wherein G35 is substituted with either an A, M, S, or T; wherein K36 is substituted with either a R, or H; wherein V37 is substituted with either an A, I, or L; wherein I38 is substituted with either an A, V, or L; wherein H39 is substituted with either a K, or R; wherein C40 is a C; wherein H41 is substituted with either a K, or R; wherein A42 is substituted with either a G, I, L, M, S, T, or V; wherein G43 is substituted with either an A, M, S, or T; wherein L44 is substituted with either an A, I, or V; wherein G45 is substituted with either an A, M, S, or T; wherein R46 is substituted with either a K, or H; wherein T47 is substituted with either an A, G, M, or S; wherein G48 is substituted with either an A, M, S, or T; wherein V49 is substituted with either an A, I, or L; wherein L50 is substituted with either an A, I, or V; wherein I51 is substituted with either an A, V, or L; wherein A52 is substituted with either a G, I, L, M, S, T, or V; wherein Y53 is either an F, or W; wherein L54 is substituted with either an A, I, or V; wherein V55 is substituted with either an A, I, or L; wherein F56 is substituted with either a W, or Y; and/or wherein A57 is substituted with either a G, I, L, M, S, T, or V of SEQ ID NO:150 in addition to any combination thereof. Other suitable substitutions within the BMY_HPP1 phosphatase active site domain are encompassed by the present invention and are referenced elsewhere herein. The present invention also encompasses the use of these BMY_HPP1 phosphatase active site domain conservative amino acid substituted polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In addition, the present invention also encompasses BMY_HPP1 polypeptides resulting from alternative initiating start codon positions of the BMY_HPP1 polynucleotide (SEQ ID NO:149).

In preferred embodiments, the following polypeptide resulting from the start codon beginning at nucleotide 31 of SEQ ID NO:149 is encompassed by the present invention:

MQVQDATRRPSAVRFLSSFLQGRRHSTSDPVLRLQQARRGSGLGSGSATKLLSSSSLQVMVAV
SSVSHAEGNPTFPERKRNLERPTPKYTKVGERLRHVIPGHMACSMACGGRACKYENPARWSE
QEQAIKGVYSSWVTDNILAMARPSSELLEKYHIIDQFLSHGIKTIINLQRPGEHASCGNPLEQES
GFTYLPEAFMEAGIYFYNFGWKDYGVASLTTILDMVKVMTFALQEGKVAIHCHAGLGRTGVL

IACYLVFATRMTADQAIIFVRAKRPNSIQTRGQLLCVREFTQFLTPLRNIFSCCDPKAHAVTLPQ
YLIRQRHLLHGYEARLLKHVPKIIHLVCKLLLDLAENRPVMMKDVSEGPGLSAEIEKTMSEMV
TMQLDKELLRHDSDVSNPPNPTAVAADFDNRGMIFSNEQQFDPLWKRRNVECLQPLTHLKRR
LSYSDSDLKRAENLLEQGETPQTVPAQILVGHKPRQQKLISHCYIPQSPEPDLHKEALVRSTLSF
WSQSKFGGLEGLKDNGSPIFHGRIIPKEAQQSGAFSADVSGSHSPGEPVSPSFANVHKDPNPAH
QQVSHCQCKTHGVGSPGSVRQNSRTPRSPLDCGSSPKAQFLVEHETQDSKDLSEAASHSALQS
ELSAEARRILAAKALANLNESVEKEELKRKVEMWQKELNSRDGAWERICGERDPFILCSLMW
SWVEQLKEPVITKEDVDMLVDRRADAAEALFLLEKGQHQTILCVLHCIVNLQTIPVDVEEAFL
AHAIKAFTKVNFDSENGPTVYNTLKKIFKHTLEEKRKMTKDGPKPGL (SEQ ID NO:175).
Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of this polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

In preferred embodiments, the following polypeptide resulting from the start codon beginning at nucleotide 208 of SEQ ID NO:149 is encompassed by the present invention:

MVAVSSVSHAEGNPTFPERKRNLERPTPKYTKVGERLRHVIPGHMACSMACG
GRACKYENPARWSEQEQAIKGVYSSWVTDNILAMARPSSELLEKYHIIDQFLS
HGIKTIINLQRPGEHASCGNPLEQESGFTYLPEAFMEAGIYFYNFGWKDYGVA
SLTTILDMVKVMTFALQEGKVAIHCHAGLGRTGVLIACYLVFATRMTADQAI
IFVRAKRPNSIQTRGQLLCVREFTQFLTPLRNIFSCCDPKAHAVTLPQYLIRQR
HLLHGYEARLLKHVPKIIHLVCKLLLDLAENRPVMMKDVSEGPGLSAEIEKT
MSEMVTMQLDKELLRHDSDVSNPPNPTAVAADFDNRGMIFSNEQQFDPLWK
RRNVECLQPLTHLKRRLSYSDSDLKRAENLLEQGETPQTVPAQILVGHKPRQ
QKLISHCYIPQSPEPDLHKEALVRSTLSFWSQSKFGGLEGLKDNGSPIFHGRIIP
KEAQQSGAFSADVSGSHSPGEPVSPSFANVHKDPNPAHQQVSHCQCKTHGVG
SPGSVRQNSRTPRSPLDCGSSPKAQFLVEHETQDSKDLSEAASHSALQSELSAE
ARRILAAKALANLNESVEKEELKRKVEMWQKELNSRDGAWERICGERDPFIL
CSLMWSWVEQLKEPVITKEDVDMLVDRRADAAEALFLLEKGQHQTILCVLH
CIVNLQTIPVDVEEAFLAHAIKAFTKVNFDSENGPTVYNTLKKIFKHTLEEKRK
MTKDGPKPGL (SEQ ID NO:176). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of this polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

In preferred embodiments, the following polypeptide resulting from the start codon beginning at nucleotide 352 of SEQ ID NO:149 is encompassed by the present

invention:

MACGGRACKYENPARWSEQEQAIKGVYSSWVTDNILAMARPSSELLEKYHII
DQFLSHGIKTIINLQRPGEHASCGNPLEQESGFTYLPEAFMEAGIYFYNFGWK
DYGVASLTTILDMVKVMTFALQEGKVAIHCHAGLGRTGVLIACYLVFATRM
TADQAIIFVRAKRPNSIQTRGQLLCVREFTQFLTPLRNIFSCCDPKAHAVTLPQ
YLIRQRHLLHGYEARLLKHVPKIIHLVCKLLLDLAENRPVMMKDVSEGPGLS
AEIEKTMSEMVTMQLDKELLRHDSDVSNPPNPTAVAADFDNRGMIFSNEQQF
DPLWKRRNVECLQPLTHLKRRLSYSDSDLKRAENLLEQGETPQTVPAQILVG
HKPRQQKLISHCYIPQSPEPDLHKEALVRSTLSFWSQSKFGGLEGLKDNGSPIF
HGRIIPKEAQQSGAFSADVSGSHSPGEPVSPSFANVHKDPNPAHQQVSHCQCK
THGVGSPGSVRQNSRTPRSPLDCGSSPKAQFLVEHETQDSKDLSEAASHSALQ
SELSAEARRILAAKALANLNESVEKEELKRKVEMWQKELNSRDGAWERICGE
RDPFILCSLMWSWVEQLKEPVITKEDVDMLVDRRADAAEALFLLEKGQHQTI
LCVLHCIVNLQTIPVDVEEAFLAHAIKAFTKVNFDSENGPTVYNTLKKIFKHTL
EEKRKMTKDGPKPGL (SEQ ID NO:177). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of this polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

In preferred embodiments, the following polypeptide resulting from the start codon beginning at nucleotide 463 of SEQ ID NO:149 is encompassed by the present invention:

MARPSSELLEKYHIIDQFLSHGIKTIINLQRPGEHASCGNPLEQESGFTYLPEAF
MEAGIYFYNFGWKDYGVASLTTILDMVKVMTFALQEGKVAIHCHAGLGRTG
VLIACYLVFATRMTADQAIIFVRAKRPNSIQTRGQLLCVREFTQFLTPLRNIFSC
CDPKAHAVTLPQYLIRQRHLLHGYEARLLKHVPKIIHLVCKLLLDLAENRPV
MMKDVSEGPGLSAEIEKTMSEMVTMQLDKELLRHDSDVSNPPNPTAVAADF
DNRGMIFSNEQQFDPLWKRRNVECLQPLTHLKRRLSYSDSDLKRAENLLEQG
ETPQTVPAQILVGHKPRQQKLISHCYIPQSPEPDLHKEALVRSTLSFWSQSKFG
GLEGLKDNGSPIFHGRIIPKEAQQSGAFSADVSGSHSPGEPVSPSFANVHKDPN
PAHQQVSHCQCKTHGVGSPGSVRQNSRTPRSPLDCGSSPKAQFLVEHETQDS
KDLSEAASHSALQSELSAEARRILAAKALANLNESVEKEELKRKVEMWQKEL
NSRDGAWERICGERDPFILCSLMWSWVEQLKEPVITKEDVDMLVDRRADAA

EALFLLEKGQHQTILCVLHCIVNLQTIPVDVEEAFLAHAIKAFTKVNFDSENGP TVYNTLKKIFKHTLEEKRKMTKDGPKPGL (SEQ ID NO:178). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of this polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

In preferred embodiments, the present invention encompasses a polynucleotide lacking the initiating start codon, in addition to, the resulting encoded polypeptide of BMY_HPP1. Specifically, the present invention encompasses the polynucleotide corresponding to nucleotides 631 thru 2448 of SEQ ID NO:149, and the polypeptide corresponding to amino acids 2 thru 607 of SEQ ID NO:150. Also encompassed are recombinant vectors comprising said encoding sequence, and host cells comprising said vector.

The present invention also provides a three-dimensional homology model of the BMY_HPP1 polypeptide (see Figure 28) representing amino acids M1 to E301 of BMY_HPP1 (SEQ ID NO:150). A three-dimensional homology model can be constructed on the basis of the known structure of a homologous protein (Greer et al, 1991, Lesk, et al, 1992, Cardozo, et al, 1995, Yuan, et al, 1995). The homology model of the BMY_HPP1 polypeptide, corresponding to amino acid residues M1 to E301 of SEQ ID NO:150, was based upon the homologous structure of 1aax, a Human Protein Tyrosine Phosphatase Complex (residues D11-N321; Protein Data Bank, PDB entry 1aax chain A; Genbank Accession No. gi|2981942; SEQ ID NO:206) and is defined by the set of structural coordinates set forth in Table VIII herein.

Homology models are useful when there is no experimental information available on the protein of interest. A 3-dimensional model can be constructed on the basis of the known structure of a homologous protein (Greer *et. al.*, 1991, Lesk, *et. al.*, 1992, Cardozo, *et. al.*, 1995, Sali, *et. al.*, 1995).

Those of skill in the art will understand that a homology model is constructed on the basis of first identifying a template, or, protein of known structure which is similar to the protein without known structure. This can be accomplished through pairwise alignment of sequences using such programs as FASTA (Pearson, *et. al.* 1990) and BLAST (Altschul, *et. al.*, 1990). In cases where sequence similarity is high (greater than 30 %), these pairwise comparison methods may be adequate. Likewise,

multiple sequence alignments or profile-based methods can be used to align a query sequence to an alignment of multiple (structurally and biochemically) related proteins. When the sequence similarity is low, more advanced techniques are used such as fold recognition (protein threading; Hendlich, *et. al.*, 1990), where the compatibility of a particular sequence with the 3-dimensional fold of a potential template protein is gauged on the basis of a knowledge-based potential. Following the initial sequence alignment, the query template can be optimally aligned by manual manipulation or by incorporation of other features (motifs, secondary structure predictions, and allowed sequence conservation). Next, structurally conserved regions can be identified and are used to construct the core secondary structure (Sali, *et. al.*, 1995) elements in the three dimensional model. Variable regions, called "unconserved regions" and loops can be added using knowledge-based techniques. The complete model with variable regions and loops can be refined performing forcefield calculations (Sali, *et. al.*, 1995, Cardozo, *et. al.*, 1995).

Protein threading and molecular modeling of BMY_HPP1 suggested that a portion of BMY_HPP1 (residues M1 to E301) had a three dimensional fold similar to that of 1aax, a Human Protein Tyrosine Phosphatase Complex (residues D11-N321; Protein Data Bank, PDB entry 1aax chain A; Genbank Accession No. gi|2981942; SEQ ID NO:206). Based on sequence, structure and known phosphatase signature sequences, BMY_HPP1 is a novel tyrosine specific phosphatase.

For BMY_HPP1, the pairwise alignment method FASTA (Pearson, *et. al.* 1990) and fold recognition methods (protein threading) generated identical sequence alignments for a portion (residues M1 to E301) of BMY_HPP1 aligned with the sequence of 1aax a tyrosine specific phosphatase (residues D11-N321 ; Protein Data Bank, PDB entry 1aax chain A; Genbank Accession No. gi|2981942; SEQ ID NO:206). The alignment of BMY_HPP1 with PDB entry 1aax is set forth in Figure 27. In this invention, the homology model of BMY_HPP1 was derived from the sequence alignment set forth in Figure 27 (residues D11-N321 of SEQ ID NO:206). An overall atomic model including plausible sidechain orientations was generated using the program LOOK (Levitt 1992). The three dimensional model for BMY_HPP1 is defined by the set of structure coordinates as set forth in Table VIII and is shown in Figure 28 rendered by backbone secondary structures.

In order to recognize errors in three-dimensional structure, knowledge based mean fields can be used to judge the quality of protein folds (Sippl 1993). The methods can be used to recognize misfolded structures as well as faulty parts of structural models. The technique generates an energy graph where the energy distribution for a given protein fold is displayed on the y-axis and residue position in the protein fold is displayed on the x-axis. The knowledge based mean fields compose a force field derived from a set of globular protein structures taken as a subset from the Protein Data Bank (Bernstein et. al. 1977). To analyze the quality of a model the energy distribution is plotted and compared to the energy distribution of the template from which the model was generated. Figure 29 shows the energy graph for the BMY_HPP1 model (dotted line) and the template (1aax, a tyrosine specific phosphatase) from which the model was generated. It is clear that the model has slightly higher energies in the C-terminal region while the N-terminal region appears to be "native-like". This graph supports the motif and sequence alignments in confirming that the three dimensional structure coordinates of BMY_HPP1 are an accurate and useful representation for the polypeptide.

The term "structure coordinates" refers to Cartesian coordinates generated from the building of a homology model.

Those of skill in the art will understand that a set of structure coordinates for a protein is a relative set of points that define a shape in three dimensions. Thus, it is possible that an entirely different set of coordinates could define a similar or identical shape. Moreover, slight variations in the individual coordinates, as emanate from generation of similar homology models using different alignment templates (i.e., other than the structure coordinates of 1aax), and/or using different methods in generating the homology model, will have minor effects on the overall shape. Variations in coordinates may also be generated because of mathematical manipulations of the structure coordinates. For example, the structure coordinates set forth in Table VIII and shown in Figure 28 could be manipulated by fractionalization of the structure coordinates; integer additions or subtractions to sets of the structure coordinates, inversion of the structure coordinates or any combination of the above.

Various computational analyses are therefore necessary to determine whether a molecule or a portion thereof is sufficiently similar to all or parts of BMY_HPP1

described above as to be considered the same. Such analyses may be carried out in current software applications, such as INSIGHTII (Accelrys Inc., San Diego, CA) version 2000 as described in the User's Guide, online (www.accelrys.com) or software applications available in the SYBYL software suite (Tripos Inc., St. Louis, MO).

Using the superimposition tool in the program INSIGHTII comparisons can be made between different structures and different conformations of the same structure. The procedure used in INSIGHTII to compare structures is divided into four steps: 1) load the structures to be compared; 2) define the atom equivalencies in these structures; 3) perform a fitting operation; and 4) analyze the results. Each structure is identified by a name. One structure is identified as the target (i.e., the fixed structure); the second structure (i.e., moving structure) is identified as the source structure. Since atom equivalency within INSIGHTII is defined by user input, for the purpose of this invention we will define equivalent atoms as protein backbone atoms (N, Cα, C and O) for all conserved residues between the two structures being compared. We will also consider only rigid fitting operations. When a rigid fitting method is used, the working structure is translated and rotated to obtain an optimum fit with the target structure. The fitting operation uses an algorithm that computes the optimum translation and rotation to be applied to the moving structure, such that the root mean square difference of the fit over the specified pairs of equivalent atom is an absolute minimum. This number, given in angstroms, is reported by INSIGHTII. For the purpose of this invention, any homology model of a BMY_HPP1 that has a root mean square deviation of conserved residue backbone atoms (N, Cα, C, O) of less than 3.0 A when superimposed on the relevant backbone atoms described by structure coordinates listed in Table VIII and shown in Figure 28 are considered identical. More preferably, the root mean square deviation is less than 2.0 Å.

This invention as embodied by the homology model enables the structure-based design of modulators of the biological function of BMY_HPP1, as well as mutants with altered biological function and/or specificity.

There is 18% sequence identity between catalytic domain of BMY_HPP1 and the Human Protein Tyrosine Phosphatase 1B (PTP1B; PDB code 1aax) as determined using the GAP program within GCG (Genetics Computing Group, Wisconsin). The structure was used as the template to generate the three dimensional model for

BMY_HPP1. For BMY_HPP1, the functionally important residues are located in a cleft near the site that in other phosphatases is shown to be the active site. The active site residues are defined by : H189-C190-G193-R196 and D 161 as well as Y162. All these residues are conserved in PTP1B (denoted by the "*" in Figure 27) and other known phosphatases. In the 1aax polypeptide, the Cysteine was mutated to a Serine to facilitate structural analysis (Jia, Z., et al., 1995). These active site residues play critical roles in the mechanism of catalysis and substrate specificity and binding.

In a preferred embodiment of the present invention, the molecule comprises the active site region defined by structure coordinates of BMY_HPP1 amino acids described above according to Table VIII, or a mutant of said molecule. The active site is defined by residues H189-C190-G193-R196 and D 161 as well as Y162 of SEQ ID NO:150. Based on the sequence alignment disclosed in Figure 27 and the structural model disclosed in Table VIII and visualized in Figure 28, D161 is identified as a general acid, Y162 as a key determinant of substrate specificity which interacts with the phosphotyrosine substrate, C190 as the catalytic Cysteine nucleophile which cleaves the phosphodiester bond, and R196 as the essential Argenine which activates the bond for cleavage as described in the literature (reviewed by Fauman and .Saper, 1996).

More preferred are molecules comprising all or any part of the active site region or a mutant or homologue of said molecule or molecular complex. By mutant or homologue of the molecule it is meant a molecule that has a root mean square deviation from the backbone atoms of said BMY_HPP1 amino acids of not more than 3.5 Angstroms.

More preferred are molecules comprising all or any part of the active site region defined as residues above or a mutant or homologue of said molecule or molecular complex. By mutant or homologue of the molecule it is meant a molecule that has a root mean square deviation from the backbone atoms of said residues in the active site region of said BMY_HPP1 of not more than 3.5 Angstroms.

The term "root mean square deviation" means the square root of the arithmetic mean of the squares of the deviations from the mean. It is a way to express the deviation or variation from a trend or object. For purposes of this invention, the "root mean square deviation" defines the variation in the backbone of a protein from the

relevant portion of the backbone of BMY_HPP1 as defined by the structure coordinates described herein.

The structure coordinates of a BMY_HPP1 homology model portion thereof are stored in a machine-readable storage medium. Such data may be used for a variety of purposes, such as drug discovery and target prioritization and validation.

Accordingly, in one embodiment of this invention is provided a machine-readable data storage medium comprising a data storage material encoded with the structure coordinates set forth in Table VIII and visualized in Figure 28.

One embodiment utilizes System 10 as disclosed in WO 98/11134, the disclosure of which is incorporated herein by reference in its entirety. Briefly, one version of these embodiments comprises a computer comprising a central processing unit ("CPU"), a working memory which may be, e.g, RAM (random-access memory) or "core" memory, mass storage memory (such as one or more disk drives or CD-ROM drives), one or more cathode-ray tube ("CRT") display terminals, one or more keyboards, one or more input lines, and one or more output lines, all of which are interconnected by a conventional bidirectional system bus.

Input hardware, coupled to the computer by input lines, may be implemented in a variety of ways. Machine-readable data of this invention may be inputted via the use of a modem or modems connected by a telephone line or dedicated data line. Alternatively or additionally, the input hardware may comprise CD-ROM drives or disk drives. In conjunction with a display terminal, keyboard may also be used as an input device.

Output hardware, coupled to the computer by output lines, may similarly be implemented by conventional devices. By way of example, output hardware may include a CRT display terminal for displaying a graphical representation of a region or domain of the present invention using a program such as QUANTA as described herein. Output hardware might also include a printer, so that hard copy output may be produced, or a disk drive, to store system output for later use.

In operation, the CPU coordinates the use of the various input and output devices, coordinates data accesses from mass storage, and accesses to and from the working memory, and determines the sequence of data processing steps. A number of programs may be used to process the machine-readable data of this invention. Such

programs are discussed in reference to the computational methods of drug discovery as described herein. Specific references to components of the hardware system are included as appropriate throughout the following description of the data storage medium.

For the purpose of the present invention, any magnetic data storage medium which can be encoded with machine-readable data would be sufficient for carrying out the storage requirements of the system. The medium could be a conventional floppy diskette or hard disk, having a suitable substrate, which may be conventional, and a suitable coating, which may be conventional, on one or both sides, containing magnetic domains whose polarity or orientation could be altered magnetically, for example. The medium may also have an opening for receiving the spindle of a disk drive or other data storage device.

The magnetic domains of the coating of a medium may be polarized or oriented so as to encode in a manner which may be conventional, machine readable data such as that described herein, for execution by a system such as the system described herein.

Another example of a suitable storage medium which could also be encoded with such machine-readable data, or set of instructions, which could be carried out by a system such as the system described herein, could be an optically-readable data storage medium. The medium could be a conventional compact disk read only memory (CD-ROM) or a rewritable medium such as a magneto-optical disk which is optically readable and magneto-optically writable. The medium preferably has a suitable substrate, which may be conventional, and a suitable coating , which may be conventional, usually of one side of substrate.

In the case of a CD-ROM, as is well known, the coating is reflective and is impressed with a plurality of pits to encode the machine-readable data. The arrangement of pits is read by reflecting laser light off the surface of the coating. A protective coating, which preferably is substantially transparent, is provided on top of the reflective coating.

In the case of a magneto-optical disk, as is well known, the coating has no pits, but has a plurality of magnetic domains whose polarity or orientation can be changed magnetically when heated above a certain temperature, as by a laser. The orientation

of the domains can be read by measuring the polarization of laser light reflected from the coating. The arrangement of the domains encodes the data as described above.

Thus, in accordance with the present invention, data capable of displaying the three dimensional structure of the BMY_HPP1 homology model, or portions thereof and their structurally similar homologues is stored in a machine-readable storage medium, which is capable of displaying a graphical three-dimensional representation of the structure. Such data may be used for a variety of purposes, such as drug discovery.

For the first time, the present invention permits the use, through homology modeling based upon the sequence of BMY_HPP1 (Figures 20A-D) of structure-based or rational drug design techniques to design, select, and synthesizes chemical entities that are capable of modulating the biological function of BMY_HPP1. Comparison of the BMY_HPP1 homology model with the structures of template phosphatases enable the use of rational or structure based drug design methods to design, select or synthesize specific chemical modulators of BMY_HPP1.

Accordingly, the present invention is also directed to the entire sequence in Figure 20A-D or any portion thereof for the purpose of generating a homology model for the purpose of three dimensional structure-based drug designs.

For purposes of this invention, we include mutants or homologues of the sequence in Figures 20A-D or any portion thereof. In a preferred embodiment, the mutants or homologues have at least 25% identity, more preferably 50% identity, more preferably 75% identity, and most preferably 90% identity to the amino acid residues in Figures 20A-D (SEQ ID NO:150).

The three-dimensional model structure of the BMY_HPP1 will also provide methods for identifying modulators of biological function. Various methods or combination thereof can be used to identify these compounds.

Structure coordinates of the active site region defined above can also be used to identify structural and chemical features. Identified structural or chemical features can then be employed to design or select compounds as potential BMY_HPP1 modulators. By structural and chemical features it is meant to include, but is not limited to, van der Waals interactions, hydrogen bonding interactions, charge interaction, hydrophobic interactions, and dipole interaction. Alternatively, or in

conjunction, the three-dimensional structural model can be employed to design or select compounds as potential BMY_HPP1 modulators. Compounds identified as potential BMY_HPP1 modulators can then be synthesized and screened in an assay characterized by binding of a test compound to the BMY_HPP1, or in characterizing BMY_HPP1 deactivation in the presence of a small molecule. Examples of assays useful in screening of potential BMY_HPP1 modulators include, but are not limited to, screening in silico, *in vitro* assays and high throughput assays. Finally, these methods may also involve modifying or replacing one or more amino acids from BMY_HPP1 according to Table VIII.

However, as will be understood by those of skill in the art upon this disclosure, other structure based design methods can be used. Various computational structure based design methods have been disclosed in the art.

For example, a number of computer modeling systems are available in which the sequence of the BMY_HPP1 and the BMY_HPP1 structure (i.e., atomic coordinates of BMY_HPP1 and/or the atomic coordinates of the active site region as provided in Table VIII) can be input. The computer system then generates the structural details of one or more these regions in which a potential BMY_HPP1 modulator binds so that complementary structural details of the potential modulators can be determined. Design in these modeling systems is generally based upon the compound being capable of physically and structurally associating with BMY_HPP1. In addition, the compound must be able to assume a conformation that allows it to associate with BMY_HPP1. Some modeling systems estimate the potential inhibitory or binding effect of a potential BMY_HPP1 modulator prior to actual synthesis and testing.

Methods for screening chemical entities or fragments for their ability to associate with a given protein target are well known. Often these methods begin by visual inspection of the binding site on the computer screen. Selected fragments or chemical entities are then positioned in one or more positions and orientations within the active site region in BMY_HPP1. Molecular docking is accomplished using software such as INSIGHTII, ICM (Molsoft LLC, La Jolla, CA), and SYBYL, following by energy minimization and molecular dynamics with standard molecular mechanic forcefields such as CHARMM and MMFF. Examples of computer

programs which assist in the selection of chemical fragment or chemical entities useful in the present invention include, but are not limited to, GRID (Goodford, 1985), AUTODOCK (Goodsell, 1990), and DOCK (Kuntz *et. al.* 1982).

Alternatively, compounds may be designed de novo using either an empty active site or optionally including some portion of a known inhibitor. Methods of this type of design include, but are not limited to LUDI (Bohm 1992), LeapFrog (Tripos Associates, St. Louis MO) and DOCK (Kuntz et. al., 1982). Programs such as DOCK (Kuntz *et. al.* 1982) can be used with the atomic coordinates from the homology model to identify potential ligands from databases or virtual databases which potentially bind the in the active site region, and which may therefore be suitable candidates for synthesis and testing. The computer programs may utilize a combination of the following steps:

(a) Selection of fragments or chemical entities from a database and then positioning the chemical entity in one or more orientations within the BMY_HPP1 active site defined by residues D161-Y162 and H189-C190-G193-R196. Characterization of the structural and chemical features of the chemical entity and active site including van der Waals interactions, hydrogen bonding interactions, charge interaction, hydrophobic bonding interaction, and dipole interactions;

(b) Search databases for molecular fragments which can be joined to or replace the docked chemical entity and spatially fit into regions defined by the said BMY_HPP1 active site;

(c) Evaluate the docked chemical entity and fragments using a combination of scoring schemes which account for van der Waals interactions, hydrogen bonding interactions, charge interaction, hydrophobic interactions; or

(d) Databases that may be used include ACD (Molecular Designs Limited), Aldrich (Aldrich Chemical Company), NCI (National Cancer Institute), Maybridge(Maybridge Chemical Company Ltd), CCDC (Cambridge Crystallographic Data Center), CAST (Chemical Abstract Service), and Derwent (Derwent Information Limited).

Upon selection of preferred chemical entities or fragments, their relationship to each other and BMY_HPP1 can be visualized and then assembled into a single potential modulator. Programs useful in assembling the individual chemical entities

include, but are not limited to SYBYL and LeapFrog (Tripos Associates, St. Louis MO), LUDI (Bohm 1992) as well as 3D Database systems (Martin 1992).

Additionally, the three-dimensional homology model of BMY_HPP1 will aid in the design of mutants with altered biological activity. Site directed mutagenesis can be used to generate proteins with similar or varying degrees of biological activity compared to native BMY_HPP1. This invention also relates to the generation of mutants or homologues of BMY_HPP1. It is clear that molecular modeling using the three dimensional structure coordinates set forth in Table VIII and visualization of the BMY_HPP1 model, Figure 28 can be utilized to design homologues or mutant polypeptides of BMY_HPP1 that have similar or altered biological activities, function or reactivities.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:149 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides consisting of a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4379 of SEQ ID NO:149, b is an integer between 15 to 4393, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:149, and where b is greater than or equal to a+14.

Features of the Polypeptide Encoded by Gene No:2

The polypeptide fragment corresponding to this gene provided as SEQ ID NO:6 (Figure 2), encoded by the polynucleotide sequence according to SEQ ID NO:5 (Figure 2), and/or encoded by the polynucleotide contained within the deposited clone, BMY_HPP2, has significant homology at the nucleotide and amino acid level to a number of phosphatases, which include, for example, the human CDC14 (also known as the cell division cycle 14, S. cerevisiae Gene A protein) homologue A (HS_CDC14A; Genbank Accession No:gi| NP_003663; SEQ ID NO:30); the human S. cerevisiae CDC14 homolog, gene B (HS_CDC14B; Genbank Accession No:gi|

NP_003662; SEQ ID NO:31); and the yeast soluble tyrosine-specific protein phosphatase Cdc14p protein (SC_CDC14; Genbank Accession No:gi| NP_002839; SEQ ID NO:32) as determined by BLASTP An alignment of the human phosphatase polypeptide with these proteins is provided in Figure 7.

BMY_HPP2 is predicted to be a phosphoprotein phosphatase based on its homology to human CDC14B as determined by BLASTP. BMY_HPP2 shows significant homology to the catalytic domains of human CDC14A and CDC14B and to yeast CDC14 including a conserved Aspartate at AA 76, a Cysteine at AA106 and an Arginine at AA 112 of BMY_HPP2 (shown in Figure 2).

Polypeptide sequences corresponding to portions of the encoded BMY_HPP2 polypeptide sequence have been described as BAA91172 (Genbank Accession No:gi 7020545). However, conceptual translation of BAA91172 indicates that the phosphatase homology is in an open reading frame that begins before the 5' end of the provided polynucleotide EST sequence, in addition to regions of the polypeptide that are homologous to known phosphatases. Thus, the Genbank record, or the sequence, provided for BAA91172 does not provide any suggestion that this clone partially encodes a phosphatase protein.

Based upon the strong homology to members of the phosphatase proteins, the polypeptide encoded by the human BMY_HPP2 phosphatase of the present invention is expected to share at least some biological activity with phosphatase proteins, preferably with members of the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases, particularly the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases referenced herein.

The present invention encompasses the use of BMY_HPP2 inhibitors and/or activators of BMY_HPP2 activity for the treatment, detection, amelioaration, or prevention of phosphatase associated disorders, including but not limited to metabolic diseases such as diabetes, in addition to neural and/or cardiovascular diseases and disorders. The present invention also encompasses the use of BMY_HPP2 inhibitors and/or activators of BMY_HPP2 activity as immunosuppressive agents, anti-inflammatory agents, and/or anti-tumor agents

The present invention encompasses the use of BMY_HPP2 phosphatase inhibitors, including, antagonists such as antisense nucleic acids, in addition to other

antagonists, as described herein, in a therapeutic regimen to diagnose, prognose, treat, ameliorate, and/or prevent diseases where a kinase activity is insufficient. One, non-limiting example of a disease which may occur due to insufficient kinase activity are certain types of diabetes, where one or more kinases involved in the insulin receptor signal pathway may have insufficient activity or insufficient expression, for example.

Moreover, the present invention encompasses the use of BMY_HPP2 phosphatase activators, and/or the use of the BMY_HPP2 phosphatase gene or protein in a gene therapy regimen, as described herein, for the diagnoses, prognoses, treatment, amelioration, and/or prevention of diseases and/or disorders where a kinase activity is overly high, such as a cancer where a kinase oncogene product has excessive activity or excessive expression.

The present invention also encompasses the use of catalytically inactive variants of BMY_HPP2 proteins, including fragments thereof, such as a protein therapeutic, or the use of the encoding polynucleotide sequence or as gene therapy, for example, in the diagnoses, prognosis, treatment, amelioration, and/or prevention of diseases or disorders where phosphatase activity is overly high.

The present invention encompasses the use of antibodies directed against the BMY_HPP2 polypeptides, including fragment and/or variants thereof, of the present invention in diagnostics, as a biomarkers, and/or as a therapeutic agents.

The present invention encompasses the use of an inactive, non-catalytic, mutant of the BMY_HPP2 phosphatase as a substrate trapping mutant to bind cellular phosphoproteins or a library of phosphopeptides to identify substrates of the BMY_HPP2 polypeptides.

The present invention encompasses the use of the BMY_HPP2 polypeptides, to identify inhibitors or activators of the BMY_HPP2 phosphatase activity using either in vitro or 'virtual' (in silico) screening methods.

One embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of the BMY_HPP2 phosphatase comprising the steps of: i.) contacting a BMY_HPP2 phosphatase inhibitor or activator labeled with an analytically detectable reagent with the BMY_HPP2 phosphatase under conditions sufficient to form a complex with the inhibitor or activator; ii.) contacting said complex with a sample containing a compound to be identified; iii) and identifying

the compound as an inhibitor or activator by detecting the ability of the test compound to alter the amount of labeled known BMY_HPP2 phosphatase inhibitor or activator in the complex.

Another embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of a BMY_HPP2 phosphatase comprising the steps of: i.) contacting the BMY_HPP2 phosphatase with a compound to be identified; and ii.) and measuring the ability of the BMY_HPP2 phosphatase to remove phosphate from a substrate.

The present invention also encomposses a method for identifying a ligand for the BMY_HPP2 phosphatase comprising the steps of: i.) contacting the BMY_HPP2 phosphatase with a series of compounds under conditions to permit binding; and ii.) detecting the presence of any ligand-bound protein.

Preferably, the above referenced methods comprise the BMY_HPP2 phosphatase in a form selected from the group consisting of whole cells, cytosolic cell fractions, membrane cell fractions, purified or partially purified forms. The invention also relates to recombinantly expressed BMY_HPP2 phosphatase in a purified, substantially purified, or unpurified state. The invention further relates to BMY_HPP2 phosphatase fused or conjugated to a protein, peptide, or other molecule or compound known in the art, or referenced herein.

The present invention also encompasses pharmaceutical composition of the BMY_HPP2 phosphatase polypeptide comprising a compound identified by above referenced methods and a pharmaceutically acceptable carrier.

Expression profiling designed to measure the steady state mRNA levels encoding the BMY_HPP2 polypeptide showed predominately high expression levels in liver and kidney; to a significant extent, in the spleen, and to a lesser extent, in lung, testis, heart, intestine, pancreas, lymph node, spinal cord, and prostate (as shown in Figure 23).

Moreover, BLAST2 searches of the LifeSeq database (Incyte Pharmaceuticals) using the full-length BMY_HPP2 polynucleotide sequence (SEQ ID NO:151) led to the determination that the BMY_HPP2 sequence is expressed significantly in lung libraries which include patients with emphysema and other pulmonary diseases. The BMY_HPP2 polynucleotide was also found to be expressed

in aorta and endothelial cells stimulated with IL-1 and TNF-alpha. These findings suggest a potential involvement of the BMY_HPP2 polynucleotides and polypeptides in the incidence of pulmonary disease and upregulation by IL-1 and TNF-alpha.

In addition, expanded expression profiling of the BMY_HPP2 polypeptide in normal tissues showed the highest level of expression in the adrenal gland, with lower but significant expression in the pineal pituitary glands suggesting a role for modulators of BMY_HPP2 activity in the treatment of endocrine disorders (as shown in Figure 30). Consistent with the expression pattern in lung libraries from the Incyte database above, high relative levels of expression were also seen in the parenchyma and bronchi of the lung, suggesting a role for modulators of BMY_HPP2 activity in the treatment of respiratory diseases such as asthma or COPD; in the kidney, suggesting a role for modulators of BMY_HPP2 activity in the treatment of kidney disorders; in the liver, suggesting a role for modulators of BMY_HPP2 activity in the treatment of liver disorders such as hepatitis or cirrhosis; in blood vessels from the choroid plexus, coronary artery and pulmonary artery, suggesting a role for modulators of BMY_HPP2 activity in the treatment of circulatory disorders such as hypertension; and in the nucleus accumbens of the brain, suggesting a role for modulators of BMY_HPP2 activity in the treatment of affective disorders such as bipolar disorder, schizophrenia and depression. In addition, the BMY_HPP2 was highly expressed in the trachea , breast and uterus and significantly expressed in many other tissues within the human body.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the predominate localized expression in adrenal gland tissue suggests the human BMY_HPP2 phosphatase polynucleotides and polypeptides, including agonists, antagonists, and/or fragments thereof, may be useful for treating, diagnosing, prognosing, amerliorating, and/or preventing endocrine disorders, which include, but are not limited to adrenocortical hyperfunction, adrenocortical hypofunction, lethargy, Congenital adrenal hyperplasia, aberrant ACTH regulation, aberrant adrenaline regulation, disorders associated with defects in P450C21, P450C18, P450C17, and P450C11 hydroxylases and in 3-hydroxysteroid dehydrogenase (3-HSD), hirsutism, oligomenorrhea, acne, virilization, oligomenorrhea, female pseudohermaphroditism, disorders associated with the

incidence of aberrant sexual characterisitics, disorders associated with aberrant cortisol secretion, hypertension, hypokalemia, hypogonadism, disorders associated with aberrant androgen secretion, adrenal virilism, Adrenal adenomas, Adrenal carcinomas, disorders associated with aberrant aldosterone secretion, aldosteronism, disorders associated with aberrant steriod biosynthesis, disorders associated with aberrant steriod transport, disorders associated with aberrant steriod secretion, disorders associated with aberrant steriod excretion, Addison's syndrome, and Cushing's syndrome.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the significant expression in liver indicates the BMY_HPP2 polynucleotides and polypeptides, in addition to, fragments and variants thereof, would be useful for the detection and treatment of liver disorders and cancers. Representative uses are described in the "Hyperproliferative Disorders", "Infectious Disease", and "Binding Activity" sections below, and elsewhere herein. Briefly, the protein can be used for the detection, treatment, amelioration, and/or prevention of hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells, cirrhosis, hepatic cysts, pyrogenic abscess, amebic abcess, hydatid cyst, cystadenocarcinoma, adenoma, focal nodular hyperplasia, hemangioma, hepatocellulae carcinoma, cholangiocarcinoma, and angiosarcoma, granulomatous liver disease, liver transplantation, hyperbilirubinemia, jaundice, parenchymal liver disease, portal hypertension, hepatobiliary disease, hepatic parenchyma, hepatic fibrosis, anemia, gallstones, cholestasis, carbon tetrachloride toxicity, beryllium toxicity, vinyl chloride toxicity, choledocholithiasis, hepatocellular necrosis, aberrant metabolism of amino acids, aberrant metabolism of carbohydrates, aberrant synthesis proteins, aberrant synthesis of glycoproteins, aberrant degradation of proteins, aberrant degradation of glycoproteins, aberrant metabolism of drugs, aberrant metabolism of hormones, aberrant degradation of drugs, aberrant degradation of drugs, aberrant regulation of lipid metabolism, aberrant regulation of cholesterol metabolism, aberrant glycogenesis, aberrant glycogenolysis, aberrant glycolysis, aberrant gluconeogenesis, hyperglycemia, glucose intolerance, hyperglycemia, decreased hepatic glucose uptake, decreased hepatic glycogen synthesis, hepatic resistance to insulin, portal-

systemic glucose shunting, peripheral insulin resistance, hormonal abnormalities, increased levels of systemic glucagon, decreased levels of systemic cortisol, increased levels of systemic insulin, hypoglycemia, decreased gluconeogenesis, decreased hepatic glycogen content, hepatic resistance to glucagon, elevated levels of systemic aromatic amino acids, decreased levels of systemic branched-chain amino acids, hepatic encephalopathy, aberrant hepatic amino acid transamination, aberrant hepatic amino acid oxidative deamination, aberrant ammonia synthesis, aberant albumin secretion, hypoalbuminemia, aberrant cytochromes b5 function, aberrant P450 function, aberrant glutathione S-acyltransferase function, aberrant cholesterol synthesis, and aberrant bile acid synthesis.

Moreover, polynucleotides and polypeptides, including fragments, agonists and/or antagonists thereof, have uses which include, directly or indirectly, treating, preventing, diagnosing, and/or prognosing the following, non-limiting, hepatic infections: liver disease caused by sepsis infection, liver disease caused by bacteremia, liver disease caused by Pneomococcal pneumonia infection, liver disease caused by Toxic shock syndrome, liver disease caused by Listeriosis, liver disease caused by Legionnaries' disease, liver disease caused by Brucellosis infection, liver disease caused by Neisseria gonorrhoeae infection, liver disease caused by Yersinia infection, liver disease caused by Salmonellosis, liver disease caused by Nocardiosis, liver disease caused by Spirochete infection, liver disease caused by Treponema pallidum infection, liver disease caused by Brrelia burgdorferi infection, liver disease caused by Leptospirosis, liver disease caused by Coxiella burnetii infection, liver disease caused by Rickettsia richettsii infection, liver disease caused by Chlamydia trachomatis infection, liver disease caused by Chlamydia psittaci infection, liver disease caused by hepatitis virus infection, liver disease caused by Epstein-Barr virus infection in addition to any other hepatic disease and/or disorder implicated by the causative agents listed above or elsewhere herein.

The strong homology to dual specificity phophatases, combined with the localized expression in kidney tissue suggests the BMY_HPP2 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing renal diseases and/or disorders, which include, but are not limited to: nephritis, renal failure, nephrotic syndrome, urinary tract infection, hematuria, proteinuria, oliguria,

polyuria, nocturia, edema, hypertension, electrolyte disorders, sterile pyuria, renal osteodystrophy, large kidneys, renal transport defects, nephrolithiasis, azotemia, anuria, urinary retention ,slowing of urinary stream, large prostate, flank tenderness, full bladder sensation after voiding, enuresis, dysuria,bacteriuria, kideny stones, glomerulonephritis, vasculitis, hemolytic uremic syndromes, thrombotic thrombocytopenic purpura, malignant hypertension, casts, tubulointerstitial kidney diseases, renal tubular acidosis, pyelonephritis, hydronephritis, nephrotic syndrome, crush syndrome, and/or renal colic, in addition to Wilm's Tumor Disease, and congenital kidney abnormalities such as horseshoe kidney, polycystic kidney, and Falconi's syndrome.for example.

The strong homology to dual specificity phosphatases, combined with the localized expression in spleen tissue, in addition to the expression in endothelial cells stimulated with IL-1 and TNF-alpha, suggests the BMY_HPP2 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing immune diseases and/or disorders. Representative uses are described in the "Immune Activity", "Chemotaxis", and "Infectious Disease" sections below, and elsewhere herein. Briefly, the strong expression in immune tissue indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells.

The BMY_HPP2 polypeptide may also be useful as a preventative agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. The BMY_HPP2 polypeptide may be useful for modulating cytokine production, antigen presentation, or other processes, such as for boosting immune responses, etc.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to

sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissuemarkers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

The significant expression of BMY_HPP2 transcripts in lung libraries as observed from electronic Northern's from the Incyte LifeSeq database suggests the potential utility for BMY_HPP2 polynucleotides and polypeptides in treating, diagnosing, prognosing, and/or preventing pulmonary diseases and disorders which include the following, not limiting examples: ARDS, emphysema, cystic fibrosis, interstitial lung disease, chronic obstructive pulmonary disease, bronchitis, lymphangioleiomyomatosis, pneumonitis, eosinophilic pneumonias, granulomatosis, pulmonary infarction, pulmonary fibrosis, pneumoconiosis, alveolar hemorrhage, neoplasms, lung abscesses, empyema, and increased susceptibility to lung infections (e.g., immumocompromised, HIV, etc.), for example.

Moreover, polynucleotides and polypeptides, including fragments, agonists and/or antagonists thereof, have uses which include, directly or indirectly, treating, preventing, diagnosing, and/or prognosing the following, non-limiting, pulmonary infections: pnemonia, bacterial pnemonia, viral pnemonia (for example, as caused by Influenza virus, Respiratory syncytial virus, Parainfluenza virus, Adenovirus, Coxsackievirus, Cytomegalovirus, Herpes simplex virus, Hantavirus, etc.), mycobacteria pnemonia (for example, as caused by Mycobacterium tuberculosis, etc.) mycoplasma pnemonia, fungal pnemonia (for example, as caused by Pneumocystis carinii, Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis, Candida sp., Cryptococcus neoformans, Aspergillus sp., Zygomycetes, etc.), Legionnaires' Disease, Chlamydia pnemonia, aspiration pnemonia, Nocordia sp. Infections, parasitic pnemonia (for example, as caused by Strongyloides, Toxoplasma gondii, etc.) necrotizing pnemonia, in addition to any other pulmonary disease and/or

disorder (e.g., non-pneumonia) implicated by the causative agents listed above or elsewhere herein.

Antisense oligonucleotides directed against BMY_HPP2 provided evidence suggesting its involvement in the regulation of mammalian cell cycle progression (see Example 56). Subjecting cells with an effective amount of a pool of five antisense oligoncleotides resulted in a significant increase in Cyclin D expression/activity providing convincing evidence that BMY_HPP2 at least regulates the activity and/or expression of Cyclin D either directly, or indirectly. Moreover, the results suggest the physiological role of BMY_HPP2 is the negative regulation of Cyclin D activity and/or expression, either directly or indirectly.

In preferred embodiments, BMY_HPP2 polynucleotides and polypeptides, including fragments thereof, are useful for treating, diagnosing, and/or ameliorating cell cycle defects, disorders related to aberrant phosphorylation, disorders related to aberrant signal transduction, proliferating disorders, and/or cancers.

Moreover, BMY_HPP2 polynucleotides and polypeptides, including fragments thereof, are useful for decreasing cellular proliferation, decreasing cellular proliferation in rapidly proliferating cells, increasing the number of cells in the G1 phase of the cell cycle, and decreasing the number of cells that progress to the S phase of the cell cycle.

In preferred embodiments, agonists directed to BMY_HPP2 are useful for decreasing cellular proliferation, decreasing cellular proliferation in rapidly proliferating cells, increasing the number of cells in the G1 phase of the cell cycle, and decreasing the number of cells that progress to the S phase of the cell cycle.

Moreover, antagonists directed against BMY_HPP2 are useful for increasing cellular proliferation, increasing cellular proliferation in rapidly proliferating cells, decreasing the number of cells in the G1 phase of the cell cycle, and increasing the number of cells that progress to the S phase of the cell cycle. Such antagonists would be particularly useful for transforming normal cells into immortalized cell lines, stimulating hematopoietic cells to grow and divide, increasing recovery rates of cancer patients that have undergone chemotherapy or other therapeutic regimen, by boosting their immune responses, etc.

The BMY_HPP2 polypeptide has been shown to comprise one glycosylation sites according to the Motif algorithm (Genetics Computer Group, Inc.). As discussed more specifically herein, protein glycosylation is thought to serve a variety of functions including: augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

Asparagine glycosylation sites have the following consensus pattern, N-{P}-[ST]-{P}, wherein N represents the glycosylation site. However, it is well known that that potential N-glycosylation sites are specific to the consensus sequence Asn-Xaa-Ser/Thr. However, the presence of the consensus tripeptide is not sufficient to conclude that an asparagine residue is glycosylated, due to the fact that the folding of the protein plays an important role in the regulation of N-glycosylation. It has been shown that the presence of proline between Asn and Ser/Thr will inhibit N-glycosylation; this has been confirmed by a recent statistical analysis of glycosylation sites, which also shows that about 50% of the sites that have a proline C-terminal to Ser/Thr are not glycosylated. Additional information relating to asparagine glycosylation may be found in reference to the following publications, which are hereby incorporated by reference herein: Marshall R.D., Annu. Rev. Biochem. 41:673-702(1972); Pless D.D., Lennarz W.J., Proc. Natl. Acad. Sci. U.S.A. 74:134-138(1977); Bause E., Biochem. J. 209:331-336(1983); Gavel Y., von Heijne G., Protein Eng. 3:433-442(1990); and Miletich J.P., Broze G.J. Jr., J. Biol. Chem... 265:11397-11404(1990).

In preferred embodiments, the following asparagine glycosylation site polypeptide is encompassed by the present invention: GVQPPNFSWVLPGR (SEQ ID NO:164). Polynucleotides encoding this polypeptide are also provided. The present invention also encompasses the use of this BMY_HPP2 asparagine glycosylation site polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

The BMY_HPP2 polypeptides of the present invention were determined to comprise several phosphorylation sites based upon the Motif algorithm (Genetics Computer Group, Inc.). The phosphorylation of such sites may regulate some

biological activity of the BMY_HPP2 polypeptide. For example, phosphorylation at specific sites may be involved in regulating the proteins ability to associate or bind to other molecules (e.g., proteins, ligands, substrates, DNA, etc.). In the present case, phosphorylation may modulate the ability of the BMY_HPP2 polypeptide to associate with other potassium channel alpha subunits, beta subunits, or its ability to modulate potassium channel function.

The BMY_HPP2 polypeptide was predicted to comprise one PKC phosphorylation site using the Motif algorithm (Genetics Computer Group, Inc.). In vivo, protein kinase C exhibits a preference for the phosphorylation of serine or threonine residues. The PKC phosphorylation sites have the following consensus pattern: [ST]-x-[RK], where S or T represents the site of phosphorylation and 'x' an intervening amino acid residue. Additional information regarding PKC phosphorylation sites can be found in Woodget J.R., Gould K.L., Hunter T., Eur. J. Biochem. 161:177-184(1986), and Kishimoto A., Nishiyama K., Nakanishi H., Uratsuji Y., Nomura H., Takeyama Y., Nishizuka Y., J. Biol. Chem... 260:12492-12499(1985); which are hereby incorporated by reference herein.

In preferred embodiments, the following PKC phosphorylation site polypeptide is encompassed by the present invention: HLVSLTERGPPHS (SEQ ID NO:165). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of these BMY_HPP2 PKC phosphorylation site polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In further confirmation of the human BMY_HPP2 polypeptide representing a novel human phosphatase polypeptide, the BMY_HPP2 polypeptide has been shown to comprise a tyrosine specific protein phosphatase active site domain according to the Motif algorithm (Genetics Computer Group, Inc.).

Tyrosine specific protein phosphatases (EC 3.1.3.48) (PTPase) are enzymes that catalyze the removal of a phosphate group attached to a tyrosine residue. These enzymes are very important in the control of cell growth, proliferation, differentiation and transformation. Multiple forms of PTPase have been characterized and can be classified into two categories: soluble PTPases and transmembrane receptor proteins that contain PTPase domain(s).

The currently known PTPases are listed below: Soluble PTPases, PTPN1 (PTP-1B), PTPN2 (T-cell PTPase; TC-PTP), PTPN3 (H1) and PTPN4 (MEG), enzymes that contain an N-terminal band 4.1-like domain and could act at junctions between the membrane and cytoskeleton, PTPN5 (STEP), PTPN6 (PTP-1C; HCP; SHP) and PTPN11 (PTP-2C; SH-PTP3; Syp), enzymes which contain two copies of the SH2 domain at its N-terminal extremity (e.g., the Drosophila protein corkscrew (gene csw) also belongs to this subgroup), PTPN7 (LC-PTP; Hematopoietic protein-tyrosine phosphatase; HePTP), PTPN8 (70Z-PEP), PTPN9 (MEG2), PTPN12 (PTP-G1; PTP-P19), Yeast PTP1, Yeast PTP2 which may be involved in the ubiquitin-mediated protein degradation pathway, Fission yeast pyp1 and pyp2 which play a role in inhibiting the onset of mitosis, Fission yeast pyp3 which contributes to the dephosphorylation of cdc2, Yeast CDC14 which may be involved in chromosome segregation, Yersinia virulence plasmid PTPAses (gene yopH), Autographa californica nuclear polyhedrosis virus 19 Kd PTPase, Dual specificity PTPases, DUSP1 (PTPN10; MAP kinase phosphatase-1; MKP-1); which dephosphorylates MAP kinase on both Thr-183 and Tyr-185, DUSP2 (PAC-1), a nuclear enzyme that dephosphorylates MAP kinases ERK1 and ERK2 on both Thr and Tyr residues, DUSP3 (VHR), DUSP4 (HVH2), DUSP5 (HVH3), DUSP6 (Pyst1; MKP-3), DUSP7 (Pyst2; MKP-X), Yeast MSG5, a PTPase that dephosphorylates MAP kinase FUS3, Yeast YVH1, Vaccinia virus H1 PTPase - a dual specificity phosphatase,

Structurally, all known receptor PTPases, are made up of a variable length extracellular domain, followed by a transmembrane region and a C-terminal catalytic cytoplasmic domain. Some of the receptor PTPases contain fibronectin type III (FN-III) repeats, immunoglobulin-like domains, MAM domains or carbonic anhydrase-like domains in their extracellular region. The cytoplasmic region generally contains two copies of the PTPAse domain. The first seems to have enzymatic activity, while the second is inactive but seems to affect substrate specificity of the first. In these domains, the catalytic cysteine is generally conserved but some other, presumably important, residues are not.

PTPase domains consist of about 300 amino acids. There are two conserved cysteines, the second one has been shown to be absolutely required for activity.

Furthermore, a number of conserved residues in its immediate vicinity have also been shown to be important.

A consensus sequence for tyrosine specific protein phophatases is provided as follows:

[LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAGP]-x-[LIVMFY], wherein C is the active site residue and "X" represents any amino acid.

Additional information related to tyrosine specific protein phosphatase domains and proteins may be found in reference to the following publications Fischer E.H., Charbonneau H., Tonks N.K., Science 253:401-406(1991); Charbonneau H., Tonks N.K., Annu. Rev. Cell Biol. 8:463-493(1992); Trowbridge I.S., J. Biol. Chem... 266:23517-23520(1991); Tonks N.K., Charbonneau H., Trends Biochem. Sci. 14:497-500(1989); and Hunter T., Cell 58:1013-1016(1989); which are hereby incorporated herein by reference in their entirety.

In preferred embodiments, the following tyrosine specific protein phosphatase active site domain polypeptide is encompassed by the present invention: GEAVGVHCALGFGRTGTMLACYL (SEQ ID NO:166). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of this tyrosine specific protein phosphatase active site domain polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

In preferred embodiments, the following N-terminal BMY_HPP2 deletion polypeptides are encompassed by the present invention: M1-K150, G2-K150, V3-K150, Q4-K150, P5-K150, P6-K150, N7-K150, F8-K150, S9-K150, W10-K150, V11-K150, L12-K150, P13-K150, G14-K150, R15-K150, L16-K150, A17-K150, G18-K150, L19-K150, A20-K150, L21-K150, P22-K150, R23-K150, L24-K150, P25-K150, A26-K150, H27-K150, Y28-K150, Q29-K150, F30-K150, L31-K150, L32-K150, D33-K150, L34-K150, G35-K150, V36-K150, R37-K150, H38-K150, L39-K150, V40-K150, S41-K150, L42-K150, T43-K150, E44-K150, R45-K150, G46-K150, P47-K150, P48-K150, H49-K150, S50-K150, D51-K150, S52-K150, C53-K150, P54-K150, G55-K150, L56-K150, T57-K150, L58-K150, H59-K150, R60-K150, L61-K150, R62-K150, I63-K150, P64-K150, D65-K150, F66-K150, C67-K150, P68-K150, P69-K150, A70-K150, P71-K150, D72-K150, Q73-K150, I74-K150, D75-K150, R76-K150, F77-K150, V78-K150, Q79-K150, I80-K150, V81-

K150, D82-K150, E83-K150, A84-K150, N85-K150, A86-K150, R87-K150, G88-K150, E89-K150, A90-K150, V91-K150, G92-K150, V93-K150, H94-K150, C95-K150, A96-K150, L97-K150, G98-K150, F99-K150, G100-K150, R101-K150, T102-K150, G103-K150, T104-K150, M105-K150, L106-K150, A107-K150, C108-K150, Y109-K150, L110-K150, V111-K150, K112-K150, E113-K150, R114-K150, G115-K150, L116-K150, A117-K150, A118-K150, G119-K150, D120-K150, A121-K150, I122-K150, A123-K150, E124-K150, I125-K150, R126-K150, R127-K150, L128-K150, R129-K150, P130-K150, G131-K150, S132-K150, I133-K150, E134-K150, T135-K150, Y136-K150, E137-K150, Q138-K150, E139-K150, K140-K150, A141-K150, V142-K150, F143-K150, and/or Q144-K150 of SEQ ID NO:152. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these N-terminal BMY_HPP2 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal BMY_HPP2 deletion polypeptides are encompassed by the present invention: M1-K150, M1-T149, M1-R148, M1-Q147, M1-Y146, M1-F145, M1-Q144, M1-F143, M1-V142, M1-A141, M1-K140, M1-E139, M1-Q138, M1-E137, M1-Y136, M1-T135, M1-E134, M1-I133, M1-S132, M1-G131, M1-P130, M1-R129, M1-L128, M1-R127, M1-R126, M1-I125, M1-E124, M1-A123, M1-I122, M1-A121, M1-D120, M1-G119, M1-A118, M1-A117, M1-L116, M1-G115, M1-R114, M1-E113, M1-K112, M1-V111, M1-L110, M1-Y109, M1-C108, M1-A107, M1-L106, M1-M105, M1-T104, M1-G103, M1-T102, M1-R101, M1-G100, M1-F99, M1-G98, M1-L97, M1-A96, M1-C95, M1-H94, M1-V93, M1-G92, M1-V91, M1-A90, M1-E89, M1-G88, M1-R87, M1-A86, M1-N85, M1-A84, M1-E83, M1-D82, M1-V81, M1-I80, M1-Q79, M1-V78, M1-F77, M1-R76, M1-D75, M1-I74, M1-Q73, M1-D72, M1-P71, M1-A70, M1-P69, M1-P68, M1-C67, M1-F66, M1-D65, M1-P64, M1-I63, M1-R62, M1-L61, M1-R60, M1-H59, M1-L58, M1-T57, M1-L56, M1-G55, M1-P54, M1-C53, M1-S52, M1-D51, M1-S50, M1-H49, M1-P48, M1-P47, M1-G46, M1-R45, M1-E44, M1-T43, M1-L42, M1-S41, M1-V40, M1-L39, M1-H38, M1-R37, M1-V36, M1-G35, M1-L34, M1-D33, M1-L32, M1-L31, M1-F30, M1-Q29, M1-Y28, M1-H27, M1-A26, M1-P25, M1-L24, M1-R23, M1-P22, M1-L21, M1-A20, M1-L19, M1-G18, M1-A17, M1-L16, M1-

R15, M1-G14, M1-P13, M1-L12, M1-V11, M1-W10, M1-S9, M1-F8, and/or M1-N7 of SEQ ID NO:152. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these C-terminal BMY_HPP2 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following BMY_HPP2 phosphatase active site domain amino acid substitutions are encompassed by the present invention: wherein M1 is substituted with either an A, C, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y; wherein G2 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V3 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein Q4 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; wherein P5 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein P6 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein N7 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein F8 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S9 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein W10 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or Y; wherein V11 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein L12 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein P13 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein G14 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein R15 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein L16 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein A17 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein G18 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L19 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein A20 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L21 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein P22 is substituted with either an A, C, D, E, F, G,

H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein R23 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein L24 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein P25 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein A26 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein H27 is substituted with either an A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Y28 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein Q29 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; wherein F30 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L31 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein L32 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein D33 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L34 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein G35 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V36 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein R37 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein H38 is substituted with either an A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L39 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein V40 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein S41 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein L42 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein T43 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein E44 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein R45 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein G46 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein P47 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein P48 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein H49 is substituted with either an A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, or Y;

wherein S50 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein D51 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S52 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein C53 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein P54 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein G55 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L56 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein T57 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein L58 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein H59 is substituted with either an A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein R60 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein L61 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein R62 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein I63 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein P64 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein D65 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein F66 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein C67 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein P68 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein P69 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein A70 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein P71 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein D72 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Q73 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; wherein I74 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein D75 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein R76 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein F77 is substituted with either an

A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V78 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein Q79 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; wherein I80 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V81 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein D82 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein E83 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A84 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein N85 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein A86 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein R87 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein G88 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein E89 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A90 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V91 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein G92 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V93 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein H94 is substituted with either an A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein C95 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A96 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L97 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein G98 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein F99 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein G100 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein R101 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein T102 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein G103 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein T104 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P,

Q, R, S, V, W, or Y; wherein M105 is substituted with either an A, C, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y; wherein L106 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein A107 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein C108 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Y109 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein L110 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; and/or wherein V111 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y of SEQ ID NO:152, in addition to any combination thereof. The present invention also encompasses the use of these BMY_HPP2 phosphatase active site domain amino acid substituted polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following BMY_HPP2 phosphatase active site domain conservative amino acid substitutions are encompassed by the present invention: wherein M1 is substituted with either an A, G, S, or T; wherein G2 is substituted with either an A, M, S, or T; wherein V3 is substituted with either an A, I, or L; wherein Q4 is substituted with a N; wherein P5 is a P; wherein P6 is a P; wherein N7 is substituted with a Q; wherein F8 is substituted with either a W, or Y; wherein S9 is substituted with either an A, G, M, or T; wherein W10 is either an F, or Y; wherein V11 is substituted with either an A, I, or L; wherein L12 is substituted with either an A, I, or V; wherein P13 is a P; wherein G14 is substituted with either an A, M, S, or T; wherein R15 is substituted with either a K, or H; wherein L16 is substituted with either an A, I, or V; wherein A17 is substituted with either a G, I, L, M, S, T, or V; wherein G18 is substituted with either an A, M, S, or T; wherein L19 is substituted with either an A, I, or V; wherein A20 is substituted with either a G, I, L, M, S, T, or V; wherein L21 is substituted with either an A, I, or V; wherein P22 is a P; wherein R23 is substituted with either a K, or H; wherein L24 is substituted with either an A, I, or V; wherein P25 is a P; wherein A26 is substituted with either a G, I, L, M, S, T, or V; wherein H27 is substituted with either a K, or R; wherein Y28 is either an F, or W; wherein Q29 is substituted with a N; wherein F30 is substituted with either a W, or Y; wherein L31 is substituted with either an A, I, or V; wherein L32 is substituted with either an A, I, or V; wherein D33 is substituted with an E;

wherein L34 is substituted with either an A, I, or V; wherein G35 is substituted with either an A, M, S, or T; wherein V36 is substituted with either an A, I, or L; wherein R37 is substituted with either a K, or H; wherein H38 is substituted with either a K, or R; wherein L39 is substituted with either an A, I, or V; wherein V40 is substituted with either an A, I, or L; wherein S41 is substituted with either an A, G, M, or T; wherein L42 is substituted with either an A, I, or V; wherein T43 is substituted with either an A, G, M, or S; wherein E44 is substituted with a D; wherein R45 is substituted with either a K, or H; wherein G46 is substituted with either an A, M, S, or T; wherein P47 is a P; wherein P48 is a P; wherein H49 is substituted with either a K, or R; wherein S50 is substituted with either an A, G, M, or T; wherein D51 is substituted with an E; wherein S52 is substituted with either an A, G, M, or T; wherein C53 is a C; wherein P54 is a P; wherein G55 is substituted with either an A, M, S, or T; wherein L56 is substituted with either an A, I, or V; wherein T57 is substituted with either an A, G, M, or S; wherein L58 is substituted with either an A, I, or V; wherein H59 is substituted with either a K, or R; wherein R60 is substituted with either a K, or H; wherein L61 is substituted with either an A, I, or V; wherein R62 is substituted with either a K, or H; wherein I63 is substituted with either an A, V, or L; wherein P64 is a P; wherein D65 is substituted with an E; wherein F66 is substituted with either a W, or Y; wherein C67 is a C; wherein P68 is a P; wherein P69 is a P; wherein A70 is substituted with either a G, I, L, M, S, T, or V; wherein P71 is a P; wherein D72 is substituted with an E; wherein Q73 is substituted with a N; wherein I74 is substituted with either an A, V, or L; wherein D75 is substituted with an E; wherein R76 is substituted with either a K, or H; wherein F77 is substituted with either a W, or Y; wherein V78 is substituted with either an A, I, or L; wherein Q79 is substituted with a N; wherein I80 is substituted with either an A, V, or L; wherein V81 is substituted with either an A, I, or L; wherein D82 is substituted with an E; wherein E83 is substituted with a D; wherein A84 is substituted with either a G, I, L, M, S, T, or V; wherein N85 is substituted with a Q; wherein A86 is substituted with either a G, I, L, M, S, T, or V; wherein R87 is substituted with either a K, or H; wherein G88 is substituted with either an A, M, S, or T; wherein E89 is substituted with a D; wherein A90 is substituted with either a G, I, L, M, S, T, or V; wherein V91 is substituted with either an A, I, or L; wherein G92 is substituted with either an A, M,

S, or T; wherein V93 is substituted with either an A, I, or L; wherein H94 is substituted with either a K, or R; wherein C95 is a C; wherein A96 is substituted with either a G, I, L, M, S, T, or V; wherein L97 is substituted with either an A, I, or V; wherein G98 is substituted with either an A, M, S, or T; wherein F99 is substituted with either a W, or Y; wherein G100 is substituted with either an A, M, S, or T; wherein R101 is substituted with either a K, or H; wherein T102 is substituted with either an A, G, M, or S; wherein G103 is substituted with either an A, M, S, or T; wherein T104 is substituted with either an A, G, M, or S; wherein M105 is substituted with either an A, G, S, or T; wherein L106 is substituted with either an A, I, or V; wherein A107 is substituted with either a G, I, L, M, S, T, or V; wherein C108 is a C; wherein Y109 is either an F, or W; wherein L110 is substituted with either an A, I, or V; and/or wherein V111 is substituted with either an A, I, or L of SEQ ID NO:152 in addition to any combination thereof. Other suitable substitutions within the BMY_HPP2 phosphatase active site domain are encompassed by the present invention and are referenced elsewhere herein. The present invention also encompasses the use of these BMY_HPP2 phosphatase active site domain conservative amino acid substituted polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the present invention encompasses a polynucleotide lacking the initiating start codon, in addition to, the resulting encoded polypeptide of BMY_HPP2. Specifically, the present invention encompasses the polynucleotide corresponding to nucleotides 92 thru 538 of SEQ ID NO:151, and the polypeptide corresponding to amino acids 2 thru 150 of SEQ ID NO:152. Also encompassed are recombinant vectors comprising said encoding sequence, and host cells comprising said vector.

The present invention also provides a three-dimensional homology model of the BMY_HPP2 polypeptide (see Figure 32) representing amino acid residues M1 to K150 of the polypeptide sequence of BMY_HPP2 (amino acid residues M1 to K150 of SEQ ID NO:152). A three-dimensional homology model can be constructed on the basis of the known structure of a homologous protein (Greer et al, 1991, Lesk, et al, 1992, Cardozo, et al, 1995, Yuan, et al, 1995). The homology model of the BMY_HPP2 polypeptide sequence (SEQ ID NO:152), was based upon the

homologous structure of 1vhr from the N-terminus of the human dual specificity phosphatase (vaccinia H1-related phosphatase VN1) (residues N31-K179; Protein Data Bank, PDB entry 1vhr chain A; Genbank Accession No. gi|1633321; SEQ ID NO:207) and is defined by the set of structural coordinates set forth in Table IX herein.

Homology models are useful when there is no experimental information available on the protein of interest. A 3-dimensional model can be constructed on the basis of the known structure of a homologous protein (Greer et al, 1991, Lesk, et al, 1992, Cardozo, et al, 1995, Sali, et al, 1995).

Those of skill in the art will understand that a homology model is constructed on the basis of first identifying a template, or, protein of known structure which is similar to the protein without known structure. This can be accomplished by through pairwise alignment of sequences using such programs as FASTA (Pearson, et al 1990) and BLAST (Altschul, et al, 1990). In cases where sequence similarity is high (greater than 30%) these pairwise comparison methods may be adequate. Likewise, multiple sequence alignments or profile-based methods can be used to align a query sequence to an alignment of multiple (structurally and biochemically) related proteins. When the sequence similarity is low, more advanced techniques are used such as fold recognition (protein threading; Hendlich, et al, 1990), where the compatibility of a particular sequence with the 3-dimensional fold of a potential template protein is gauged on the basis of a knowledge-based potential. Following the initial sequence alignment, the query template can be optimally aligned by manual manipulation or by incorporation of other features (motifs, secondary structure predictions, and allowed sequence conservation). Next, structurally conserved regions can be identified and used to construct the core secondary structure (Sali, et al, 1995). Loops can be added using knowledge-based techniques, and refined performing forcefield calculations (Sali, et al, 1995, Cardozo, et al, 1995).

For BMY_HPP2 the pairwise alignment method FASTA (Pearson, et al 1990) and fold recognition methods (protein threading) generated identical sequence alignments for a portion (residues M1 to K150 of SEQ ID NO:152) of BMY_HPP2 aligned with the sequence of 1vhr from the N-terminus of the human dual specificity phosphatase (vaccinia H1-related phosphatase VN1) (residues N31-K179; Protein

Data Bank, PDB entry 1vhr chain A; Genbank Accession No. gi|1633321; SEQ ID NO:207). The alignment of BMY-HPP2 with PDB entry 1vhr is set forth in Figure 31. In this invention, the homology model of BMY_HPP2 was derived from the sequence alignment set forth in Figure 31, and hence an overall atomic model including plausible sidechain orientations using the program LOOK (Levitt, 1992). The three dimensional model for BMY-HPP2 is defined by the set of structure coordinates as set forth in Table IX and visualized in Figure 32.

In order to recognize errors in three-dimensional structures knowledge based mean fields can be used to judge the quality of protein folds (Sippl 1993). The methods can be used to recognize misfolded structures as well as faulty parts of structural models. The technique generates an energy graph where the energy distribution for a given protein fold is displayed on the y-axis and residue position in the protein fold is displayed on the x-axis. The knowledge based mean fields compose a force field derived from a set of globular protein structures taken as a subset from the Protein Data Bank (Bernstein et. al. 1977). To analyze the quality of a model the energy distribution is plotted and compared to the energy distribution of the template from which the model was generated. Figure 33 shows the energy graph for theBMY_HPP2 model (dotted line) and the template (1vhr, a dual-specificity phosphatase) from which the model was generated. It is clear that the model and template have similar energies over the aligned region, suggesting thatBMY_HPP2 is in a "native-like" conformation. This graph supports the motif and sequence alignments in confirming that the three dimensional structure coordinates ofBMY_HPP2 are an accurate and useful representation for the polypeptide.

The term "structure coordinates" refers to Cartesian coordinates generated from the building of a homology model.

Those of skill in the art will understand that a set of structure coordinates for a protein is a relative set of points that define a shape in three dimensions. Thus, it is possible that an entirely different set of coordinates could define a similar or identical shape. Moreover, slight variations in the individual coordinates, as emanate from generation of similar homology models using different alignment templates (i.e., other than the structure coordinates of 1vhr), and/or using different methods in generating the homology model, will have minor effects on the overall shape. Variations in

coordinates may also be generated because of mathematical manipulations of the structure coordinates. For example, the structure coordinates set forth in Table IX and visualized in Figure 32 could be manipulated by fractionalization of the structure coordinates; integer additions or subtractions to sets of the structure coordinates, inversion of the structure coordinates or any combination of the above.

Various computational analyses are therefore necessary to determine whether a molecule or a portion thereof is sufficiently similar to all or parts of BMY_HPP2 described above as to be considered the same. Such analyses may be carried out in current software applications, such as INSIGHTII (Molecular Simulations Inc., San Diego, CA) version 2000 and as described in the accompanying User's Guide.

Using the superimposition tool in the program INSIGHTII comparisons can be made between different structures and different conformations of the same structure. The procedure used in INSIGHTII to compare structures is divided into four steps: 1) load the structures to be compared; 2) define the atom equivalencies in these structures; 3) perform a fitting operation; and 4) analyze the results. Each structure is identified by a name. One structure is identified as the target (i.e., the fixed structure); the second structure (i.e., moving structure) is identified as the source structure. Since atom equivalency within INSIGHTII is defined by user input, for the purpose of this invention we will define equivalent atoms as protein backbone atoms (N, Cα, C and O) for all conserved residues between the two structures being compared. We will also consider only rigid fitting operations. When a rigid fitting method is used, the working structure is translated and rotated to obtain an optimum fit with the target structure. The fitting operation uses an algorithm that computes the optimum translation and rotation to be applied to the moving structure, such that the root mean square difference of the fit over the specified pairs of equivalent atom is an absolute minimum. This number, given in angstroms, is reported by INSIGHTII. For the purpose of this invention, any homology model of a BMY_HPP2 that has a root mean square deviation of conserved residue backbone atoms (N, Cα, C, O) of less than 3.0 A when superimposed on the relevant backbone atoms described by structure coordinates listed in Table IX and visualized in Figure 32 are considered identical. More preferably, the root mean square deviation is less than 2.0 Å.

This invention as embodied by the homology model enables the structure-based design of modulators of the biological function of BMY_HPP2, as well as mutants with altered biological function and/or specificity.

There is 23% sequence identity between catalytic domain of BMY_HPP2 and the human dual specificity phosphatase VHR (Yuvaniyama, J., et al., 1996; PDB identifier 1vhr) which was used as the template for 3D model generation as determined by the GAP program within GCG (Genetics Computer Group, Wisconsin). For the BMY_HPP2 the functionally important residues are located in a cleft comprised of residues D65, H94-C95-X-X-G98-X-X-R101 (the 'active site'). All these residues are conserved in 1vhr (D92, H123-C124-X-X-G127-X-X-R130). Based on the sequence alignment disclosed in Figure 31 and the structural model disclosed in Table IX and visualized in Figure 32, D65 is identified as a general acid, C95 as the catalytic Cysteine nucleophile which cleaves the phosphodiester bond, and R101 as the essential Argenine which activates the bond for cleavage as described in the literature (reviewed by Fauman and Saper, 1996).

In a preferred embodiment of the present invention, the molecule comprises the cleft region defined by structure coordinates of BMY_HPP2 amino acids described above according to Table IX, or a mutant of said molecule.

More preferred are molecules comprising all or any part of the cleft or a mutant or homologue of said molecule or molecular complex. By mutant or homologue of the molecule it is meant a molecule that has a root mean square deviation from the backbone atoms of said BMY_HPP2 amino acids of not more than 3.5 Angstroms.

The term "root mean square deviation" means the square root of the arithmetic mean of the squares of the deviations from the mean. It is a way to express the deviation or variation from a trend or object. For purposes of this invention, the "root mean square deviation" defines the variation in the backbone of a protein from the relevant portion of the backbone of BMY_HPP2 as defined by the structure coordinates described herein.

The structure coordinates of a BMY_HPP2 homology model portions thereof are stored in a machine-readable storage medium. Such data may be used for a variety of purposes, such as drug discovery.

Accordingly, in one embodiment of this invention is provided a machine-readable data storage medium comprising a data storage material encoded with the structure coordinates set forth in Table IX

One embodiment utilizes System 10 as disclosed in WO 98/11134, the disclosure of which is incorporated herein by reference in its entirety. Briefly, one version of these embodiments comprises a computer comprising a central processing unit ("CPU"), a working memory which may be, e.g, RAM (random-access memory) or "core" memory, mass storage memory (such as one or more disk drives or CD-ROM drives), one or more cathode-ray tube ("CRT") display terminals, one or more keyboards, one or more input lines, and one or more output lines, all of which are interconnected by a conventional bidirectional system bus.

Input hardware, coupled to the computer by input lines, may be implemented in a variety of ways. Machine-readable data of this invention may be inputted via the use of a modem or modems connected by a telephone line or dedicated data line. Alternatively or additionally, the input hardware may comprise CD-ROM drives or disk drives. In conjunction with a display terminal, keyboard may also be used as an input device.

Output hardware, coupled to the computer by output lines, may similarly be implemented by conventional devices. By way of example, output hardware may include a CRT display terminal for displaying a graphical representation of a region or domain of the present invention using a program such as QUANTA as described herein. Output hardware might also include a printer, so that hard copy output may be produced, or a disk drive, to store system output for later use.

In operation, the CPU coordinates the use of the various input and output devices, coordinates data accesses from mass storage, and accesses to and from the working memory, and determines the sequence of data processing steps. A number of programs may be used to process the machine-readable data of this invention. Such programs are discussed in reference to the computational methods of drug discovery as described herein. Specific references to components of the hardware system are included as appropriate throughout the following description of the data storage medium.

For the purpose of the present invention, any magnetic data storage medium which can be encoded with machine-readable data would be sufficient for carrying out the storage requirements of the system. The medium could be a conventional floppy diskette or hard disk, having a suitable substrate, which may be conventional, and a suitable coating, which may be conventional, on one or both sides, containing magnetic domains whose polarity or orientation could be altered magnetically, for example. The medium may also have an opening for receiving the spindle of a disk drive or other data storage device.

The magnetic domains of the coating of a medium may be polarized or oriented so as to encode in a manner which may be conventional, machine readable data such as that described herein, for execution by a system such as the system described herein.

Another example of a suitable storage medium which could also be encoded with such machine-readable data, or set of instructions, which could be carried out by a system such as the system described herein, could be an optically-readable data storage medium. The medium could be a conventional compact disk read only memory (CD-ROM) or a rewritable medium such as a magneto-optical disk which is optically readable and magneto-optically writable. The medium preferably has a suitable substrate, which may be conventional, and a suitable coating , which may be conventional, usually of one side of substrate.

In the case of a CD-ROM, as is well known, the coating is reflective and is impressed with a plurality of pits to encode the machine-readable data. The arrangement of pits is read by reflecting laser light off the surface of the coating. A protective coating, which preferably is substantially transparent, is provided on top of the reflective coating.

In the case of a magneto-optical disk, as is well known, the coating has no pits, but has a plurality of magnetic domains whose polarity or orientation can be changed magnetically when heated above a certain temperature, as by a laser. The orientation of the domains can be read by measuring the polarization of laser light reflected from the coating. The arrangement of the domains encodes the data as described above.

Thus, in accordance with the present invention, data capable of displaying the three dimensional structure of the BMY_HPP2 homology model, or portions thereof

and their structurally similar homologues is stored in a machine-readable storage medium, which is capable of displaying a graphical three-dimensional representation of the structure. Such data may be used for a variety of purposes, such as drug discovery.

For the first time, the present invention permits the use, through homology modeling based upon the sequence of BMY_HPP2 (Figure 21; SEQ ID NO:152) of structure-based or rational drug design techniques to design, select, and synthesize chemical entities that are capable of modulating the biological function of BMY_HPP2.

Accordingly, the present invention is also directed to the entire sequence in Figure 21 or any portion thereof for the purpose of generating a homology model for the purpose of 3D structure-based drug design.

For purposes of this invention, we include mutants or homologues of the sequence in Figure 21 or any portion thereof. In a preferred embodiment, the mutants or homologues have at least 25% identity, more preferably 50% identity, more preferably 75% identity, and most preferably 90% identity to the amino acid residues in Figure 21.

The three-dimensional model structure of the BMY_HPP2 will also provide methods for identifying modulators of biological function. Various methods or combination thereof can be used to identify these compounds.

Structure coordinates of the catalytic region defined above can also be used to identify structural and chemical features. Identified structural or chemical features can then be employed to design or select compounds as potential BMY_HPP2 modulators. By structural and chemical features it is meant to include, but is not limited to, van der Waals interactions, hydrogen bonding interactions, charge interaction, hydrophobic bonding interaction, and dipole interaction. Alternatively, or in conjunction, the three-dimensional structural model can be employed to design or select compounds as potential BMY_HPP2 modulators. Compounds identified as potential BMY_HPP2 modulators can then be synthesized and screened in an assay characterized by binding of a test compound to the BMY_HPP2, or in characterizing BMY_HPP2 deactivation in the presence of a small molecule. Examples of assays useful in screening of potential BMY_HPP2 modulators include, but are not limited

to, screening in silico, *in vitro* assays and high throughput assays. Finally, these methods may also involve modifying or replacing one or more amino acids from BMY_HPP2 according to Table IX.

However, as will be understood by those of skill in the art upon this disclosure, other structure based design methods can be used. Various computational structure based design methods have been disclosed in the art.

For example, a number of computer modeling systems are available in which the sequence of the BMY_HPP2 and the BMY_HPP2 structure (i.e., atomic coordinates of BMY_HPP2 and/or the atomic coordinates of the active site as provided in Table IX) can be input. This computer system then generates the structural details of one or more these regions in which a potential BMY_HPP2 modulator binds so that complementary structural details of the potential modulators can be determined. Design in these modeling systems is generally based upon the compound being capable of physically and structurally associating with BMY_HPP2. In addition, the compound must be able to assume a conformation that allows it to associate with BMY_HPP2. Some modeling systems estimate the potential inhibitory or binding effect of a potential BMY_HPP2 modulator prior to actual synthesis and testing.

Methods for screening chemical entities or fragments for their ability to associate with a given protein target are also well known. Often these methods begin by visual inspection of the binding site on the computer screen. Selected fragments or chemical entities are then positioned in one or more of the active site region in BMY_HPP2. Docking is accomplished using software such as INSIGHTII, QUANTA and SYBYL, following by energy minimization and molecular dynamics with standard molecular mechanic forcefields such as CHARMM and AMBER. Examples of computer programs which assist in the selection of chemical fragment or chemical entities useful in the present invention include, but are not limited to, GRID (Goodford, 1985), AUTODOCK (Goodsell, 1990), and DOCK (Kuntz et al. 1982).

Upon selection of preferred chemical entities or fragments, their relationship to each other and BMY_HPP2 can be visualized and then assembled into a single potential modulator. Programs useful in assembling the individual chemical entities

include, but are not limited to SYBYL and LeapFrog (Tripos Associates, St. Louis MO), LUDI (Bohm 1992) and 3D Database systems (Martin 1992).

Alternatively, compounds may be designed de novo using either an empty active site or optionally including some portion of a known inhibitor. Methods of this type of design include, but are not limited to LUDI (Bohm 1992) and LeapFrog (Tripos Associates, St. Louis MO).

In addition, BMY_HPP2 is overall well suited to modern methods including combinatorial chemistry.

Programs such as DOCK (Kuntz et al. 1982) can be used with the atomic coordinates from the homology model to identify potential ligands from databases or virtual databases which potentially bind the in the metal binding region, and which may therefore be suitable candidates for synthesis and testing.

Additionally, the three-dimensional homology model of BMY_HPP2 will aid in the design of mutants with altered biological activity.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:151 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides consisting of a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 864 of SEQ ID NO:151, b is an integer between 15 to 878, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:151, and where b is greater than or equal to a+14.

Features of the Polypeptide Encoded by Gene No:3

The polypeptide fragment corresponding to this gene provided as SEQ ID NO:8 (Figure 3), encoded by the polynucleotide sequence according to SEQ ID NO:7 (Figure 3), and/or encoded by the polynucleotide contained within the deposited clone, BMY_HPP3, has significant homology at the nucleotide and amino acid level to a number of phosphatases, which include, for example, the human protein tyrosine phosphatase PTPCAAX1 protein (HS_PTPCAAX1; Genbank Accession No:gi|

AAB40597; SEQ ID NO:33); the human protein tyrosine phosphatase PTPCAAX2 (HS_PTPCAAX2; Genbank Accession No:gi| AAB40598; SEQ ID NO:34); the mouse prenylated protein tyrosine phosphatase (MM_PTPCAAX; Genbank Accession No:gi| JC5981; SEQ ID NO:35); and the Drosophila PRL-1 protein (DM_PRL1; Genbank Accession No:gi| AAF53506; SEQ ID NO:36) as determined by BLASTP. An alignment of the human phosphatase polypeptide with these proteins is provided in Figure 8.

BMY_HPP3 is predicted to be a prenylated phosphoprotein phosphatase based on its similarity to drosophila, mouse and human prenylated phosphotyrosine phosphatases (PTPCAAX proteins). Among the conserved catalytic residues, there is a conserved Aspartate ("D") and a conserved nucleophilic Cysteine ("C") as shown in Figure 8. At the C-terminus, a consensus prenylation site is conserved in BMY-HPP3 suggesting that the protein could be post-translationally modified by farnesylation or geranylation.

Preferred polynucleotides of the present invention comprise the following nucleic acid sequence:

ATGGCTAGAATGAACCTCCCTGCTTCTGTGGACATTGCATACAAAAATGT
GAGATTTCTTATTACACACAACCCAACCAATACCTACTTTAATAGATTCTT
ACAGGAACTTAAGCAGGATGGAGTTACCACCATAGTAAGAGTATGAAAA
GCAACTTACAACATTGCTCTTTTAGAGAAGGGAAGCATCCAGGTTCCGGA
CTGGCCTTTTGATGATGGTACAGCACCATCCAGCCAGATAATTGATAACTG
GTTAAAACTTATGAAAAATAAATTTCATGAAGATCCTGGTTGTTGTATTGC
AATTCACTGTGTTGTAGGTTTTGGGTGAGCTCCAGTTGCTAGTTGCCCTAG
CTTTAATTGAAGGTGGAATGAAATATGAAAATGTAGTACAGTTCATCAGA
TAAAAGTGACATGGAACTTTTAACAGCAAACAACTTTTGTATTTGGAGAA
ATATTGTCTTAAAATATGCTTGCACCTCAGAAATCCCAGAAATAACTGTTT
CCTTCAG (SEQ ID NO: 83). Polypeptides encoding by these polynucleotides are also provided.

Preferred polypeptides of the present invention comprise the following amino acid sequence:

MARMNLPASVDIAYKNVRFLITHNPTNTYFNRFLQELKQDGVTTIVRVKATY
NIALLEKGSIQVPDWPFDDGTAPSSQIIDNWLKLMKNKFHEDPGCCIAIHCVV

GFGELQLLVALALIEGGMKYENVVQFIRKHGTFNSKQLLYLEKYCLKICLHLR NPRNNCFLQ (SEQ ID NO:84). Polynucleotides encoding these polypeptides are also provided.

Based upon the strong homology to members of the phosphatase proteins, the polypeptide encoded by the human BMY_HPP3 phosphatase of the present invention is expected to share at least some biological activity with phosphatase proteins, preferably with members of the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases, particularly the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases referenced herein.

The present invention encompasses the use of BMY_HPP3 inhibitors and/or activators of BMY_HPP3 activity for the treatment, detection, amelioaration, or prevention of phosphatase associated disorders, including but not limited to metabolic diseases such as diabetes, in addition to neural and/or cardiovascular diseases and disorders. The present invention also encompasses the use of BMY_HPP3 inhibitors and/or activators of BMY_HPP3 activity as immunosuppressive agents, anti-inflammatory agents, and/or anti-tumor agents

The present invention encompasses the use of BMY_HPP3 phosphatase inhibitors, including, antagonists such as antisense nucleic acids, in addition to other antagonists, as described herein, in a therapeutic regimen to diagnose, prognose, treat, ameliorate, and/or prevent diseases where a kinase activity is insufficient. One, non-limiting example of a disease which may occur due to insufficient kinase activity are certain types of diabetes, where one or more kinases involved in the insulin receptor signal pathway may have insufficient activity or insufficient expression, for example.

Moreover, the present invention encompasses the use of BMY_HPP3 phosphatase activators, and/or the use of the BMY_HPP3 phosphatase gene or protein in a gene therapy regimen, as described herein, for the diagnoses, prognoses, treatment, amelioration, and/or prevention of diseases and/or disorders where a kinase activity is overly high, such as a cancer where a kinase oncogene product has excessive activity or excessive expression.

The present invention also encompasses the use of catalytically inactive variants of BMY_HPP3 proteins, including fragments thereof, such as a protein therapeutic, or the use of the encoding polynucleotide sequence or as gene therapy,

for example, in the diagnoses, prognosis, treatment, amelioration, and/or prevention of diseases or disorders where phosphatase activity is overly high.

The present invention encompasses the use of antibodies directed against the BMY_HPP3 polypeptides, including fragment and/or variants thereof, of the present invention in diagnostics, as a biomarkers, and/or as a therapeutic agents.

The present invention encompasses the use of an inactive, non-catalytic, mutant of the BMY_HPP3 phosphatase as a substrate trapping mutant to bind cellular phosphoproteins or a library of phosphopeptides to identify substrates of the BMY_HPP3 polypeptides.

The present invention encompasses the use of the BMY_HPP3 polypeptides, to identify inhibitors or activators of the BMY_HPP3 phosphatase activity using either in vitro or 'virtual' (in silico) screening methods.

One embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of the BMY_HPP3 phosphatase comprising the steps of: i.) contacting a BMY_HPP3 phosphatase inhibitor or activator labeled with an analytically detectable reagent with the BMY_HPP3 phosphatase under conditions sufficient to form a complex with the inhibitor or activator; ii.) contacting said complex with a sample containing a compound to be identified; iii) and identifying the compound as an inhibitor or activator by detecting the ability of the test compound to alter the amount of labeled known BMY_HPP3 phosphatase inhibitor or activator in the complex.

Another embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of a BMY_HPP3 phosphatase comprising the steps of: i.) contacting the BMY_HPP3 phosphatase with a compound to be identified; and ii.) and measuring the ability of the BMY_HPP3 phosphatase to remove phosphate from a substrate.

The present invention also encomposses a method for identifying a ligand for the BMY_HPP3 phosphatase comprising the steps of: i.) contacting the BMY_HPP3 phosphatase with a series of compounds under conditions to permit binding; and ii.) detecting the presence of any ligand-bound protein.

Preferably, the above referenced methods comprise the BMY_HPP3 phosphatase in a form selected from the group consisting of whole cells, cytosolic cell

fractions, membrane cell fractions, purified or partially purified forms. The invention also relates to recombinantly expressed BMY_HPP3 phosphatase in a purified, substantially purified, or unpurified state. The invention further relates to BMY_HPP3 phosphatase fused or conjugated to a protein, peptide, or other molecule or compound known in the art, or referenced herein.

The present invention also encompasses pharmaceutical composition of the BMY_HPP3 phosphatase polypeptide comprising a compound identified by above referenced methods and a pharmaceutically acceptable carrier.

Features of the Polypeptide Encoded by Gene No:4

The polypeptide fragment corresponding to this gene provided as SEQ ID NO:10 (Figure 4), encoded by the polynucleotide sequence according to SEQ ID NO:9 (Figure 4), and/or encoded by the polynucleotide contained within the deposited clone, BMY_HPP4, has significant homology at the nucleotide and amino acid level to a number of phosphatases, which include, for example,the mouse osteotesticular protein tyrosine phosphatase (MM_OST-PTP; Genbank Accession No:gi| AAG28768; SEQ ID NO:37); and the rat protein-tyrosine-phosphatase (RN_PTP-OST; Genbank Accession No:gi| A55148; SEQ ID NO:38) as determined by BLASTP. An alignment of the human phosphatase polypeptide with these proteins is provided in Figure 9.

BMY_HPP4 is predicted to be a phosphoprotein phosphatase based on its homology to rat osteotesticular receptor protein-tyrosine-phosphatase precursor (Genbank ID 1083770) and to mouse receptor protein-tyrosine-phosphatase precursor (Genbank ID 11066925). The BMY_HPP4 polypeptide has been shown to comprise a conserved Aspartate ("D") at amino acid 182 of SEQ ID NO:10 (Figure 4), a catalytic Cysteine ("C") at amino acid 216 of SEQ ID NO:10 (Figure 4), and a conserved Argenine ("R") at amino acid 227 of SEQ ID NO:10 (Figure 4).

The predicted exon structure of the BMY_HPP4 gene is provided in Table V. The 'Start' and 'End' designations refer to the respective nucleotide positions of the BMY_HPP4 as they appear for BAC AL 354751. The numbering begins at the start of BAC AL354751; nucleotide 71352 in the BAC is equivalent to nucleotide 1 of the BMY_HPP4 transcript (SEQ ID NO:9; Figure 4).

Based upon the strong homology to members of the phosphatase proteins, the polypeptide encoded by the human BMY_HPP4 phosphatase of the present invention is expected to share at least some biological activity with phosphatase proteins, preferably with members of the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases, particularly the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases referenced herein.

The present invention encompasses the use of BMY_HPP4 inhibitors and/or activators of BMY_HPP4 activity for the treatment, detection, amelioaration, or prevention of phosphatase associated disorders, including but not limited to metabolic diseases such as diabetes, in addition to neural and/or cardiovascular diseases and disorders. The present invention also encompasses the use of BMY_HPP4 inhibitors and/or activators of BMY_HPP4 activity as immunosuppressive agents, anti-inflammatory agents, and/or anti-tumor agents

The present invention encompasses the use of BMY_HPP4 phosphatase inhibitors, including, antagonists such as antisense nucleic acids, in addition to other antagonists, as described herein, in a therapeutic regimen to diagnose, prognose, treat, ameliorate, and/or prevent diseases where a kinase activity is insufficient. One, non-limiting example of a disease which may occur due to insufficient kinase activity are certain types of diabetes, where one or more kinases involved in the insulin receptor signal pathway may have insufficient activity or insufficient expression, for example.

Moreover, the present invention encompasses the use of BMY_HPP4 phosphatase activators, and/or the use of the BMY_HPP4 phosphatase gene or protein in a gene therapy regimen, as described herein, for the diagnoses, prognoses, treatment, amelioration, and/or prevention of diseases and/or disorders where a kinase activity is overly high, such as a cancer where a kinase oncogene product has excessive activity or excessive expression.

The present invention also encompasses the use of catalytically inactive variants of BMY_HPP4 proteins, including fragments thereof, such as a protein therapeutic, or the use of the encoding polynucleotide sequence or as gene therapy, for example, in the diagnoses, prognosis, treatment, amelioration, and/or prevention of diseases or disorders where phosphatase activity is overly high.

The present invention encompasses the use of antibodies directed against the BMY_HPP4 polypeptides, including fragment and/or variants thereof, of the present invention in diagnostics, as a biomarkers, and/or as a therapeutic agents.

The present invention encompasses the use of an inactive, non-catalytic, mutant of the BMY_HPP4 phosphatase as a substrate trapping mutant to bind cellular phosphoproteins or a library of phosphopeptides to identify substrates of the BMY_HPP4 polypeptides.

The present invention encompasses the use of the BMY_HPP4 polypeptides, to identify inhibitors or activators of the BMY_HPP4 phosphatase activity using either in vitro or 'virtual' (in silico) screening methods.

One embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of the BMY_HPP4 phosphatase comprising the steps of: i.) contacting a BMY_HPP4 phosphatase inhibitor or activator labeled with an analytically detectable reagent with the BMY_HPP4 phosphatase under conditions sufficient to form a complex with the inhibitor or activator; ii.) contacting said complex with a sample containing a compound to be identified; iii) and identifying the compound as an inhibitor or activator by detecting the ability of the test compound to alter the amount of labeled known BMY_HPP4 phosphatase inhibitor or activator in the complex.

Another embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of a BMY_HPP4 phosphatase comprising the steps of: i.) contacting the BMY_HPP4 phosphatase with a compound to be identified; and ii.) and measuring the ability of the BMY_HPP4 phosphatase to remove phosphate from a substrate.

The present invention also encomposses a method for identifying a ligand for the BMY_HPP4 phosphatase comprising the steps of: i.) contacting the BMY_HPP4 phosphatase with a series of compounds under conditions to permit binding; and ii.) detecting the presence of any ligand-bound protein.

Preferably, the above referenced methods comprise the BMY_HPP4 phosphatase in a form selected from the group consisting of whole cells, cytosolic cell fractions, membrane cell fractions, purified or partially purified forms. The invention also relates to recombinantly expressed BMY_HPP4 phosphatase in a purified,

substantially purified, or unpurified state. The invention further relates to BMY_HPP4 phosphatase fused or conjugated to a protein, peptide, or other molecule or compound known in the art, or referenced herein.

The present invention also encompasses pharmaceutical composition of the BMY_HPP4 phosphatase polypeptide comprising a compound identified by above referenced methods and a pharmaceutically acceptable carrier.

Expression profiling of the BMY_HPP4 polypeptide in normal tissues showed that BMY_HPP4 is expressed at higher levels in the cerebellum than in any other tissue, suggesting a role for modulators of BMY_HPP4 activity in the treatment of neurological disorders such as depression, bipolar disorder, schizophrenia, dementia and cognitive disorders (as shown in Figure 34). BMY_HPP4 was also expressed at lower levels in other subregions of the brain. In addition, BMY_HPP4 was expressed at significant levels in the pineal and pituitary glands, suggesting a role for modulators of BMY_HPP4 activity in endocrine disorders.

The strong homology to dual specificity phophatases, combined with the localized expression in cerebellum suggests the BMY_HPP4 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing neurodegenerative disease states, behavioral disorders, or inflammatory conditions. Representative uses are described in the "Regeneration" and "Hyperproliferative Disorders" sections below, in the Examples, and elsewhere herein. Briefly, the uses include, but are not limited to the detection, treatment, and/or prevention of Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue

markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

The strong homology to dual specificity phophatases, combined with the localized expression in pineal and pituitary glands suggests the BMY_HPP4 polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing endocrine diseases and/or disorders, which include, but are not limited to, the following: aberrant growth hormone synthesis and/or secretion, aberrant prolactin synthesis and/or secretion, aberrant luteinizing hormone synthesis and/or secretion, aberrant follicle-stimulating hormone synthesis and/or secretion, aberrant thyroid-stimulating hormone synthesis and/or secretion, aberrant adrenocorticotropin synthesis and/or secretion, aberrant vasopressin secretion, aberrant oxytocin secretion, aberrant growth, aberrant lactation, aberrant sexual characteristic development, aberrant testosterone synthesis and/or secretion, aberrant estrogen synthesis and/or secretion, aberrant water homeostasis, hypogonadism, Addison's disease, hypothyroidism, Cushing's disease, agromegaly, gigantism, lethargy, osteoporosis, aberrant calcium homeostasis, aberrant potassium homeostasis, reproductive disorders, and developmental disorders.

Features of the Polypeptide Encoded by Gene No:5

The polypeptide corresponding to this gene provided as SEQ ID NO:42 (Figure 5), encoded by the polynucleotide sequence according to SEQ ID NO:41 (Figure 5), and/or encoded by the polynucleotide contained within the deposited clone, BMY_HPP5, has significant homology at the nucleotide and amino acid level to a number of phosphatases, which include, for example, the human dual specificity phosphatase 8 (hs_dspp8; Genbank Accession No:gi| NP_004411; SEQ ID NO:39); and the mouse neuronal tyrosine/threonine phosphatase 1 (r mm_npp1; Genbank Accession No:gi| NP_032774; SEQ ID NO:40) as determined by BLASTP. An alignment of the human phosphatase polypeptide with these proteins is provided in Figures 10A-B.

The determined nucleotide sequence of the BMY_HPP5 cDNA in Figures 5A-E (SEQ ID NO:41) contains an open reading frame encoding a protein of about 665 amino acid residues, with a deduced molecular weight of about 73kDa. The amino acid sequence of the predicted BMY_HPP5 polypeptide is shown in Figures 5A-E (SEQ ID NO:42). The BMY_HPP5 protein shown in Figures 5A-E was determined to share significant identity and similarity to several known phosphatases, particularly, dual-specificity protein phosphatases. Specifically, the BMY_HPP5 protein shown in Figures 5A-E was determined to be about 46% identical and 58% similar to the human dual specificity phosphatase 8 (HS_DSPP8; Genbank Accession No: gi| NP_004411; SEQ ID NO:39); and about 43% identical and 56% similar to the mouse neuronal tyrosine/threonine phosphatase 1 (MM_NPP1; Genbank Accession No: gi| NP_032774; SEQ ID NO:40), as shown in Figure 12.

BMY_HPP5 is predicted to encode a phosphoprotein phosphatase based on its homology to known dual-specificity protein phosphatases including human dual-specificity protein phosphatase 8 (GI 4758212) and mouse neuronal tyrosine/threonine phosphatase 1 (GI 6679156) (Figures 10A-B). The BMY_HPP5 polypeptide was determined to comprise conserved residues, which include, the catalytic Aspartate ("D") at amino acid 212, and a conserved Cysteine ("C") at amino acid 244, and Arginine ("R") at amino acid 249 of SEQ ID NO:42 (Figures 5A-E).

Based upon the strong homology to members of the phosphatase proteins, the polypeptide encoded by the human BMY_HPP5 phosphatase of the present invention is expected to share at least some biological activity with phosphatase proteins, preferably with members of the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases, particularly the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases referenced herein.

Expression profiling designed to measure the steady state mRNA levels encoding the human phosphatase polypeptide, BMY_HPP5, showed predominately high expression levels in the testis and spinal cord, and to a lesser extent, in bone marrow, brain, liver, and thymus. (See Figure 11).

Moreover, expanded expression profiling of the BMY_HPP5 polypeptide in normal human tissues showed the highest levels of expression in the adrenal, pineal and pituitary glands suggesting that modulators of BMY_HPP5 activity could be

useful in the treatment of endocrine disorders (as shown in Figure 35). BMY_HPP5 also expressed at high levels in the cerebellum, suggesting a role for modulators of BMY_HPP5 activity in the treatment of neurological disorders such as depression, bipolar disorder, schizophrenia, dementia and cognitive disorders; in the prostate, suggesting a role for modulators of BMY_HPP5 activity in the treatment of prostate cancer or benign prostatic hyperplasia; in the testis, suggesting a role for modulators of BMY_HPP5 activity in the treatment of male infertility caused by defective or insufficient spermatogenesis, as a contraceptive agent, or in the treatment of testicular cancer. BMYBMY_HPP5 was also expressed at a lower but significant level in many other normal human tissues.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the predominate localized expression in adrenal gland tissue suggests the human BMY_HPP5 phosphatase polynucleotides and polypeptides, including antagonists, and/or fragments thereof, may be useful for treating, diagnosing, prognosing, ameliorating, and/or preventing endocrine disorders, which include, but are mot limited to adrenocortical hyperfunction, adrenocortical hypofunction, lethargy. Congenital adrenal hyperplasia, aberrant ACTH regulation, aberrant adrenaline regulation, disorders associated with defects in P450C21, P450C18, P450C17, and P450C11 hydroxylases and in 3-hydroxysteroid dehydrogenase (3-HSD), hirsutism, oligomenorrhea, acne, virilization, oligomenorrhea, female pseudohermaphroditism, disorders associated with the incidence of aberrant sexual characterisitics, disorders associated with aberrant cortisol secretion, hypertension, hypokalemia, hypogonadism, disorders associated with aberrant androgen secretion, adrenal virilism, Adrenal adenomas, Adrenal carcinomas, disorders associated with aberrant aldosterone secretion, aldosteronism, disorders associated with aberrant steriod biosynthesis, disorders associated with aberrant steriod transport, disorders associated with aberrant steriod secretion, disorders associated with aberrant steriod excretion, Addison's syndrome, and Cushing's syndrome.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the predominate localized expression in pituitary gland tissue suggests the BMY_HPP5 polynucleotides and polypeptides may be useful in

treating, diagnosing, prognosing, and/or preventing endocrine diseases and/or disorders, which include, but are not limited to, the following: aberrant growth hormone synthesis and/or secretion, aberrant prolactin synthesis and/or secretion, aberrant luteinizing hormone synthesis and/or secretion, aberrant follicle-stimulating hormone synthesis and/or secretion, aberrant thyroid-stimulating hormone synthesis and/or secretion, aberrant adrenocorticotropin synthesis and/or secretion, aberrant vasopressin secretion, aberrant oxytocin secretion, aberrant growth, aberrant lactation, aberrant sexual characteristic development, aberrant testosterone synthesis and/or secretion, aberrant estrogen synthesis and/or secretion, aberrant water homeostasis, hypogonadism, Addison's disease, hypothyroidism, Cushing's disease, agromegaly, gigantism, lethargy, osteoporosis, aberrant calcium homeostasis, aberrant potassium homeostasis, reproductive disorders, developmental disorders, and depression related to low incident light levels.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the predominate localized expression in testis tissue suggests the human BMY_HPP5 phosphatase polynucleotides and polypeptides, including antagonists, and/or fragments thereof, may be useful for treating, diagnosing, prognosing, and/or preventing male reproductive disorders, such as, for example, male infertility, impotence, and/or testicular cancer. This gene product may also be useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. The testes are also a site of active gene expression of transcripts that is expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. If fact, increased expression of certain phosphatases have been identified as tumor markers for testicular cancer (see, for example, Koshida, K., Nishino, A., Yamamoto, H., Uchibayashi, T., Naito, K.,Hisazumi, H., Hirano, K., Hayashi, Y., Wahren, B., Andersson, L, J. Urol., 146(1):57-60, (1991); and Klein, EA, Urol. Clin. North. Am., 20(1):67-73, (1993)).

Alternatively, the strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the significant localized expression in spinal

cord and brain tissue suggests the human phosphatase polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing neural diseases and/or disorders. Representative uses are described in the "Neurological Diseases" section below, and elsewhere herein. Briefly, the expression in neural tissue indicates a role in Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, meningitis, encephalitis, demyelinating diseases, peripheral neuropathies, neoplasia, trauma, congenital malformations, spinal dyphida, spinal cord injuries, ischemia and infarction, aneurysms, hemorrhages, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, depression, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, elevated expression of this gene product in regions of the brain indicates it plays a role in normal neural function. Potentially, this gene product is involved in synapse formation, neurotransmission, learning, cognition, homeostasis, or neuronal differentiation or survival. Furthermore, the protein may also be used to determine biological activity, to raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

Moreover, the tissue distribution in liver indicates the protein product of this clone would be useful for the detection and treatment of liver disorders and cancers. Representative uses are described in the "Hyperproliferative Disorders", "Infectious Disease", and "Binding Activity" sections below, and elsewhere herein. Briefly, the protein can be used for the detection, treatment, and/or prevention of hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells. In addition the expression in fetus would suggest a useful role for the protein product in developmental abnormalities, fetal deficiencies, pre-natal disorders and various would-healing diseases and/or tissue trauma.

Moreover, human phosphatase polynucleotides and polypeptides, including fragments and agonists thereof, may have uses which include treating, diagnosing,

prognosing, and/or preventing hyperproliferative disorders, particularly of the renal, neural, and reproductive systems. Such disorders may include, for example, cancers, and metastasis.

The human phosphatase polynucleotides and polypeptides, including fragments and agonists thereof, may have uses which include, either directly or indirectly, for boosting immune responses.

The human phosphatase polynucleotides and polypeptides, including fragments and /or antagonists thereof, may have uses which include identification of modulators of human phosphatase function including antibodies (for detection or neutralization), naturally-occurring modulators and small molecule modulators. Antibodies to domains of the human phosphatase protein could be used as diagnostic agents of cardiovascular and inflammatory conditions in patients, are useful in monitoring the activation of signal transduction pathways, and can be used as a biomarker for the involvement of phosphatases in disease states, and in the evaluation of inhibitors of phosphatases in vivo.

Human phosphatase polypeptides and polynucleotides have additional uses which include diagnosing diseases related to the over and/or under expression of human phosphatase by identifying mutations in the human phosphatase gene by using human phosphatase sequences as probes or by determining human phosphatase protein or mRNA expression levels. Human phosphatase polypeptides may be useful for screening compounds that affect the activity of the protein. Human phosphatase peptides can also be used for the generation of specific antibodies and as bait in yeast two hybrid screens to find proteins the specifically interact with human phosphatase (described elsewhere herein).

Although it is believed the encoded polypeptide may share at least some biological activities with phosphatase proteins (particularly dual specificity proteins), a number of methods of determining the exact biological function of this clone are either known in the art or are described elsewhere herein. Briefly, the function of this clone may be determined by applying microarray methodology. Nucleic acids corresponding to the human phosphatase polynucleotides, in addition to, other clones of the present invention, may be arrayed on microchips for expression profiling. Depending on which polynucleotide probe is used to hybridize to the slides, a change

in expression of a specific gene may provide additional insight into the function of this gene based upon the conditions being studied. For example, an observed increase or decrease in expression levels when the polynucleotide probe used comes from diseased heart tissue, as compared to, normal tissue might indicate a function in modulating cardiac function, for example. In the case of human BMY_HPP5 phosphatase, testis, spinal cord, brain, liver, bone marrow, and thymus tissue should be used, for example, to extract RNA to prepare the probe.

In addition, the function of the protein may be assessed by applying quantitative PCR methodology, for example. Real time quantitative PCR would provide the capability of following the expression of the human phosphatase gene throughout development, for example. Quantitative PCR methodology requires only a nominal amount of tissue from each developmentally important step is needed to perform such experiments. Therefore, the application of quantitative PCR methodology to refining the biological function of this polypeptide is encompassed by the present invention. In the case of human phosphatase, a disease correlation related to human phosphatase may be made by comparing the mRNA expression level of human phosphatase in normal tissue, as compared to diseased tissue (particularly diseased tissue isolated from the following: testis, spinal cord, brain, liver, bone marrow, and thymus tissue). Significantly higher or lower levels of human phosphatase expression in the diseased tissue may suggest human phosphatase plays a role in disease progression, and antagonists against human phosphatase polypeptides would be useful therapeutically in treating, preventing, and/or ameliorating the disease. Alternatively, significantly higher or lower levels of human phosphatase expression in the diseased tissue may suggest human phosphatase plays a defensive role against disease progression, and agonists of human phosphatase polypeptides may be useful therapeutically in treating, preventing, and/or ameliorating the disease. Also encompassed by the present invention are quantitative PCR probes corresponding to the polynucleotide sequence provided as SEQ ID NO:41 (Figures 4A-D).

The function of the protein may also be assessed through complementation assays in yeast. For example, in the case of the human phosphatase, transforming yeast deficient in purinergic receptor activity, for example, and assessing their ability

to grow would provide convincing evidence the human phosphatase polypeptide has purinergic receptor activity. Additional assay conditions and methods that may be used in assessing the function of the polynucleotides and polypeptides of the present invention are known in the art, some of which are disclosed elsewhere herein.

Alternatively, the biological function of the encoded polypeptide may be determined by disrupting a homologue of this polypeptide in Mice and/or rats and observing the resulting phenotype. Such knock-out experiments are known in the art, some of which are disclosed elsewhere herein.

Moreover, the biological function of this polypeptide may be determined by the application of antisense and/or sense methodology and the resulting generation of transgenic mice and/or rats. Expressing a particular gene in either sense or antisense orientation in a transgenic mouse or rat could lead to respectively higher or lower expression levels of that particular gene. Altering the endogenous expression levels of a gene can lead to the observation of a particular phenotype that can then be used to derive indications on the function of the gene. The gene can be either over-expressed or under expressed in every cell of the organism at all times using a strong ubiquitous promoter, or it could be expressed in one or more discrete parts of the organism using a well characterized tissue-specific promoter (e.g., a kidney, lung, spinal cord, or testes tissue specific promoter), or it can be expressed at a specified time of development using an inducible and/or a developmentally regulated promoter.

In the case of human phosphatase transgenic mice or rats, if no phenotype is apparent in normal growth conditions, observing the organism under diseased conditions (renal, pulmonary, neurological, or reproductive disorders, in addition to cancers, etc.) may lead to understanding the function of the gene. Therefore, the application of antisense and/or sense methodology to the creation of transgenic mice or rats to refine the biological function of the polypeptide is encompassed by the present invention.

In preferred embodiments, the following N-terminal deletion mutants are encompassed by the present invention: M1-S665, A2-S665, H3-S665, E4-S665, M5-S665, I6-S665, G7-S665, T8-S665, Q9-S665, I10-S665, V11-S665, T12-S665, E13-S665, R14-S665, L15-S665, V16-S665, A17-S665, L18-S665, L19-S665, E20-S665, S21-S665, G22-S665, T23-S665, E24-S665, K25-S665, V26-S665, L27-S665, L28-

S665, I29-S665, D30-S665, S31-S665, R32-S665, P33-S665, F34-S665, V35-S665, E36-S665, Y37-S665, N38-S665, T39-S665, S40-S665, H41-S665, I42-S665, L43-S665, E44-S665, A45-S665, I46-S665, N47-S665, I48-S665, N49-S665, C50-S665, S51-S665, K52-S665, L53-S665, M54-S665, K55-S665, R56-S665, R57-S665, L58-S665, Q59-S665, Q60-S665, D61-S665, K62-S665, V63-S665, L64-S665, I65-S665, T66-S665, E67-S665, L68-S665, I69-S665, Q70-S665, H71-S665, S72-S665, A73-S665, K74-S665, H75-S665, K76-S665, V77-S665, D78-S665, I79-S665, D80-S665, C81-S665, S82-S665, Q83-S665, K84-S665, V85-S665, V86-S665, V87-S665, Y88-S665, D89-S665, Q90-S665, S91-S665, S92-S665, Q93-S665, D94-S665, V95-S665, A96-S665, S97-S665, L98-S665, S99-S665, S100-S665, D101-S665, C102-S665, F103-S665, L104-S665, T105-S665, V106-S665, L107-S665, L108-S665, G109-S665, K110-S665, L111-S665, E112-S665, K113-S665, S114-S665, F115-S665, N116-S665, S117-S665, V118-S665, H119-S665, L120-S665, L121-S665, A122-S665, G123-S665, G124-S665, F125-S665, A126-S665, E127-S665, F128-S665, S129-S665, R130-S665, C131-S665, F132-S665, P133-S665, G134-S665, L135-S665, C136-S665, E137-S665, G138-S665, K139-S665, S140-S665, T141-S665, L142-S665, V143-S665, P144-S665, T145-S665, C146-S665, I147-S665, S148-S665, Q149-S665, P150-S665, C151-S665, L152-S665, P153-S665, V154-S665, A155-S665, N156-S665, I157-S665, G158-S665, P159-S665, T160-S665, R161-S665, I162-S665, L163-S665, P164-S665, N165-S665, L166-S665, Y167-S665, L168-S665, G169-S665, C170-S665, Q171-S665, R172-S665, D173-S665, V174-S665, L175-S665, N176-S665, K177-S665, E178-S665, L179-S665, M180-S665, Q181-S665, Q182-S665, N183-S665, G184-S665, I185-S665, G186-S665, Y187-S665, V188-S665, L189-S665, N190-S665, A191-S665, S192-S665, N193-S665, T194-S665, C195-S665, P196-S665, K197-S665, P198-S665, D199-S665, F200-S665, I201-S665, P202-S665, E203-S665, S204-S665, H205-S665, F206-S665, L207-S665, R208-S665, V209-S665, P210-S665, V211-S665, N212-S665, D213-S665, S214-S665, F215-S665, C216-S665, E217-S665, K218-S665, I219-S665, L220-S665, P221-S665, W222-S665, L223-S665, D224-S665, K225-S665, S226-S665, V227-S665, D228-S665, F229-S665, I230-S665, E231-S665, K232-S665, A233-S665, K234-S665, A235-S665, S236-S665, N237-S665, G238-S665, C239-S665, V240-S665, L241-S665, V242-S665, H243-S665, C244-S665, L245-S665, A246-S665,

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sequences encoding these polypeptides are also provided. The present invention also encompasses the use of the human BMY_HPP5 phosphatase N-terminal deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal deletion mutants are encompassed by the present invention: M1-S665, M1-V664, M1-E663, M1-I662, M1-I661, M1-E660, M1-M659, M1-S658, M1-G657, M1-S656, M1-F655, M1-S654, M1-S653, M1-Q652, M1-S651, M1-G650, M1-V649, M1-K648, M1-G647, M1-L646, M1-E645, M1-E644, M1-R643, M1-S642, M1-R641, M1-N640, M1-E639, M1-S638, M1-M637, M1-I636, M1-S635, M1-E634, M1-G633, M1-F632, M1-E631, M1-M630, M1-Q629, M1-C628, M1-S627, M1-R626, M1-R625, M1-K624, M1-F623, M1-Q622, M1-K621, M1-E620, M1-F619, M1-P618, M1-S617, M1-E616, M1-E615, M1-H614, M1-W613, M1-S612, M1-R611, M1-R610, M1-S609, M1-D608, M1-A607, M1-R606, M1-D605, M1-S604, M1-P603, M1-K602, M1-Q601, M1-R600, M1-R599, M1-R598, M1-V597, M1-S596, M1-Y595, M1-V594, M1-Q593, M1-D592, M1-G591, M1-C590, M1-T589, M1-P588, M1-L587, M1-Q586, M1-S585, M1-C584, M1-S583, M1-Y582, M1-A581, M1-S580, M1-Y579, M1-S578, M1-A577, M1-S576, M1-G575, M1-G574, M1-Y573, M1-I572, M1-A571, M1-S570, M1-A569, M1-S568, M1-Y567, M1-F566, M1-H565, M1-S564, M1-S563, M1-E562, M1-T561, M1-A560, M1-F559, M1-Y558, M1-W557, M1-S556, M1-S555, M1-T554, M1-L553, M1-S552, M1-P551, M1-T550, M1-S549, M1-T548, M1-Q547, M1-P546, M1-A545, M1-L544, M1-I543, M1-D542, M1-S541, M1-H540, M1-W539, M1-G538, M1-K537, M1-L536, M1-G535, M1-L534, M1-G533, M1-A532, M1-S531, M1-K530, M1-T529, M1-L528, M1-H527, M1-Q526, M1-Q525, M1-S524, M1-T523, M1-S522, M1-L521, M1-G520, M1-F519, M1-L518, M1-F517, M1-S516, M1-T515, M1-H514, M1-Y513, M1-N512, M1-D511, M1-E510, M1-V509, M1-S508, M1-G507, M1-S506, M1-R505, M1-H504, M1-L503, M1-P502, M1-S501, M1-L500, M1-L499, M1-S498, M1-R497, M1-Q496, M1-A495, M1-T494, M1-G493, M1-S492, M1-S491, M1-S490, M1-T489, M1-R488, M1-V487, M1-S486, M1-H485, M1-L484, M1-R483, M1-K482, M1-S481, M1-Q480, M1-S479, M1-D478, M1-S477, M1-P476, M1-R475, M1-A474, M1-T473, M1-Q472, M1-L471, M1-K470, M1-K469, M1-P468, M1-I467, M1-S466, M1-A465, M1-E464, M1-E463,

M1-K462, M1-D461, M1-P460, M1-S459, M1-T458, M1-E457, M1-P456, M1-T455, M1-Q454, M1-E453, M1-S452, M1-L451, M1-E450, M1-Q449, M1-V448, M1-P447, M1-S446, M1-F445, M1-Q444, M1-C443, M1-L442, M1-K441, M1-N440, M1-T439, M1-G438, M1-D437, M1-L436, M1-T435, M1-T434, M1-S433, M1-P432, M1-K431, M1-Y430, M1-Y429, M1-E428, M1-L427, M1-A426, M1-D425, M1-E424, M1-S423, M1-S422, M1-S421, M1-F420, M1-G419, M1-H418, M1-L417, M1-S416, M1-A415, M1-A414, M1-M413, M1-S412, M1-A411, M1-S410, M1-Y409, M1-S408, M1-V407, M1-S406, M1-K405, M1-I404, M1-D403, M1-L402, M1-S401, M1-F400, M1-S399, M1-R398, M1-K397, M1-L396, M1-K395, M1-N394, M1-S393, M1-D392, M1-E391, M1-L390, M1-R389, M1-D388, M1-A387, M1-S386, M1-L385, M1-H384, M1-L383, M1-G382, M1-S381, M1-L380, M1-A379, M1-Q378, M1-V377, M1-L376, M1-P375, M1-S374, M1-D373, M1-E372, M1-L371, M1-L370, M1-S369, M1-P368, M1-Q367, M1-V366, M1-S365, M1-P364, M1-V363, M1-S362, M1-P361, M1-V360, M1-S359, M1-A358, M1-P357, M1-H356, M1-V355, M1-P354, M1-R353, M1-Q352, M1-G351, M1-A350, M1-A349, M1-E348, M1-S347, M1-T346, M1-A345, M1-S344, M1-D343, M1-A342, M1-C341, M1-P340, M1-P339, M1-S338, M1-L337, M1-P336, M1-T335, M1-E334, M1-S333, M1-K332, M1-Q331, M1-G330, M1-G329, M1-E328, M1-S327, M1-V326, M1-A325, M1-P324, M1-V323, M1-P322, M1-E321, M1-N320, M1-P319, M1-K318, M1-E317, M1-L316, M1-P315, M1-L314, M1-L313, M1-K312, M1-L311, M1-K310, M1-S309, M1-K308, M1-P307, M1-G306, M1-S305, M1-A304, M1-G303, M1-T302, M1-Q301, M1-N300, M1-K299, M1-I298, M1-K297, M1-K296, M1-E295, M1-Y294, M1-A293, M1-L292, M1-L291, M1-Q290, M1-G289, M1-L288, M1-F287, M1-N286, M1-F285, M1-N284, M1-P283, M1-S282, M1-I281, M1-T280, M1-P279, M1-R278, M1-K277, M1-E276, M1-K275, M1-V274, M1-F273, M1-R272, M1-Y271, M1-A270, M1-E269, M1-D268, M1-L267, M1-S266, M1-M265, M1-D264, M1-M263, M1-R262, M1-K261, M1-M260, M1-I259, M1-Y258, M1-A257, M1-I256, M1-A255, M1-I254, M1-T253, M1-A252, M1-S251, M1-R250, M1-S249, M1-I248, M1-G247, M1-A246, M1-L245, M1-C244, M1-H243, M1-V242, M1-L241, M1-V240, M1-C239, M1-G238, M1-N237, M1-S236, M1-A235, M1-K234, M1-A233, M1-K232, M1-E231, M1-I230, M1-F229, M1-D228, M1-V227, M1-S226, M1-K225, M1-D224, M1-L223, M1-W222, M1-P221, M1-L220, M1-I219,

M1-K218, M1-E217, M1-C216, M1-F215, M1-S214, M1-D213, M1-N212, M1-V211, M1-P210, M1-V209, M1-R208, M1-L207, M1-F206, M1-H205, M1-S204, M1-E203, M1-P202, M1-I201, M1-F200, M1-D199, M1-P198, M1-K197, M1-P196, M1-C195, M1-T194, M1-N193, M1-S192, M1-A191, M1-N190, M1-L189, M1-V188, M1-Y187, M1-G186, M1-I185, M1-G184, M1-N183, M1-Q182, M1-Q181, M1-M180, M1-L179, M1-E178, M1-K177, M1-N176, M1-L175, M1-V174, M1-D173, M1-R172, M1-Q171, M1-C170, M1-G169, M1-L168, M1-Y167, M1-L166, M1-N165, M1-P164, M1-L163, M1-I162, M1-R161, M1-T160, M1-P159, M1-G158, M1-I157, M1-N156, M1-A155, M1-V154, M1-P153, M1-L152, M1-C151, M1-P150, M1-Q149, M1-S148, M1-I147, M1-C146, M1-T145, M1-P144, M1-V143, M1-L142, M1-T141, M1-S140, M1-K139, M1-G138, M1-E137, M1-C136, M1-L135, M1-G134, M1-P133, M1-F132, M1-C131, M1-R130, M1-S129, M1-F128, M1-E127, M1-A126, M1-F125, M1-G124, M1-G123, M1-A122, M1-L121, M1-L120, M1-H119, M1-V118, M1-S117, M1-N116, M1-F115, M1-S114, M1-K113, M1-E112, M1-L111, M1-K110, M1-G109, M1-L108, M1-L107, M1-V106, M1-T105, M1-L104, M1-F103, M1-C102, M1-D101, M1-S100, M1-S99, M1-L98, M1-S97, M1-A96, M1-V95, M1-D94, M1-Q93, M1-S92, M1-S91, M1-Q90, M1-D89, M1-Y88, M1-V87, M1-V86, M1-V85, M1-K84, M1-Q83, M1-S82, M1-C81, M1-D80, M1-I79, M1-D78, M1-V77, M1-K76, M1-H75, M1-K74, M1-A73, M1-S72, M1-H71, M1-Q70, M1-I69, M1-L68, M1-E67, M1-T66, M1-I65, M1-L64, M1-V63, M1-K62, M1-D61, M1-Q60, M1-Q59, M1-L58, M1-R57, M1-R56, M1-K55, M1-M54, M1-L53, M1-K52, M1-S51, M1-C50, M1-N49, M1-I48, M1-N47, M1-I46, M1-A45, M1-E44, M1-L43, M1-I42, M1-H41, M1-S40, M1-T39, M1-N38, M1-Y37, M1-E36, M1-V35, M1-F34, M1-P33, M1-R32, M1-S31, M1-D30, M1-I29, M1-L28, M1-L27, M1-V26, M1-K25, M1-E24, M1-T23, M1-G22, M1-S21, M1-E20, M1-L19, M1-L18, M1-A17, M1-V16, M1-L15, M1-R14, M1-E13, M1-T12, M1-V11, M1-I10, M1-Q9, M1-T8, and/or M1-G7 of SEQ ID NO:42. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of the human BMY_HPP5 phosphatase C-terminal deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The present invention also encompasses immunogenic and/or antigenic epitopes of the human BMY_HPP5 phosphatase polypeptide.

The human phosphatase polypeptides of the present invention were determined to comprise several phosphorylation sites based upon the Motif algorithm (Genetics Computer Group, Inc.). The phosphorylation of such sites may regulate some biological activity of the human phosphatase polypeptide. For example, phosphorylation at specific sites may be involved in regulating the proteins ability to associate or bind to other molecules (e.g., proteins, ligands, substrates, DNA, etc.). In the present case, phosphorylation may modulate the ability of the human phosphatase polypeptide to associate with other polypeptides, particularly cognate ligand for human phosphatase, or its ability to modulate certain cellular signal pathways.

Specifically, the BMY_HPP5 polypeptide was predicted to comprise one tyrosine phosphorylation site using the Motif algorithm (Genetics Computer Group, Inc.). Such sites are phosphorylated at the tyrosine amino acid residue. The consensus pattern for tyrosine phosphorylation sites are as follows: [RK]-x(2)-[DE]-x(3)-Y, or [RK]-x(3)-[DE]-x(2)-Y, where Y represents the phosphorylation site and 'x' represents an intervening amino acid residue. Additional information specific to tyrosine phosphorylation sites can be found in Patschinsky T., Hunter T., Esch F.S., Cooper J.A., Sefton B.M., Proc. Natl. Acad. Sci. U.S.A. 79:973-977(1982); Hunter T., J. Biol. Chem... 257:4843-4848(1982), and Cooper J.A., Esch F.S., Taylor S.S., Hunter T., J. Biol. Chem... 259:7835-7841(1984), which are hereby incorporated herein by reference.

In preferred embodiments, the following tyrosine phosphorylation site polypeptides are encompassed by the present invention: NGCVLVHCLAGISRSATIAIAYI (SEQ ID NO:103). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of the human BMY_HPP5 tyrosine phosphorylation site polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The human phosphatase polypeptide was predicted to comprise twelve PKC phosphorylation sites using the Motif algorithm (Genetics Computer Group, Inc.). In vivo, protein kinase C exhibits a preference for the phosphorylation of serine or threonine residues. The PKC phosphorylation sites have the following consensus pattern: [ST]-x-[RK], where S or T represents the site of phosphorylation and 'x' an intervening amino acid residue. Additional information regarding PKC

phosphorylation sites can be found in Woodget J.R., Gould K.L., Hunter T., Eur. J. Biochem. 161:177-184(1986), and Kishimoto A., Nishiyama K., Nakanishi H., Uratsuji Y., Nomura H., Takeyama Y., Nishizuka Y., J. Biol. Chem... 260:12492-12499(1985); which are hereby incorporated by reference herein.

In preferred embodiments, the following PKC phosphorylation site polypeptides are encompassed by the present invention: GTQIVTERLVALL (SEQ ID NO:91),

LLESGTEKVLLID (SEQ ID NO:92), ELIQHSAKHKVDI (SEQ ID NO:93), VDIDCSQKVVVYD (SEQ ID NO:94), DRLEDSNKLKRSF (SEQ ID NO:95), TTLDGTNKLCQFS (SEQ ID NO:96), PKKLQTARPSDSQ (SEQ ID NO: 97), PSDSQSKRLHSVR (SEQ ID NO:98), SKRLHSVRTSSSG (SEQ ID NO:99), GDQVYSVRRRQKP (SEQ ID NO:100), RRQKPSDRADSRR (SEQ ID NO:101), and/or SDRADSRRSWHEE (SEQ ID NO:102). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of the human BMY_HPP5 phosphatase PKC phosphorylation site polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The human phosphatase polypeptide has been shown to comprise six glycosylation sites according to the Motif algorithm (Genetics Computer Group, Inc.). As discussed more specifically herein, protein glycosylation is thought to serve a variety of functions including: augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

Asparagine phosphorylation sites have the following consensus pattern, N-{P}-[ST]-{P}, wherein N represents the glycosylation site. However, it is well known that that potential N-glycosylation sites are specific to the consensus sequence Asn-Xaa-Ser/Thr. However, the presence of the consensus tripeptide is not sufficient to conclude that an asparagine residue is glycosylated, due to the fact that the folding of the protein plays an important role in the regulation of N-glycosylation. It has been shown that the presence of proline between Asn and Ser/Thr will inhibit N-glycosylation; this has been confirmed by a recent statistical analysis of glycosylation sites, which also shows that about 50% of the sites that have a proline C-terminal to

Ser/Thr are not glycosylated. Additional information relating to asparagine glycosylation may be found in reference to the following publications, which are hereby incorporated by reference herein: Marshall R.D., Annu. Rev. Biochem. 41:673-702(1972); Pless D.D., Lennarz W.J., Proc. Natl. Acad. Sci. U.S.A. 74:134-138(1977); Bause E., Biochem. J. 209:331-336(1983); Gavel Y., von Heijne G., Protein Eng. 3:433-442(1990); and Miletich J.P., Broze G.J. Jr., J. Biol. Chem... 265:11397-11404(1990).

In preferred embodiments, the following asparagine glycosylation site polypeptides are encompassed by the present invention: PFVEYNTSHILEAI (SEQ ID NO:85), EAININCSKLMKRR (SEQ ID NO:86), IGYVLNASNTCPKP (SEQ ID NO:87), LRVPVNDSFCEKIL (SEQ ID NO:88), EKKIKNQTGASGPK (SEQ ID NO:89), and/or SIMSENRSREELGK (SEQ ID NO:90). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of the human BMY_HPP5 phosphatase asparagine glycosylation site polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The present invention encompasses the use of BMY_HPP5 inhibitors and/or activators of BMY_HPP5 activity for the treatment, detection, amelioration, or prevention of phosphatase associated disorders, including but not limited to metabolic diseases such as diabetes, in addition to neural and/or cardiovascular diseases and disorders. The present invention also encompasses the use of BMY_HPP5 inhibitors and/or activators of BMY_HPP5 activity as immunosuppressive agents, anti-inflammatory agents, and/or anti-tumor agents

The present invention encompasses the use of BMY_HPP5 phosphatase inhibitors, including, antagonists such as antisense nucleic acids, in addition to other antagonists, as described herein, in a therapeutic regimen to diagnose, prognose, treat, ameliorate, and/or prevent diseases where a kinase activity is insufficient. One, non-limiting example of a disease which may occur due to insufficient kinase activity are certain types of diabetes, where one or more kinases involved in the insulin receptor signal pathway may have insufficient activity or insufficient expression, for example.

Moreover, the present invention encompasses the use of BMY_HPP5 phosphatase activators, and/or the use of the BMY_HPP5 phosphatase gene or protein in a gene therapy regimen, as described herein, for the diagnoses, prognoses,

treatment, amelioration, and/or prevention of diseases and/or disorders where a kinase activity is overly high, such as a cancer where a kinase oncogene product has excessive activity or excessive expression.

The present invention also encompasses the use of catalytically inactive variants of BMY_HPP5 proteins, including fragments thereof, such as a protein therapeutic, or the use of the encoding polynucleotide sequence or as gene therapy, for example, in the diagnoses, prognosis, treatment, amelioration, and/or prevention of diseases or disorders where phosphatase activity is overly high.

The present invention encompasses the use of antibodies directed against the BMY_HPP5 polypeptides, including fragment and/or variants thereof, of the present invention in diagnostics, as a biomarkers, and/or as a therapeutic agents.

The present invention encompasses the use of an inactive, non-catalytic, mutant of the BMY_HPP5 phosphatase as a substrate trapping mutant to bind cellular phosphoproteins or a library of phosphopeptides to identify substrates of the BMY_HPP5 polypeptides.

The present invention encompasses the use of the BMY_HPP5 polypeptides, to identify inhibitors or activators of the BMY_HPP5 phosphatase activity using either in vitro or 'virtual' (in silico) screening methods.

One embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of the BMY_HPP5 phosphatase comprising the steps of: i.) contacting a BMY_HPP5 phosphatase inhibitor or activator labeled with an analytically detectable reagent with the BMY_HPP5 phosphatase under conditions sufficient to form a complex with the inhibitor or activator; ii.) contacting said complex with a sample containing a compound to be identified; iii) and identifying the compound as an inhibitor or activator by detecting the ability of the test compound to alter the amount of labeled known BMY_HPP5 phosphatase inhibitor or activator in the complex.

Another embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of a BMY_HPP5 phosphatase comprising the steps of: i.) contacting the BMY_HPP5 phosphatase with a compound to be identified; and ii.) and measuring the ability of the BMY_HPP5 phosphatase to remove phosphate from a substrate.

The present invention also encomposses a method for identifying a ligand for the BMY_HPP5 phosphatase comprising the steps of: i.) contacting the BMY_HPP5 phosphatase with a series of compounds under conditions to permit binding; and ii.) detecting the presence of any ligand-bound protein.

Preferably, the above referenced methods comprise the BMY_HPP5 phosphatase in a form selected from the group consisting of whole cells, cytosolic cell fractions, membrane cell fractions, purified or partially purified forms. The invention also relates to recombinantly expressed BMY_HPP5 phosphatase in a purified, substantially purified, or unpurified state. The invention further relates to BMY_HPP5 phosphatase fused or conjugated to a protein, peptide, or other molecule or compound known in the art, or referenced herein.

The present invention also encompasses a pharmaceutical composition of the BMY_HPP5 phosphatase polypeptide comprising a compound identified by above referenced methods and a pharmaceutically acceptable carrier.

In preferred embodiments, the present invention encompasses a polynucleotide lacking the initiating start codon, in addition to, the resulting encoded polypeptide of BMY_HPP5. Specifically, the present invention encompasses the polynucleotide corresponding to nucleotides 473 thru 2464 of SEQ ID NO:41, and the polypeptide corresponding to amino acids 2 thru 665 of SEQ ID NO:42. Also encompassed are recombinant vectors comprising said encoding sequence, and host cells comprising said vector.

The present invention also provides a three-dimensional homology model of the BMY_HPP5 polypeptide (see Figure 38) representing amino acids N157 to I300 of BMY_HPP5 (SEQ ID NO:42). A three-dimensional homology model can be constructed on the basis of the known structure of a homologous protein (Greer et al, 1991, Lesk, et al, 1992, Cardozo, et al, 1995, Yuan, et al, 1995). The homology model of the BMY_HPP5 polypeptide, corresponding to amino acid residues N157 to I300 of SEQ ID NO:42, was based upon the homologous structure of 1vhr from the N-terminus of human dual specificity phosphatase MAP Kinase phosphatase 3 (also called PYST1) (residues A204-L347; Protein Data Bank, PDB entry 1mkp chain A Genbank Accession No. gi|5822131; SEQ ID NO:208) (Stewart, A. E. , et al., 1999) and is defined by the set of structural coordinates set forth in Table X herein.

Homology models are useful when there is no experimental information available on the protein of interest. A 3-dimensional model can be constructed on the basis of the known structure of a homologous protein (Greer et al, 1991, Lesk, et al, 1992, Cardozo, et al, 1995, Sali, et al, 1995).

Those of skill in the art will understand that a homology model is constructed on the basis of first identifying a template, or, protein of known structure which is similar to the protein without known structure. This can be accomplished by through pairwise alignment of sequences using such programs as FASTA (Pearson, et al 1990) and BLAST (Altschul, et al, 1990). In cases where sequence similarity is high (greater than 30 %) these pairwise comparison methods may be adequate. Likewise, multiple sequence alignments or profile-based methods can be used to align a query sequence to an alignment of multiple (structurally and biochemically) related proteins. When the sequence similarity is low, more advanced techniques are used such as fold recognition (protein threading; Hendlich, et al, 1990), where the compatibility of a particular sequence with the 3-dimensional fold of a potential template protein is gauged on the basis of a knowledge-based potential. Following the initial sequence alignment, the query template can be optimally aligned by manual manipulation or by incorporation of other features (motifs, secondary structure predictions, and allowed sequence conservation). Next, structurally conserved regions can be identified and used to construct the core secondary structure (Sali, et al, 1995). Loops can be added using knowledge-based techniques, and refined performing force field calculations (Sali, et al, 1995; Cardozo, et al, 1995).

For BMY_HPP5 the pairwise alignment method FASTA (Pearson, et al 1990) and fold recognition methods (protein threading) generated identical sequence alignments for a portion (residues N157 to I300 of SEQ ID NO:42) of BMY_HPP5 aligned with the sequence of the human dual specificity phosphatase MAP Kinase phosphatase 3 (also called PYST1) (residues A204-L347; Protein Data Bank, PDB entry 1mkp chain A; Genbank Accession No. gi|5822131; SEQ ID NO:208) (Stewart, A. E. , et al., 1999). The alignment of BMY_HPP5 with PDB entry 1mkp is set forth in Figure 37. In this invention, the homology model of BMY_HPP5 was derived from the sequence alignment set forth in Figure 37, and thence an overall atomic model including plausible sidechain orientations using the program LOOK (Levitt, 1992).

The three dimensional model for BMY_HPP5 is defined by the set of structure coordinates as set forth in Table X and visualized in Figure 38.

In order to recognize errors in three-dimensional structures knowledge based mean fields can be used to judge the quality of protein folds (Sippl 1993). The methods can be used to recognize misfolded structures as well as faulty parts of structural models. The technique generates an energy graph where the energy distribution for a given protein fold is displayed on the y-axis and residue position in the protein fold is displayed on the x-axis. The knowledge based mean fields compose a force field derived from a set of globular protein structures taken as a subset from the Protein Data Bank (Bernstein et. al. 1977). To analyze the quality of a model the energy distribution is plotted and compared to the energy distribution of the template from which the model was generated. Figure 39 shows the energy graph for theBMY_HPP5 model (dotted line) and the template (1mkp, a dual-specificity phosphatase) from which the model was generated. It is clear that the model and template have similar energies over the aligned region, suggesting thatBMY_HPP5 is in a "native-like" conformation. This graph supports the motif and sequence alignments in confirming that the three dimensional structure coordinates ofBMY_HPP5 are an accurate and useful representation for the polypeptide.

The term "structure coordinates" refers to Cartesian coordinates generated from the building of a homology model.

Those of skill in the art will understand that a set of structure coordinates for a protein is a relative set of points that define a shape in three dimensions. Thus, it is possible that an entirely different set of coordinates could define a similar or identical shape. Moreover, slight variations in the individual coordinates, as emanate from generation of similar homology models using different alignment templates (i.e., other than the structure coordinates of 1mkp), and/or using different methods in generating the homology model, will have minor effects on the overall shape. Variations in coordinates may also be generated because of mathematical manipulations of the structure coordinates. For example, the structure coordinates set forth in Table X and visualized in Figure 38 could be manipulated by fractionalization of the structure coordinates; integer additions or subtractions to sets of the structure coordinates, inversion of the structure coordinates or any combination of the above.

Various computational analyses are therefore necessary to determine whether a molecule or a portion thereof is sufficiently similar to all or parts of BMY_HPP5 described above as to be considered the same. Such analyses may be carried out in current software applications, such as INSIGHTII (Molecular Simulations Inc., San Diego, CA) version 2000 and as described in the accompanying User's Guide.

Using the superimposition tool in the program INSIGHTII comparisons can be made between different structures and different conformations of the same structure. The procedure used in INSIGHTII to compare structures is divided into four steps: 1) load the structures to be compared; 2) define the atom equivalencies in these structures; 3) perform a fitting operation; and 4) analyze the results. Each structure is identified by a name. One structure is identified as the target (i.e., the fixed structure); the second structure (i.e., moving structure) is identified as the source structure. Since atom equivalency within INSIGHTII is defined by user input, for the purpose of this invention we will define equivalent atoms as protein backbone atoms (N, Cα, C and O) for all conserved residues between the two structures being compared. We will also consider only rigid fitting operations. When a rigid fitting method is used, the working structure is translated and rotated to obtain an optimum fit with the target structure. The fitting operation uses an algorithm that computes the optimum translation and rotation to be applied to the moving structure, such that the root mean square difference of the fit over the specified pairs of equivalent atom is an absolute minimum. This number, given in angstroms, is reported by INSIGHTII. For the purpose of this invention, any homology model of a BMY_HPP5 that has a root mean square deviation of conserved residue backbone atoms (N, Cα, C, O) of less than 3.0 A when superimposed on the relevant backbone atoms described by structure coordinates listed in Table X and visualized in Figure 38 are considered identical. More preferably, the root mean square deviation is less than 2.0 Å.

This invention as embodied by the homology model enables the structure-based design of modulators of the biological function of BMY_HPP5, as well as mutants with altered biological function and/or specificity.

There is 40% sequence identity between catalytic domain of BMY_HPP5 and 1mkp which was used as the template for 3D model generation. For the BMY_HPP5 the functionally important residues are located in a cleft comprised of residues D213,

H243, C244, R250, and S251. All these residues are conserved in 1mkp (for structure determination studies the cysteine was mutated to a serine in 1mkp). Based on the sequence alignment disclosed in Figure 37 and the structural model disclosed in Table X and visualized in Figure 38, D213 is identified as a general acid, C244 as the catalytic Cysteine nucleophile which cleaves the phosphodiester bond, and R250 as the essential Argenine which activates the bond for cleavage as described in the literature (reviewed by Fauman and .Saper, 1996). Other important residues include F287 which imparts substrate specificity onto the enzyme. All of these residues are conserved.

In a preferred embodiment of the present invention, the molecule comprises the cleft region defined by structure coordinates of BMY_HPP5 amino acids described above according to Table X, or a mutant of said molecule.

More preferred are molecules comprising all or any part of the cleft or a mutant or homologue of said molecule or molecular complex. By mutant or homologue of the molecule it is meant a molecule that has a root mean square deviation from the backbone atoms of said BMY_HPP5 amino acids of not more than 3.5 Angstroms.

The term "root mean square deviation" means the square root of the arithmetic mean of the squares of the deviations from the mean. It is a way to express the deviation or variation from a trend or object. For purposes of this invention, the "root mean square deviation" defines the variation in the backbone of a protein from the relevant portion of the backbone of BMY_HPP5 as defined by the structure coordinates described herein.

The structure coordinates of a BMY_HPP5 homology model portions thereof are stored in a machine-readable storage medium. Such data may be used for a variety of purposes, such as drug discovery.

Accordingly, in one embodiment of this invention is provided a machine-readable data storage medium comprising a data storage material encoded with the structure coordinates set forth in Table X.

One embodiment utilizes System 10 as disclosed in WO 98/11134, the disclosure of which is incorporated herein by reference in its entirety. Briefly, one version of these embodiments comprises a computer comprising a central processing

unit ("CPU"), a working memory which may be, e.g, RAM (random-access memory) or "core" memory, mass storage memory (such as one or more disk drives or CD-ROM drives), one or more cathode-ray tube ("CRT") display terminals, one or more keyboards, one or more input lines, and one or more output lines, all of which are interconnected by a conventional bidirectional system bus.

Input hardware, coupled to the computer by input lines, may be implemented in a variety of ways. Machine-readable data of this invention may be inputted via the use of a modem or modems connected by a telephone line or dedicated data line. Alternatively or additionally, the input hardware may comprise CD-ROM drives or disk drives. In conjunction with a display terminal, keyboard may also be used as an input device.

Output hardware, coupled to the computer by output lines, may similarly be implemented by conventional devices. By way of example, output hardware may include a CRT display terminal for displaying a graphical representation of a region or domain of the present invention using a program such as QUANTA as described herein. Output hardware might also include a printer, so that hard copy output may be produced, or a disk drive, to store system output for later use.

In operation, the CPU coordinates the use of the various input and output devices, coordinates data accesses from mass storage, and accesses to and from the working memory, and determines the sequence of data processing steps. A number of programs may be used to process the machine-readable data of this invention. Such programs are discussed in reference to the computational methods of drug discovery as described herein. Specific references to components of the hardware system are included as appropriate throughout the following description of the data storage medium.

For the purpose of the present invention, any magnetic data storage medium which can be encoded with machine-readable data would be sufficient for carrying out the storage requirements of the system. The medium could be a conventional floppy diskette or hard disk, having a suitable substrate, which may be conventional, and a suitable coating, which may be conventional, on one or both sides, containing magnetic domains whose polarity or orientation could be altered magnetically, for

example. The medium may also have an opening for receiving the spindle of a disk drive or other data storage device.

The magnetic domains of the coating of a medium may be polarized or oriented so as to encode in a manner which may be conventional, machine readable data such as that described herein, for execution by a system such as the system described herein.

Another example of a suitable storage medium which could also be encoded with such machine-readable data, or set of instructions, which could be carried out by a system such as the system described herein, could be an optically-readable data storage medium. The medium could be a conventional compact disk read only memory (CD-ROM) or a rewritable medium such as a magneto-optical disk which is optically readable and magneto-optically writable. The medium preferably has a suitable substrate, which may be conventional, and a suitable coating , which may be conventional, usually of one side of substrate.

In the case of a CD-ROM, as is well known, the coating is reflective and is impressed with a plurality of pits to encode the machine-readable data. The arrangement of pits is read by reflecting laser light off the surface of the coating. A protective coating, which preferably is substantially transparent, is provided on top of the reflective coating.

In the case of a magneto-optical disk, as is well known, the coating has no pits, but has a plurality of magnetic domains whose polarity or orientation can be changed magnetically when heated above a certain temperature, as by a laser. The orientation of the domains can be read by measuring the polarization of laser light reflected from the coating. The arrangement of the domains encodes the data as described above.

Thus, in accordance with the present invention, data capable of displaying the three dimensional structure of the BMY_HPP5 homology model, or portions thereof and their structurally similar homologues is stored in a machine-readable storage medium, which is capable of displaying a graphical three-dimensional representation of the structure. Such data may be used for a variety of purposes, such as drug discovery.

For the first time, the present invention permits the use, through homology modeling based upon the sequence of BMY_HPP5 (Figures 5A-D; SEQ ID NO:42) of

structure-based or rational drug design techniques to design, select, and synthesize chemical entities that are capable of modulating the biological function of BMY_HPP5.

Accordingly, the present invention is also directed to the entire sequence in Figures 5A-D or any portion thereof for the purpose of generating a homology model for the purpose of 3D structure-based drug design.

For purposes of this invention, we include mutants or homologues of the sequence in Figures 5A-D or any portion thereof. In a preferred embodiment, the mutants or homologues have at least 25% identity, more preferably 50% identity, more preferably 75% identity, and most preferably 90% identity to the amino acid residues in Figures 5A-D.

The three-dimensional model structure of the BMY_HPP5 will also provide methods for identifying modulators of biological function. Various methods or combination thereof can be used to identify these compounds.

Structure coordinates of the catalytic region defined above can also be used to identify structural and chemical features. Identified structural or chemical features can then be employed to design or select compounds as potential BMY_HPP5 modulators. By structural and chemical features it is meant to include, but is not limited to, van der Waals interactions, hydrogen bonding interactions, charge interaction, hydrophobic bonding interaction, and dipole interaction. Alternatively, or in conjunction, the three-dimensional structural model can be employed to design or select compounds as potential BMY_HPP5 modulators. Compounds identified as potential BMY_HPP5 modulators can then be synthesized and screened in an assay characterized by binding of a test compound to the BMY_HPP5, or in characterizing BMY_HPP5 deactivation in the presence of a small molecule. Examples of assays useful in screening of potential BMY_HPP5 modulators include, but are not limited to, screening in silico, *in vitro* assays and high throughput assays. Finally, these methods may also involve modifying or replacing one or more amino acids from BMY_HPP5 according to Table X.

However, as will be understood by those of skill in the art upon this disclosure, other structure based design methods can be used. Various computational structure based design methods have been disclosed in the art.

For example, a number of computer modeling systems are available in which the sequence of the BMY_HPP5 and the BMY_HPP5 structure (i.e., atomic coordinates of BMY_HPP5 and/or the atomic coordinates of the active site as provided in Table X) can be input. This computer system then generates the structural details of one or more these regions in which a potential BMY_HPP5 modulator binds so that complementary structural details of the potential modulators can be determined. Design in these modeling systems is generally based upon the compound being capable of physically and structurally associating with BMY_HPP5. In addition, the compound must be able to assume a conformation that allows it to associate with BMY_HPP5. Some modeling systems estimate the potential inhibitory or binding effect of a potential BMY_HPP5 modulator prior to actual synthesis and testing.

Methods for screening chemical entities or fragments for their ability to associate with a given protein target are also well known. Often these methods begin by visual inspection of the binding site on the computer screen. Selected fragments or chemical entities are then positioned in one or more of the active site region in BMY_HPP5. Docking is accomplished using software such as INSIGHTII, QUANTA and SYBYL, following by energy minimization and molecular dynamics with standard molecular mechanic forcefields such as CHARMM and AMBER. Examples of computer programs which assist in the selection of chemical fragment or chemical entities useful in the present invention include, but are not limited to, GRID (Goodford, 1985), AUTODOCK (Goodsell, 1990), and DOCK (Kuntz et al. 1982).

Upon selection of preferred chemical entities or fragments, their relationship to each other and BMY_HPP5 can be visualized and then assembled into a single potential modulator. Programs useful in assembling the individual chemical entities include, but are not limited to SYBYL and LeapFrog (Tripos Associates, St. Louis MO), LUDI (Bohm 1992) and 3D Database systems (Martin 1992).

Alternatively, compounds may be designed de novo using either an empty active site or optionally including some portion of a known inhibitor. Methods of this type of design include, but are not limited to LUDI (Bohm 1992) and LeapFrog (Tripos Associates, St. Louis MO).

In addition, BMY_HPP5 is overall well suited to modern methods including combinatorial chemistry.

Programs such as DOCK (Kuntz et al. 1982) can be used with the atomic coordinates from the homology model to identify potential ligands from databases or virtual databases which potentially bind the in the metal binding region, and which may therefore be suitable candidates for synthesis and testing.

Additionally, the three-dimensional homology model of BMY_HPP5 will aid in the design of mutants with altered biological activity.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO: 41 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides consisting of a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 5097 of SEQ ID NO:41, b is an integer between 15 to 5111, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a+14.

Features of the Polypeptide Encoded by Gene No:6

The development of inflammatory disease is characterized by infiltration of circulating blood cells across the endothelium into the tissue. A number of key events occur in the endothelial cells that mediate this "gateway" function. The endothelial cells express receptors and chemokines that sequentially tether the leukocytes, activate them, cause them to tightly adhere, and aid in their transmigration through endothelial cell junctions. This process is initiated by the production of early inflammatory mediators such as TNF-α. The coordinated expression of receptors and chemokines is mediated by intracellular signaling molecules including kinases, scaffolding proteins, and transcription factors. These molecules thus form a signaling cascade that may be a "master switch" for the development of inflammatory processes. Components of this cascade such as the transcription factor NF-kB are

known. However, there are many other components that have not yet been identified. The analysis of genes that are differentially expressed in TNF-α-activated endothelium may help to identify other components of this "master switch" cascade.

To this end, the RNA expressed in TNF-α-stimulated human lung microvascular endothelial cells has been analyzed to identify gene products involved in regulatory events. Resting cells were stimulated for 1 h with TNF-α, and the RNA was isolated from the cells. Complementary DNA (cDNA) was created from the isolated RNA. The cDNAs that were upregulated in response to TNFα were identified using subtractive hybridization methodology.

A novel dual specificity phosphatase (DSP), RET31 (Regulated in Endothelial cells treated with TNF-α clone 31) (Figures 13A-F) was identified from the TNF-α treated endothelial subtraction library. The dual specificity phosphatase catalytic (DSPc) domain for RET 31 was identified using the DSPc PFAM-HMM (PF00782). A search for homologues identified three other DSPs that contain extensive homology to RET31 (Figures 14A-C). RET31, DUS8, DUSP6 and MAP-kinase phosphatase 5 are shown in a multiple sequence alignment comparing the DSPc domains of these four proteins (Figure 17).

RET31 was confirmed to be up-regulated by TNF-α, reaching a peak of expression at 6 h by northern blot analysis (Figure 15). RET31 mRNA was virtually undetectable in brain, spleen, and peripheral blood leukocytes by Northern blot analysis.

RET31 is believed to represent a novel splice variant of the BMY_HPP5 polypeptide of the present invention. The sequence for RET31 differs in the 5' end from that of BMY_HPP5. However, comparison of the tissue expression of RET31 and BMY_HPP5 showed significant differential expression despite their significant identity. Specifically, the tissue expression of BMY_HPP5 by PCR analysis (as described elsewhere herein) suggested that there were significant levels of RET31 in the brain. The reason for such disparate expression profiles is unclear but may be related to the use of separate pools of RNA or to the use of alternate probes.

In all tissues that expressed significant levels of RET31, there was a primary hybridizing band and a secondary band of lower molecular weight. It is not clear

whether this represents splice variants of the same gene or whether there is a homologue present.

The polypeptide corresponding to this gene provided as SEQ ID NO:108 (Figure 13A-F), encoded by the polynucleotide sequence according to SEQ ID NO:109 (Figure 13A-F), and/or encoded by the polynucleotide contained within the deposited clone, RET31, has significant homology at the nucleotide and amino acid level to a number of phosphatases, which include, for example, the human protein-tyrosine phosphatase DUS8 protein, also referred to as hVH-5 (DUS8; Genbank Accession No:gi|U27193; SEQ ID NO:110); the human dual specificity MAP kinase DUSP6 protein (DUSP6; Genbank Accession No:gi|AB013382; SEQ ID NO:111); and the human map kinase phosphatase MKP-5 protein (MKP-5; Genbank Accession No:gi|AB026436; SEQ ID NO:112) as determined by BLASTP. An alignment of the human phosphatase polypeptide with these proteins is provided in Figures 14A-C.

The human protein-tyrosine phosphatase DUS8 protein (also referred to as hVH-5) is thought to be a member of a subset of protein tyrosine phosphatases that regulate mitogen-activated protein kinase. The catalytic region of hVH-5 was expressed as a fusion protein and was shown to hydrolyze p-nitrophenylphosphate and inactivate mitogen-activated protein kinase, thus proving that hVH-5 possessed phosphatase activity. Moreover, expression of hVH-5 transcripts were induced in PC12 cells upon nerve growth factor and insulin treatment in a manner characteristic of an immediate-early gene, suggesting a possible role in the signal transduction cascade (The J. Neurochem. 65 (4), 1823-1833 (1995)).

The dual specificity MAP kinase DUSP6 protein is believed to be implicated in pancreatic carcinogensis based upon its encoding polynucleotide mapping to chromosome locus12q21, one of the regions of frequent allelic loss in pancreatic cancer, in addition to, its reduced expressions amonst several pancreatic cancer cell lines (Cytogenet. Cell Genet. 82 (3-4), 156-159 (1998)).

The human map kinase phosphatase MKP-5 protein was determined to belong to a group of dual specificity protein phosphatases that negatively regulate members of the mitogen-activated protein kinase (MAPK) superfamily, which consists of three major subfamilies, MAPK/extracellular signal-regulated kinase (ERK), stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK), and p38. Members

of this group show distinct substrate specificities for MAPKs, different tissue distribution and subcellular localization, and different modes of inducibility of their expression by extracellular stimuli. MKP-5 was shown to bind to p38 and SAPK/JNK, but not to MAPK/ERK, and inactivate p38 and SAPK/JNK, but not MAPK/ERK. p38 was determined to be the preferred substrate for MKP-5. MKP-5 mRNA was widely expressed in various tissues and organs, and its expression in cultured cells was inducible by stress stimuli. Thus, MKP-5 is thought to represent a type of dual specificity phosphatase specific for p38 and SAPK/JNK (J Biol Chem., 274(28):19949-56, (1999)).

The determined nucleotide sequence of the RET31 cDNA in Figures 13A-F (SEQ ID NO:41) contains an open reading frame encoding a protein of about 665 amino acid residues, with a deduced molecular weight of about 73.1kDa. The amino acid sequence of the predicted RET31 polypeptide is shown in Figures 13A-F (SEQ ID NO:42). The RET31 protein shown in Figures 13A-F was determined to share significant identity and similarity to several known phosphatases, particularly, dual-specificity protein phosphatases. Specifically, the RET31 protein shown in Figures 13A-F was determined to be about 50.3% identical and 56.8% similar to human protein-tyrosine phosphatase DUS8 protein (DUS8; Genbank Accession No:gi|U27193; SEQ ID NO:110); to be about 36.5% identical and 48.3% similar to the human dual specificity MAP kinase DUSP6 protein (DUSP6; Genbank Accession No:gi|AB013382; SEQ ID NO:111); and to be about 34.3% identical and 47.2% similar to the human map kinase phosphatase MKP-5 protein (MKP-5; Genbank Accession No:gi|AB026436; SEQ ID NO:112), as shown in Figure 12.

Based upon the strong homology to members of the phosphatase proteins, the polypeptide encoded by the human RET31 phosphatase of the present invention is expected to share at least some biological activity with phosphatase proteins, preferably with members of the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases, particularly the novel phosphotyrosine/dual-specificity (P-Tyr, P-Ser and P-Thr) phosphatases referenced herein.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the predominant localized expression in adrenal gland tissue suggests the human RET31 phosphatase polynucleotides and polypeptides,

including antagonists, and/or fragments thereof, may be useful for treating, diagnosing, prognosing, ameliorating, and/or preventing endocrine disorders, which include, but are mot limited to adrenocortical hyperfunction, adrenocortical hypofunction, lethargy. Congenital adrenal hyperplasia, aberrant ACTH regulation, aberrant adrenaline regulation, disorders associated with defects in P450C21, P450C18, P450C17, and P450C11 hydroxylases and in 3-hydroxysteroid dehydrogenase (3-HSD), hirsutism, oligomenorrhea, acne, virilization, female pseudohermaphroditism, disorders associated with the incidence of aberrant sexual characterisitics, disorders associated with aberrant cortisol secretion, hypertension, hypokalemia, hypogonadism, disorders associated with aberrant androgen secretion, adrenal virilism, Adrenal adenomas, Adrenal carcinomas, disorders associated with aberrant aldosterone secretion, aldosteronism, disorders associated with aberrant steriod biosynthesis, disorders associated with aberrant steriod transport, disorders associated with aberrant steriod secretion, disorders associated with aberrant steriod excretion, Addison's syndrome, and Cushing's syndrome.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the localized expression in testis and prostate tissue suggests the human RET31 phosphatase polynucleotides and polypeptides, including antagonists, and/or fragments thereof, may be useful for treating, diagnosing, prognosing, and/or preventing male reproductive disorders, such as, for example, male infertility, impotence, prostate cancer, ejaculatory disorders, and/or testicular cancer. This gene product may also be useful in assays designed to identify binding agents, as such agents (antagonists) are useful as male contraceptive agents. The testes are also a site of active gene expression of transcripts that is expressed, particularly at low levels, in other tissues of the body. Therefore, this gene product may be expressed in other specific tissues or organs where it may play related functional roles in other processes, such as hematopoiesis, inflammation, bone formation, and kidney function, to name a few possible target indications. If fact, increased expression of certain phosphatases have been identified as tumor markers for testicular cancer (see, for example, Koshida, K., Nishino, A., Yamamoto, H., Uchibayashi, T., Naito, K.,Hisazumi, H., Hirano, K., Hayashi, Y., Wahren, B., Andersson, L, J. Urol., 146(1):57-60, (1991); and Klein, EA, Urol. Clin. North. Am., 20(1):67-73, (1993)).

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the significant localized expression in ovary and placental tissue suggests the human phosphatase polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing reproductive disorders.

In preferred embodiments, RET31 polynucleotides and polypeptides including agonists and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing the following, non-limiting, diseases or disorders of the uterus: dysfunctional uterine bleeding, amenorrhea, primary dysmenorrhea, sexual dysfunction, infertility, pelvic inflammatory disease, endometriosis, placental aromatase deficiency, premature menopause, and placental dysfunction.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the significant localized expression in skeletal tissue suggests the human phosphatase polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing muscle diseases and/or disorders, which include but are not limited to, musculodegenerative disorders, multiple sclerosis, atrophy, ticks.

Alternatively, the strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the significant localized expression in liver tissue suggests the human phosphatase polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing hepatic diseases and/or disorders. Representative uses are described in the "Hyperproliferative Disorders", "Infectious Disease", and "Binding Activity" sections below, and elsewhere herein. Briefly, the protein can be used for the detection, treatment, and/or prevention of hepatoblastoma, jaundice, hepatitis, liver metabolic diseases and conditions that are attributable to the differentiation of hepatocyte progenitor cells, cirrhosis, hepatic cysts, pyrogenic abscess, amebic abcess, hydatid cyst, cystadenocarcinoma, adenoma, focal nodular hyperplasia, hemangioma, hepatocellulae carcinoma, cholangiocarcinoma, angiosarcoma, and granulomatous liver disease. In addition the protein product is useful for treating developmental abnormalities, fetal deficiencies, pre-natal disorders and various would-healing diseases and/or tissue trauma.

Moreover, polynucleotides and polypeptides, including fragments and/or antagonists thereof, have uses which include, directly or indirectly, treating, preventing, diagnosing, and/or prognosing the following, non-limiting, hepatic infections: liver disease caused by sepsis infection, liver disease caused by bacteremia, liver disease caused by Pneomococcal pneumonia infection, liver disease caused by Toxic shock syndrome, liver disease caused by Listeriosis, liver disease caused by Legionnaries' disease, liver disease caused by Brucellosis infection, liver disease caused by Neisseria gonorrhoeae infection, liver disease caused by Yersinia infection, liver disease caused by Salmonellosis, liver disease caused by Nocardiosis, liver disease caused by Spirochete infection, liver disease caused by Treponema pallidum infection, liver disease caused by Brrelia burgdorferi infection, liver disease caused by Leptospirosis, liver disease caused by Coxiella burnetii infection, liver disease caused by Rickettsia richettsii infection, liver disease caused by Chlamydia trachomatis infection, liver disease caused by Chlamydia psittaci infection, in addition to any other hepatic disease and/or disorder implicated by the causative agents listed above or elsewhere herein.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the significant localized expression in placental tissue suggests the human phosphatase polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing a variety of vascular disorders and conditions, which include, but are not limited to miscrovascular disease, vascular leak syndrome, aneurysm, stroke, embolism, thrombosis, coronary artery disease, arteriosclerosis, and/or atherosclerosis. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissue markers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the predominate localized expression in pancreas tissue suggests the human RET31 phosphatase polynucleotides and polypeptides, including antagonists, and/or fragments thereof, may be useful for treating, diagnosing,

prognosing, and/or preventing pancreatic, in addition to metabolic and gastrointestinal disorders.

In preferred embodiments, RET31 polynucleotides and polypeptides including agonists, antagonists, and fragments thereof, have uses which include treating, diagnosing, prognosing, and/or preventing the following, non-limiting, diseases or disorders of the pancreas: diabetes mellitus, diabetes, type 1 diabetes, type 2 diabetes, adult onset diabetes, indications related to islet cell transplantation, indications related to pancreatic transplantation, pancreatitis, pancreatic cancer, pancreatic exocrine insufficiency, alcohol induced pancreatitis, maldigestion of fat, maldigestion of protein, hypertriglyceridemia, vitamin b12 malabsorption, hypercalcemia, hypocalcemia, hyperglycemia, ascites, pleural effusions, abdominal pain, pancreatic necrosis, pancreatic abscess, pancreatic pseudocyst, gastrinomas, pancreatic islet cell hyperplasia, multiple endocrine neoplasia type 1 (men 1) syndrome, insulitis, amputations, diabetic neuropathy, pancreatic auto-immune disease, genetic defects of -cell function, HNF-1 aberrations (formerly MODY3), glucokinase aberrations (formerly MODY2), HNF-4 aberrations (formerly MODY1), mitochondrial DNA aberrations, genetic defects in insulin action, type a insulin resistance, leprechaunism, Rabson-Mendenhall syndrome, lipoatrophic diabetes, pancreatectomy, cystic fibrosis, hemochromatosis, fibrocalculous pancreatopathy, endocrinopathies, acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma, drug- or chemical-induced diabetes such as from the following drugs: Vacor, Pentamdine, Nicotinic acid, Glucocorticoids, Thyroid hormone, Diazoxide, Adrenergic agonists, Thiazides, Dilantin, and Interferon, pancreatic infections, congential rubella, cytomegalovirus, uncommon forms of immune-mediated diabetes, “stiff-man" syndrome, anti-insulin receptor antibodies, in addition to other genetic syndromes sometimes associated with diabetes which include, for example, Down's syndrome, Klinefelter's syndrome, Turner's syndrome, Wolfram's syndrome, Friedrich's ataxia, Huntington's chorea, Lawrence Moon Beidel syndrome, Myotonic dystrophy, Porphyria, and Prader Willi syndrome, and/or Gestational diabetes mellitus (GDM).

The strong homology to phosphatases, particularly dual-specificity phosphatases, combined with the predominate localized expression in thymus tissue

suggests the human RET31 phosphatase polynucleotides and polypeptides, including antagonists, and/or fragments thereof, may be useful for treating, diagnosing, prognosing, and/or preventing immune and hematopoietic disorders. Representative uses are described in the "Immune Activity", "Chemotaxis", and "Infectious Disease" sections below, and elsewhere herein. Briefly, the strong expression in immune tissue indicates a role in regulating the proliferation; survival; differentiation; and/or activation of hematopoietic cell lineages, including blood stem cells.

The RET31 polypeptide may also be useful as a preventative agent for immunological disorders including arthritis, asthma, immunodeficiency diseases such as AIDS, leukemia, rheumatoid arthritis, granulomatous disease, inflammatory bowel disease, sepsis, acne, neutropenia, neutrophilia, psoriasis, hypersensitivities, such as T-cell mediated cytotoxicity; immune reactions to transplanted organs and tissues, such as host-versus-graft and graft-versus-host diseases, or autoimmunity disorders, such as autoimmune infertility, lense tissue injury, demyelination, systemic lupus erythematosis, drug induced hemolytic anemia, rheumatoid arthritis, Sjogren's disease, and scleroderma. Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product may be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

The RET31 polypeptide may be useful for modulating cytokine production, antigen presentation, or other processes, such as for boosting immune responses, etc. Expression in cells of lymphoid origin, indicates the natural gene product would be involved in immune functions.

Moreover, the protein may represent a secreted factor that influences the differentiation or behavior of other blood cells, or that recruits hematopoietic cells to sites of injury. Thus, this gene product is thought to be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types. Furthermore, the protein may also be used to determine biological activity, raise antibodies, as tissuemarkers, to isolate cognate ligands or receptors, to identify agents that modulate their interactions, in addition to its use as a nutritional supplement. Protein, as well as, antibodies directed against the

protein may show utility as a tumor marker and/or immunotherapy targets for the above listed tissues.

The human phosphatase polynucleotides and polypeptides, including fragments and agonists thereof, may have uses which include, either directly or indirectly, for boosting immune responses.

The strong homology to phosphatases, particularly dual-specificity phosphatases, suggests the human phosphatase polynucleotides and polypeptides may be useful in treating, diagnosing, prognosing, and/or preventing a variety of disorders and conditions, particularly inflammatory conditions, which include, but are not limited to rheumatoid arthritis, juvenile arthritis, psoriasis, asthma, ischemia-repurfusion, multiple sclerosis, rejection of organ or tissue transplants, chronic obstructive pulmonary disease, inflammatory bowel disease, Chrohn's disease, ulcerative colitis, inacute respiratory distress syndrome, systemic lupus erythematosis, cystic fibrosis, autoimmune diseases, cancers, tumors, and neoplasms.

The human phosphatase polynucleotides and polypeptides, including fragments and /or antagonists thereof, may have uses which include identification of modulators of human phosphatase function including antibodies (for detection or neutralization), naturally-occurring modulators and small molecule modulators. Antibodies to domains of the human phosphatase protein could be used as diagnostic agents of cardiovascular and inflammatory conditions in patients, are useful in monitoring the activation of signal transduction pathways, and can be used as a biomarker for the involvement of phosphatases in disease states, and in the evaluation of inhibitors of phosphatases in vivo.

Human phosphatase polypeptides and polynucleotides have additional uses which include diagnosing diseases related to the over and/or under expression of human phosphatase by identifying mutations in the human phosphatase gene by using human phosphatase sequences as probes or by determining human phosphatase protein or mRNA expression levels. Human phosphatase polypeptides may be useful for screening compounds that affect the activity of the protein. Human phosphatase peptides can also be used for the generation of specific antibodies and as bait in yeast two hybrid screens to find proteins the specifically interact with human phosphatase (described elsewhere herein).

Immunohistochemistry analysis of the protein localization of the RET31 polypeptide (see Example 58) in normal and diseased tissues determined that RET31 was strongly expressed in normal respiratory epithelial cell bodies, type I and II pneumocytes, lung neutrophils, lung mast cells, lung macrophages, in comparison to the same in asthmatic patients which showed less staining. These results suggest that RET31 polypeptides and polynucleotides, including fragments thereof, may be useful for the treatment of pulmonary disorders. The decreased staining in diseased lung tissues suggests RET31 is essential for normal cell maintainance and homeostasis, and is downregulated in transformed, or rapidly proliferating cells. Thus, agonists of RET31 polypeptides and polynucleotides may be particularly useful for the treatment of pulmonary disorders.

Immunohistochemistry analysis of the protein localization of the RET31 polypeptide (see Example 58) in normal and diseased tissues determined that RET31 was also strongly expressed in chondrocytes and rimming osteoblasts in degenerative arthritis, in addition to hematopoeitic cell tissue. Moreover, melanocytes were strongly positive, as was skin with psoriasis. These results suggest that RET31 may be involved in inflammatory responses of certain diseases and/or disorders. Thus, RET31 polypeptides and polynucleotides, including fragments thereof, may be useful for the treatment of inflammatory disorders, particularly inflammatory disorders of the skin and bone, such as, psoriasis and arthritis, for example. Moreover, antagonists of RET31 polypeptides and polynucleotides may be useful for the treatment of inflammatory disorders, particularly inflammatory disorders of the skin and bone, such as, psoriasis and arthritis, for example.

Assays designed to assess the phosphatase activity of the RET31 polypeptide have been performed and prove that RET31 does indeed have phosphatase activity as described in Example 57 herein (see Figure 36). The observed phosphatase activity was specific to RET31 as GST alone did not result in any observed activity. In addition, the observed phosphatase activity was specifically inhibited by the known phosphatase active site inhibitor, vanadate.

In addition to assaying the full-length RET31 polypeptide (SEQ ID NO:109), a C-terminal deletion of RET31 was also assayed corresponding to amino acids M1 to

T302 of SEQ ID NO:109). The M1 to T302 deletion mutant had an unexpected five fold increase in phosphatase activity relative to the full-length protein.

A phosphatase with a sequence similar to the RET31 polypeptide has been reported as MKP7 (Masuda et al., JBC *276*, 39002-39011; and Tanoue et al., JBC., *276*, 26269-26639). These authors reported that the phosphatase could bind to and dephosphorylate the p38 kinase and the Jnk kinase in cells, resulting in the inactivation of these kinases. Activation of p38 kinase is known to be important in the induction of apoptosis (Herlaar and Brown, Molecular Medicine Today *5*, 439-447). One pathway where p38 has been reported to be important is in paclitaxel (Taxol®) induced apoptosis in tumor cells (Seidman et al., Experimental Cell Research *268*, 84-92). Similarly, activation of the Jnk kinase has also been reported to be important in the induction of apoptosis (Chang and Karin, Nature *410*, 37-40), including in paclitaxel induced apoptosis (Lee et al., JBC., *273*, 28253-28260). Therefore, inhibitors of RET31 should induce apoptosis in tumor cells by increasing the activation of p38 and Jnk kinases in the cells by preventing the dephosphorylation of these kinases. This would be particularly advantageous when combined with a chemotherapeutic drug, such as paclitaxel, that activates p38 and/or Jnk kinases to help induce apoptosis. Such a use represents a novel utility of RET31 antagonists and which has not be appreciated by Masuda et al., nor by Tanoue et al. Indeed, Masuda et al. teach that MKP7 may be a tumor suppressor gene, in which case inhibition of MKP7 would increase malignancies, which teaches away from our intended use for RET31 inhibitors.

In preferred embodiments, the present invention encompasses the use of inhibitors of RET31 for the treatment of cancer. Per the teachings described herein, inhibitors of RET31 may include small molecule inhibitors of RET31 activity, inhibitors that prevent RET31 from binding to p38 and/or Jnk kinases, antisense oligonucleotides to RET31, and antibodies directed against RET31. Such RET31 inhibitors would be particularly useful in malignancies where RET31 was overexpressed relative to normal tissues. In addition to the use of RET31 inhibitors as single agents, inhibitors of RET31 would be of particular use in combination with paclitaxel and other chemotherapeutic agents that induce Jnk and/or p38 dependent apoptosis in tumor cells for the treatment of malignancies. Other chemotherapeutic

agents that may induce the activation of Jnk and/or p38 leading to apoptosis that would be of use in combination with inhibitors of RET31 include but are not limited to RRR-alpha-tocopherol succinate, DA-125 [(8S,10S)-8-(3-aminopropanoyloxyacetyl)-10-[(2,6-dideoxy-2-fluoro-alpha-L-talopyranosyl) oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-5,12-naphthacene-dione hydrochloride] a novel anthracycline derivative, cisplatin, tamoxifen, sulindac sulfone, sulindac, arsenic trioxide, actinomycin D, docetaxel (Taxotere), vinblastine, vincristine, nocodazole, colchicines, and other microtubule-interfering agents.

Although it is believed the encoded polypeptide may share at least some biological activities with phosphatase proteins (particularly dual specificity proteins), a number of methods of determining the exact biological function of this clone are either known in the art or are described elsewhere herein. Briefly, the function of this clone may be determined by applying microarray methodology. Nucleic acids corresponding to the human phosphatase polynucleotides, in addition to, other clones of the present invention, may be arrayed on microchips for expression profiling. Depending on which polynucleotide probe is used to hybridize to the slides, a change in expression of a specific gene may provide additional insight into the function of this gene based upon the conditions being studied. For example, an observed increase or decrease in expression levels when the polynucleotide probe used comes from diseased testis tissue, as compared to, normal tissue might indicate a function in modulating testis function, for example. In the case of human RET31 phosphatase, adrenal gland, testis, prostate, ovary, skeletal muscle, liver, placenta, pancreas, thymus, small intestine, thyroid, heart, kidney, and/or lung tissue should be used, for example, to extract RNA to prepare the probe.

In addition, the function of the protein may be assessed by applying quantitative PCR methodology, for example. Real time quantitative PCR would provide the capability of following the expression of the human phosphatase gene throughout development, for example. Quantitative PCR methodology requires only a nominal amount of tissue from each developmentally important step is needed to perform such experiments. Therefore, the application of quantitative PCR methodology to refining the biological function of this polypeptide is encompassed by the present invention. In the case of human phosphatase, a disease correlation related

to human phosphatase may be made by comparing the mRNA expression level of human phosphatase in normal tissue, as compared to diseased tissue (particularly diseased tissue isolated from the following: adrenal gland, testis, prostate, ovary, skeletal muscle, liver, placenta, pancreas, thymus, small intestine, thyroid, heart, kidney, and/or lung tissue). Significantly higher or lower levels of human phosphatase expression in the diseased tissue may suggest human phosphatase plays a role in disease progression, and antagonists against human phosphatase polypeptides would be useful therapeutically in treating, preventing, and/or ameliorating the disease. Alternatively, significantly higher or lower levels of human phosphatase expression in the diseased tissue may suggest human phosphatase plays a defensive role against disease progression, and agonists of human phosphatase polypeptides may be useful therapeutically in treating, preventing, and/or ameliorating the disease. Also encompassed by the present invention are quantitative PCR probes corresponding to the polynucleotide sequence provided as SEQ ID NO:108 (Figures 13A-F).

The function of the protein may also be assessed through complementation assays in yeast. For example, in the case of the human phosphatase, transforming yeast deficient in dual-specificity phosphatase activity, for example, and assessing their ability to grow would provide convincing evidence the human phosphatase polypeptide has dual-specificity phosphatase activity. Additional assay conditions and methods that may be used in assessing the function of the polynucleotides and polypeptides of the present invention are known in the art, some of which are disclosed elsewhere herein.

Alternatively, the biological function of the encoded polypeptide may be determined by disrupting a homologue of this polypeptide in Mice and/or rats and observing the resulting phenotype. Such knock-out experiments are known in the art, some of which are disclosed elsewhere herein.

Moreover, the biological function of this polypeptide may be determined by the application of antisense and/or sense methodology and the resulting generation of transgenic mice and/or rats. Expressing a particular gene in either sense or antisense orientation in a transgenic mouse or rat could lead to respectively higher or lower expression levels of that particular gene. Altering the endogenous expression levels of a gene can lead to the observation of a particular phenotype that can then be used to

derive indications on the function of the gene. The gene can be either over-expressed or under expressed in every cell of the organism at all times using a strong ubiquitous promoter, or it could be expressed in one or more discrete parts of the organism using a well characterized tissue-specific promoter (e.g., a adrenal gland, testis, prostate, ovary, skeletal muscle, liver, placenta, pancreas, thymus, small intestine, thyroid, heart, kidney, and/or lung tissue specific promoter), or it can be expressed at a specified time of development using an inducible and/or a developmentally regulated promoter.

In the case of human phosphatase transgenic mice or rats, if no phenotype is apparent in normal growth conditions, observing the organism under diseased conditions (metabolic, reproductive, immune, hematopoietic, cardiovascular, hepatic, or pulmonary disorders, in addition to cancers, etc.) may lead to understanding the function of the gene. Therefore, the application of antisense and/or sense methodology to the creation of transgenic mice or rats to refine the biological function of the polypeptide is encompassed by the present invention.

In preferred embodiments, the following N-terminal RET31 deletion polypeptides are encompassed by the present invention: M1-S665, A2-S665, H3-S665, E4-S665, M5-S665, I6-S665, G7-S665, T8-S665, Q9-S665, I10-S665, V11-S665, T12-S665, E13-S665, R14-S665, L15-S665, V16-S665, A17-S665, L18-S665, L19-S665, E20-S665, S21-S665, G22-S665, T23-S665, E24-S665, K25-S665, V26-S665, L27-S665, L28-S665, I29-S665, D30-S665, S31-S665, R32-S665, P33-S665, F34-S665, V35-S665, E36-S665, Y37-S665, N38-S665, T39-S665, S40-S665, H41-S665, I42-S665, L43-S665, E44-S665, A45-S665, I46-S665, N47-S665, I48-S665, N49-S665, C50-S665, S51-S665, K52-S665, L53-S665, M54-S665, K55-S665, R56-S665, R57-S665, L58-S665, Q59-S665, Q60-S665, D61-S665, K62-S665, V63-S665, L64-S665, I65-S665, T66-S665, E67-S665, L68-S665, I69-S665, Q70-S665, H71-S665, S72-S665, A73-S665, K74-S665, H75-S665, K76-S665, V77-S665, D78-S665, I79-S665, D80-S665, C81-S665, S82-S665, Q83-S665, K84-S665, V85-S665, V86-S665, V87-S665, Y88-S665, D89-S665, Q90-S665, S91-S665, S92-S665, Q93-S665, D94-S665, V95-S665, A96-S665, S97-S665, L98-S665, S99-S665, S100-S665, D101-S665, C102-S665, F103-S665, L104-S665, T105-S665, V106-S665, L107-S665, L108-S665, G109-S665, K110-S665, L111-S665, E112-S665, K113-S665,

S114-S665, F115-S665, N116-S665, S117-S665, V118-S665, H119-S665, L120-S665, L121-S665, A122-S665, G123-S665, G124-S665, F125-S665, A126-S665, E127-S665, F128-S665, S129-S665, R130-S665, C131-S665, F132-S665, P133-S665, G134-S665, L135-S665, C136-S665, E137-S665, G138-S665, K139-S665, S140-S665, T141-S665, L142-S665, V143-S665, P144-S665, T145-S665, C146-S665, I147-S665, S148-S665, Q149-S665, P150-S665, C151-S665, L152-S665, P153-S665, V154-S665, A155-S665, N156-S665, I157-S665, G158-S665, P159-S665, T160-S665, R161-S665, I162-S665, L163-S665, P164-S665, N165-S665, L166-S665, Y167-S665, L168-S665, G169-S665, C170-S665, Q171-S665, R172-S665, D173-S665, V174-S665, L175-S665, N176-S665, K177-S665, E178-S665, L179-S665, I180-S665, Q181-S665, Q182-S665, N183-S665, G184-S665, I185-S665, G186-S665, Y187-S665, V188-S665, L189-S665, N190-S665, A191-S665, S192-S665, Y193-S665, T194-S665, C195-S665, P196-S665, K197-S665, P198-S665, D199-S665, F200-S665, I201-S665, P202-S665, E203-S665, S204-S665, H205-S665, F206-S665, L207-S665, R208-S665, V209-S665, P210-S665, V211-S665, N212-S665, D213-S665, S214-S665, F215-S665, C216-S665, E217-S665, K218-S665, I219-S665, L220-S665, P221-S665, W222-S665, L223-S665, D224-S665, K225-S665, S226-S665, V227-S665, D228-S665, F229-S665, I230-S665, E231-S665, K232-S665, A233-S665, K234-S665, A235-S665, S236-S665, N237-S665, G238-S665, C239-S665, V240-S665, L241-S665, V242-S665, H243-S665, C244-S665, L245-S665, A246-S665, G247-S665, I248-S665, S249-S665, R250-S665, S251-S665, A252-S665, T253-S665, I254-S665, A255-S665, I256-S665, A257-S665, Y258-S665, I259-S665, M260-S665, K261-S665, R262-S665, M263-S665, D264-S665, M265-S665, S266-S665, L267-S665, D268-S665, E269-S665, A270-S665, Y271-S665, R272-S665, F273-S665, V274-S665, K275-S665, E276-S665, K277-S665, R278-S665, P279-S665, T280-S665, I281-S665, S282-S665, P283-S665, N284-S665, F285-S665, N286-S665, F287-S665, L288-S665, G289-S665, Q290-S665, L291-S665, L292-S665, D293-S665, Y294-S665, E295-S665, K296-S665, K297-S665, I298-S665, K299-S665, N300-S665, Q301-S665, T302-S665, G303-S665, A304-S665, S305-S665, G306-S665, P307-S665, K308-S665, S309-S665, K310-S665, L311-S665, K312-S665, L313-S665, L314-S665, H315-S665, L316-S665, E317-S665, K318-S665, P319-S665, N320-S665, E321-S665,

P322-S665, V323-S665, P324-S665, A325-S665, V326-S665, S327-S665, E328-S665, G329-S665, G330-S665, Q331-S665, K332-S665, S333-S665, E334-S665, T335-S665, P336-S665, L337-S665, S338-S665, P339-S665, P340-S665, C341-S665, A342-S665, D343-S665, S344-S665, A345-S665, T346-S665, S347-S665, E348-S665, A349-S665, A350-S665, G351-S665, Q352-S665, R353-S665, P354-S665, V355-S665, H356-S665, P357-S665, A358-S665, S359-S665, V360-S665, P361-S665, S362-S665, V363-S665, P364-S665, S365-S665, V366-S665, Q367-S665, P368-S665, S369-S665, L370-S665, L371-S665, E372-S665, D373-S665, S374-S665, P375-S665, L376-S665, V377-S665, Q378-S665, A379-S665, L380-S665, S381-S665, G382-S665, L383-S665, H384-S665, L385-S665, S386-S665, A387-S665, D388-S665, R389-S665, L390-S665, E391-S665, D392-S665, S393-S665, N394-S665, K395-S665, L396-S665, K397-S665, R398-S665, S399-S665, F400-S665, S401-S665, L402-S665, D403-S665, I404-S665, K405-S665, S406-S665, V407-S665, S408-S665, Y409-S665, S410-S665, A411-S665, S412-S665, M413-S665, A414-S665, A415-S665, S416-S665, L417-S665, H418-S665, G419-S665, F420-S665, S421-S665, S422-S665, S423-S665, E424-S665, D425-S665, A426-S665, L427-S665, E428-S665, Y429-S665, Y430-S665, K431-S665, P432-S665, S433-S665, T434-S665, T435-S665, L436-S665, D437-S665, G438-S665, T439-S665, N440-S665, K441-S665, L442-S665, C443-S665, Q444-S665, F445-S665, S446-S665, P447-S665, V448-S665, Q449-S665, E450-S665, L451-S665, S452-S665, E453-S665, Q454-S665, T455-S665, P456-S665, E457-S665, T458-S665, S459-S665, P460-S665, D461-S665, K462-S665, E463-S665, E464-S665, A465-S665, S466-S665, I467-S665, P468-S665, K469-S665, K470-S665, L471-S665, Q472-S665, T473-S665, A474-S665, R475-S665, P476-S665, S477-S665, D478-S665, S479-S665, Q480-S665, S481-S665, K482-S665, R483-S665, L484-S665, H485-S665, S486-S665, V487-S665, R488-S665, T489-S665, S490-S665, S491-S665, S492-S665, G493-S665, T494-S665, A495-S665, Q496-S665, R497-S665, S498-S665, L499-S665, L500-S665, S501-S665, P502-S665, L503-S665, H504-S665, R505-S665, S506-S665, G507-S665, S508-S665, V509-S665, E510-S665, D511-S665, N512-S665, Y513-S665, H514-S665, T515-S665, S516-S665, F517-S665, L518-S665, F519-S665, G520-S665, L521-S665, S522-S665, T523-S665, S524-S665, Q525-S665, Q526-S665, H527-S665, L528-S665, T529-S665, K530-

S665, S531-S665, A532-S665, G533-S665, L534-S665, G535-S665, L536-S665, K537-S665, G538-S665, W539-S665, H540-S665, S541-S665, D542-S665, I543-S665, L544-S665, A545-S665, P546-S665, Q547-S665, T548-S665, S549-S665, T550-S665, P551-S665, S552-S665, L553-S665, T554-S665, S555-S665, S556-S665, W557-S665, Y558-S665, F559-S665, A560-S665, T561-S665, E562-S665, S563-S665, S564-S665, H565-S665, F566-S665, Y567-S665, S568-S665, A569-S665, S570-S665, A571-S665, I572-S665, Y573-S665, G574-S665, G575-S665, S576-S665, A577-S665, S578-S665, Y579-S665, S580-S665, A581-S665, Y582-S665, S583-S665, C584-S665, S585-S665, Q586-S665, L587-S665, P588-S665, T589-S665, C590-S665, G591-S665, D592-S665, Q593-S665, V594-S665, Y595-S665, S596-S665, V597-S665, R598-S665, R599-S665, R600-S665, Q601-S665, K602-S665, P603-S665, S604-S665, D605-S665, R606-S665, A607-S665, D608-S665, S609-S665, R610-S665, R611-S665, S612-S665, W613-S665, H614-S665, E615-S665, E616-S665, S617-S665, P618-S665, F619-S665, E620-S665, K621-S665, Q622-S665, F623-S665, K624-S665, R625-S665, R626-S665, S627-S665, C628-S665, Q629-S665, M630-S665, E631-S665, F632-S665, G633-S665, E634-S665, S635-S665, I636-S665, M637-S665, S638-S665, E639-S665, N640-S665, R641-S665, S642-S665, R643-S665, E644-S665, E645-S665, L646-S665, G647-S665, K648-S665, V649-S665, G650-S665, S651-S665, Q652-S665, S653-S665, S654-S665, F655-S665, S656-S665, G657-S665, S658-S665, and/or M659-S665 of SEQ ID NO:109. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these N-terminal RET31 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal RET31 deletion polypeptides are encompassed by the present invention: M1-S665, M1-V664, M1-E663, M1-I662, M1-I661, M1-E660, M1-M659, M1-S658, M1-G657, M1-S656, M1-F655, M1-S654, M1-S653, M1-Q652, M1-S651, M1-G650, M1-V649, M1-K648, M1-G647, M1-L646, M1-E645, M1-E644, M1-R643, M1-S642, M1-R641, M1-N640, M1-E639, M1-S638, M1-M637, M1-I636, M1-S635, M1-E634, M1-G633, M1-F632, M1-E631, M1-M630, M1-Q629, M1-C628, M1-S627, M1-R626, M1-R625, M1-K624, M1-F623, M1-Q622, M1-K621, M1-E620, M1-F619, M1-P618,

M1-S617, M1-E616, M1-E615, M1-H614, M1-W613, M1-S612, M1-R611, M1-R610, M1-S609, M1-D608, M1-A607, M1-R606, M1-D605, M1-S604, M1-P603, M1-K602, M1-Q601, M1-R600, M1-R599, M1-R598, M1-V597, M1-S596, M1-Y595, M1-V594, M1-Q593, M1-D592, M1-G591, M1-C590, M1-T589, M1-P588, M1-L587, M1-Q586, M1-S585, M1-C584, M1-S583, M1-Y582, M1-A581, M1-S580, M1-Y579, M1-S578, M1-A577, M1-S576, M1-G575, M1-G574, M1-Y573, M1-I572, M1-A571, M1-S570, M1-A569, M1-S568, M1-Y567, M1-F566, M1-H565, M1-S564, M1-S563, M1-E562, M1-T561, M1-A560, M1-F559, M1-Y558, M1-W557, M1-S556, M1-S555, M1-T554, M1-L553, M1-S552, M1-P551, M1-T550, M1-S549, M1-T548, M1-Q547, M1-P546, M1-A545, M1-L544, M1-I543, M1-D542, M1-S541, M1-H540, M1-W539, M1-G538, M1-K537, M1-L536, M1-G535, M1-L534, M1-G533, M1-A532, M1-S531, M1-K530, M1-T529, M1-L528, M1-H527, M1-Q526, M1-Q525, M1-S524, M1-T523, M1-S522, M1-L521, M1-G520, M1-F519, M1-L518, M1-F517, M1-S516, M1-T515, M1-H514, M1-Y513, M1-N512, M1-D511, M1-E510, M1-V509, M1-S508, M1-G507, M1-S506, M1-R505, M1-H504, M1-L503, M1-P502, M1-S501, M1-L500, M1-L499, M1-S498, M1-R497, M1-Q496, M1-A495, M1-T494, M1-G493, M1-S492, M1-S491, M1-S490, M1-T489, M1-R488, M1-V487, M1-S486, M1-H485, M1-L484, M1-R483, M1-K482, M1-S481, M1-Q480, M1-S479, M1-D478, M1-S477, M1-P476, M1-R475, M1-A474, M1-T473, M1-Q472, M1-L471, M1-K470, M1-K469, M1-P468, M1-I467, M1-S466, M1-A465, M1-E464, M1-E463, M1-K462, M1-D461, M1-P460, M1-S459, M1-T458, M1-E457, M1-P456, M1-T455, M1-Q454, M1-E453, M1-S452, M1-L451, M1-E450, M1-Q449, M1-V448, M1-P447, M1-S446, M1-F445, M1-Q444, M1-C443, M1-L442, M1-K441, M1-N440, M1-T439, M1-G438, M1-D437, M1-L436, M1-T435, M1-T434, M1-S433, M1-P432, M1-K431, M1-Y430, M1-Y429, M1-E428, M1-L427, M1-A426, M1-D425, M1-E424, M1-S423, M1-S422, M1-S421, M1-F420, M1-G419, M1-H418, M1-L417, M1-S416, M1-A415, M1-A414, M1-M413, M1-S412, M1-A411, M1-S410, M1-Y409, M1-S408, M1-V407, M1-S406, M1-K405, M1-I404, M1-D403, M1-L402, M1-S401, M1-F400, M1-S399, M1-R398, M1-K397, M1-L396, M1-K395, M1-N394, M1-S393, M1-D392, M1-E391, M1-L390, M1-R389, M1-D388, M1-A387, M1-S386, M1-L385, M1-H384, M1-L383, M1-G382, M1-S381, M1-L380, M1-A379, M1-Q378, M1-V377, M1-L376, M1-P375, M1-S374, M1-

D373, M1-E372, M1-L371, M1-L370, M1-S369, M1-P368, M1-Q367, M1-V366, M1-S365, M1-P364, M1-V363, M1-S362, M1-P361, M1-V360, M1-S359, M1-A358, M1-P357, M1-H356, M1-V355, M1-P354, M1-R353, M1-Q352, M1-G351, M1-A350, M1-A349, M1-E348, M1-S347, M1-T346, M1-A345, M1-S344, M1-D343, M1-A342, M1-C341, M1-P340, M1-P339, M1-S338, M1-L337, M1-P336, M1-T335, M1-E334, M1-S333, M1-K332, M1-Q331, M1-G330, M1-G329, M1-E328, M1-S327, M1-V326, M1-A325, M1-P324, M1-V323, M1-P322, M1-E321, M1-N320, M1-P319, M1-K318, M1-E317, M1-L316, M1-H315, M1-L314, M1-L313, M1-K312, M1-L311, M1-K310, M1-S309, M1-K308, M1-P307, M1-G306, M1-S305, M1-A304, M1-G303, M1-T302, M1-Q301, M1-N300, M1-K299, M1-I298, M1-K297, M1-K296, M1-E295, M1-Y294, M1-D293, M1-L292, M1-L291, M1-Q290, M1-G289, M1-L288, M1-F287, M1-N286, M1-F285, M1-N284, M1-P283, M1-S282, M1-I281, M1-T280, M1-P279, M1-R278, M1-K277, M1-E276, M1-K275, M1-V274, M1-F273, M1-R272, M1-Y271, M1-A270, M1-E269, M1-D268, M1-L267, M1-S266, M1-M265, M1-D264, M1-M263, M1-R262, M1-K261, M1-M260, M1-I259, M1-Y258, M1-A257, M1-I256, M1-A255, M1-I254, M1-T253, M1-A252, M1-S251, M1-R250, M1-S249, M1-I248, M1-G247, M1-A246, M1-L245, M1-C244, M1-H243, M1-V242, M1-L241, M1-V240, M1-C239, M1-G238, M1-N237, M1-S236, M1-A235, M1-K234, M1-A233, M1-K232, M1-E231, M1-I230, M1-F229, M1-D228, M1-V227, M1-S226, M1-K225, M1-D224, M1-L223, M1-W222, M1-P221, M1-L220, M1-I219, M1-K218, M1-E217, M1-C216, M1-F215, M1-S214, M1-D213, M1-N212, M1-V211, M1-P210, M1-V209, M1-R208, M1-L207, M1-F206, M1-H205, M1-S204, M1-E203, M1-P202, M1-I201, M1-F200, M1-D199, M1-P198, M1-K197, M1-P196, M1-C195, M1-T194, M1-Y193, M1-S192, M1-A191, M1-N190, M1-L189, M1-V188, M1-Y187, M1-G186, M1-I185, M1-G184, M1-N183, M1-Q182, M1-Q181, M1-I180, M1-L179, M1-E178, M1-K177, M1-N176, M1-L175, M1-V174, M1-D173, M1-R172, M1-Q171, M1-C170, M1-G169, M1-L168, M1-Y167, M1-L166, M1-N165, M1-P164, M1-L163, M1-I162, M1-R161, M1-T160, M1-P159, M1-G158, M1-I157, M1-N156, M1-A155, M1-V154, M1-P153, M1-L152, M1-C151, M1-P150, M1-Q149, M1-S148, M1-I147, M1-C146, M1-T145, M1-P144, M1-V143, M1-L142, M1-T141, M1-S140, M1-K139, M1-G138, M1-E137, M1-C136, M1-L135, M1-G134, M1-P133, M1-F132, M1-C131, M1-R130, M1-S129, M1-F128,

M1-E127, M1-A126, M1-F125, M1-G124, M1-G123, M1-A122, M1-L121, M1-L120, M1-H119, M1-V118, M1-S117, M1-N116, M1-F115, M1-S114, M1-K113, M1-E112, M1-L111, M1-K110, M1-G109, M1-L108, M1-L107, M1-V106, M1-T105, M1-L104, M1-F103, M1-C102, M1-D101, M1-S100, M1-S99, M1-L98, M1-S97, M1-A96, M1-V95, M1-D94, M1-Q93, M1-S92, M1-S91, M1-Q90, M1-D89, M1-Y88, M1-V87, M1-V86, M1-V85, M1-K84, M1-Q83, M1-S82, M1-C81, M1-D80, M1-I79, M1-D78, M1-V77, M1-K76, M1-H75, M1-K74, M1-A73, M1-S72, M1-H71, M1-Q70, M1-I69, M1-L68, M1-E67, M1-T66, M1-I65, M1-L64, M1-V63, M1-K62, M1-D61, M1-Q60, M1-Q59, M1-L58, M1-R57, M1-R56, M1-K55, M1-M54, M1-L53, M1-K52, M1-S51, M1-C50, M1-N49, M1-I48, M1-N47, M1-I46, M1-A45, M1-E44, M1-L43, M1-I42, M1-H41, M1-S40, M1-T39, M1-N38, M1-Y37, M1-E36, M1-V35, M1-F34, M1-P33, M1-R32, M1-S31, M1-D30, M1-I29, M1-L28, M1-L27, M1-V26, M1-K25, M1-E24, M1-T23, M1-G22, M1-S21, M1-E20, M1-L19, M1-L18, M1-A17, M1-V16, M1-L15, M1-R14, M1-E13, M1-T12, M1-V11, M1-I10, M1-Q9, M1-T8, and/or M1-G7 of SEQ ID NO:109. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these C-terminal RET31 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The present invention also encompasses immunogenic and/or antigenic epitopes of the human RET31 phosphatase polypeptide.

The human phosphatase polypeptides of the present invention were determined to comprise several phosphorylation sites based upon the Motif algorithm (Genetics Computer Group, Inc.). The phosphorylation of such sites may regulate some biological activity of the human phosphatase polypeptide. For example, phosphorylation at specific sites may be involved in regulating the proteins ability to associate or bind to other molecules (e.g., proteins, ligands, substrates, DNA, etc.). In the present case, phosphorylation may modulate the ability of the human phosphatase polypeptide to associate with other polypeptides, particularly cognate ligand for human phosphatase, or its ability to modulate certain cellular signal pathways.

The human phosphatase polypeptide was predicted to comprise twelve PKC phosphorylation sites using the Motif algorithm (Genetics Computer Group, Inc.). In vivo, protein kinase C exhibits a preference for the phosphorylation of serine or

threonine residues. The PKC phosphorylation sites have the following consensus pattern: [ST]-x-[RK], where S or T represents the site of phosphorylation and 'x' an intervening amino acid residue. Additional information regarding PKC phosphorylation sites can be found in Woodget J.R., Gould K.L., Hunter T., Eur. J. Biochem. 161:177-184(1986), and Kishimoto A., Nishiyama K., Nakanishi H., Uratsuji Y., Nomura H., Takeyama Y., Nishizuka Y., J. Biol. Chem... 260:12492-12499(1985); which are hereby incorporated by reference herein.

In preferred embodiments, the following PKC phosphorylation site polypeptides are encompassed by the present invention: GTQIVTERLVALL (SEQ ID NO:116), LLESGTEKVLLID (SEQ ID NO:117), ELIQHSAKHKVDI (SEQ ID NO:118), VDIDCSQKVVVYD (SEQ ID NO:119), DRLEDSNKLKRSF (SEQ ID NO:120), TTLDGTNKLCQFS (SEQ ID NO:121), PKKLQTARPSDSQ (SEQ ID NO:122), PSDSQSKRLHSVR (SEQ ID NO:123), SKRLHSVRTSSSG (SEQ ID NO:124), GDQVYSVRRRQKP (SEQ ID NO:125), RRQKPSDRADSRR (SEQ ID NO:126), and/or SDRADSRRSWHEE (SEQ ID NO:127). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of the human RET31 phosphatase PKC phosphorylation site polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

The human phosphatase polypeptide has been shown to comprise six glycosylation sites according to the Motif algorithm (Genetics Computer Group, Inc.). As discussed more specifically herein, protein glycosylation is thought to serve a variety of functions including: augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion.

Asparagine phosphorylation sites have the following consensus pattern, N-{P}-[ST]-{P}, wherein N represents the glycosylation site. However, it is well known that that potential N-glycosylation sites are specific to the consensus sequence Asn-Xaa-Ser/Thr. However, the presence of the consensus tripeptide is not sufficient to conclude that an asparagine residue is glycosylated, due to the fact that the folding of the protein plays an important role in the regulation of N-glycosylation. It has been shown that the presence of proline between Asn and Ser/Thr will inhibit N-

glycosylation; this has been confirmed by a recent statistical analysis of glycosylation sites, which also shows that about 50% of the sites that have a proline C-terminal to Ser/Thr are not glycosylated. Additional information relating to asparagine glycosylation may be found in reference to the following publications, which are hereby incorporated by reference herein: Marshall R.D., Annu. Rev. Biochem. 41:673-702(1972); Pless D.D., Lennarz W.J., Proc. Natl. Acad. Sci. U.S.A. 74:134-138(1977); Bause E., Biochem. J. 209:331-336(1983); Gavel Y., von Heijne G., Protein Eng. 3:433-442(1990); and Miletich J.P., Broze G.J. Jr., J. Biol. Chem... 265:11397-11404(1990).

In preferred embodiments, the following asparagine glycosylation site polypeptides are encompassed by the present invention: PFVEYNTSHILEAI (SEQ ID NO:128), EAININCSKLMKRR (SEQ ID NO:129), IGYVLNASYTCPKP (SEQ ID NO:130), LRVPVNDSFCEKIL (SEQ ID NO:131), EKKIKNQTGASGPK (SEQ ID NO:132), and/or SIMSENRSREELGK (SEQ ID NO:133). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use of the human RET31 phosphatase asparagine glycosylation site polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In confirmation of the human RET31 representing a novel human phosphatase polypeptide, the RET31 polypeptide has been shown to comprise a dual specificity phosphatase catalytic domain as identified by the BLAST2 algorithm using the DSPc PFAM HMM (PF00782) as a query sequence.

The catalytic residue of the human RET31 polypeptide is represented by an acitve site cysteine located at amino acid residue 244 of SEQ ID NO:109 (Figures 13A-F).

In preferred embodiments, the following human RET31 DSPc domain polypeptide is encompassed by the present invention: GPTRILPNLYLGCQRDVLNKELIQQNGIGYVLNASYTCPKPDFIPESHFLRVPV NDSFCEKILPWLDKSVDFIEKAKASNGCVLVHCLAGISRSATIAIAYIMKRMD MSLDEAYRFVKEKRPTISPNFNFLGQLLDYEKK (SEQ ID NO:134). Polynucleotides encoding this polypeptide are also provided. The present invention

also encompasses the use of this human RET31 DSPc domain polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

In preferred embodiments, the following human RET31 DSPc domain amino acid substitutions are encompassed by the present invention: wherein G158 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein P159 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein T160 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein R161 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein I162 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L163 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein P164 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein N165 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein L166 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein Y167 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein L168 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein G169 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein C170 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Q171 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; wherein R172 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein D173 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V174 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein L175 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein N176 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein K177 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein E178 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L179 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein I180 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Q181 is substituted with either an A, C, D, E, F, G, H, I,

K, L, M, N, P, R, S, T, V, W, or Y; wherein Q182 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; wherein N183 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein G184 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein I185 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein G186 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Y187 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein V188 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein L189 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein N190 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein A191 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S192 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein Y193 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein T194 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein C195 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein P196 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein K197 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein P198 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein D199 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein F200 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein I201 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein P202 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein E203 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S204 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein H205 is substituted with either an A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein F206 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L207 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein R208 is substituted with either an A, C, D, E, F,

G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein V209 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein P210 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein V211 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein N212 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein D213 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S214 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein F215 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein C216 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein E217 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein K218 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein I219 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L220 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein P221 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein W222 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or Y; wherein L223 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein D224 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein K225 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S226 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein V227 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein D228 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein F229 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein I230 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein E231 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein K232 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A233 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein K234 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A235 is substituted with either a C, D, E, F, G,

H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S236 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein N237 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein G238 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein C239 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V240 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein L241 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein V242 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein H243 is substituted with either an A, C, D, E, F, G, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein C244 is substituted with either an A, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L245 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein A246 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein G247 is substituted with either an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein I248 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S249 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein R250 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein S251 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein A252 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein T253 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein I254 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A255 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein I256 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A257 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Y258 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein I259 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein M260 is substituted with either an A, C, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y; wherein K261 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein R262 is substituted with either an A, C, D,

E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein M263 is substituted with either an A, C, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y; wherein D264 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein M265 is substituted with either an A, C, D, E, F, G, H, I, K, L, N, P, Q, R, S, T, V, W, or Y; wherein S266 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein L267 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein D268 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein E269 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein A270 is substituted with either a C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Y271 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein R272 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein F273 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein V274 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, W, or Y; wherein K275 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein E276 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein K277 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; wherein R278 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, S, T, V, W, or Y; wherein P279 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein T280 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, V, W, or Y; wherein I281 is substituted with either an A, C, D, E, F, G, H, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein S282 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, T, V, W, or Y; wherein P283 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, Q, R, S, T, V, W, or Y; wherein N284 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein F285 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein N286 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, P, Q, R, S, T, V, W, or Y; wherein F287 is substituted with either an A, C, D, E, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein L288 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein G289 is substituted with either

an A, C, D, E, F, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Q290 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, R, S, T, V, W, or Y; wherein L291 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein L292 is substituted with either an A, C, D, E, F, G, H, I, K, M, N, P, Q, R, S, T, V, W, or Y; wherein D293 is substituted with either an A, C, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein Y294 is substituted with either an A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, or W; wherein E295 is substituted with either an A, C, D, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, or Y; wherein K296 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y; and/or wherein K297 is substituted with either an A, C, D, E, F, G, H, I, L, M, N, P, Q, R, S, T, V, W, or Y of SEQ ID NO:109, in addition to any combination thereof. The present invention also encompasses the use of these human RET31 DSPc domain amino acid substituted polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following human RET31 DSPc domain conservative amino acid substitutions are encompassed by the present invention: wherein G158 is substituted with either an A, M, S, or T; wherein P159 is a P; wherein T160 is substituted with either an A, G, M, or S; wherein R161 is substituted with either a K, or H; wherein I162 is substituted with either an A, V, or L; wherein L163 is substituted with either an A, I, or V; wherein P164 is a P; wherein N165 is substituted with a Q; wherein L166 is substituted with either an A, I, or V; wherein Y167 is either an F, or W; wherein L168 is substituted with either an A, I, or V; wherein G169 is substituted with either an A, M, S, or T; wherein C170 is a C; wherein Q171 is substituted with a N; wherein R172 is substituted with either a K, or H; wherein D173 is substituted with an E; wherein V174 is substituted with either an A, I, or L; wherein L175 is substituted with either an A, I, or V; wherein N176 is substituted with a Q; wherein K177 is substituted with either a R, or H; wherein E178 is substituted with a D; wherein L179 is substituted with either an A, I, or V; wherein I180 is substituted with either an A, V, or L; wherein Q181 is substituted with a N; wherein Q182 is substituted with a N; wherein N183 is substituted with a Q; wherein G184 is substituted with either an A, M, S, or T; wherein I185 is substituted with either an A, V, or L; wherein G186 is substituted with either an A, M, S, or T;

wherein Y187 is either an F, or W; wherein V188 is substituted with either an A, I, or L; wherein L189 is substituted with either an A, I, or V; wherein N190 is substituted with a Q; wherein A191 is substituted with either a G, I, L, M, S, T, or V; wherein S192 is substituted with either an A, G, M, or T; wherein Y193 is either an F, or W; wherein T194 is substituted with either an A, G, M, or S; wherein C195 is a C; wherein P196 is a P; wherein K197 is substituted with either a R, or H; wherein P198 is a P; wherein D199 is substituted with an E; wherein F200 is substituted with either a W, or Y; wherein I201 is substituted with either an A, V, or L; wherein P202 is a P; wherein E203 is substituted with a D; wherein S204 is substituted with either an A, G, M, or T; wherein H205 is substituted with either a K, or R; wherein F206 is substituted with either a W, or Y; wherein L207 is substituted with either an A, I, or V; wherein R208 is substituted with either a K, or H; wherein V209 is substituted with either an A, I, or L; wherein P210 is a P; wherein V211 is substituted with either an A, I, or L; wherein N212 is substituted with a Q; wherein D213 is substituted with an E; wherein S214 is substituted with either an A, G, M, or T; wherein F215 is substituted with either a W, or Y; wherein C216 is a C; wherein E217 is substituted with a D; wherein K218 is substituted with either a R, or H; wherein I219 is substituted with either an A, V, or L; wherein L220 is substituted with either an A, I, or V; wherein P221 is a P; wherein W222 is either an F, or Y; wherein L223 is substituted with either an A, I, or V; wherein D224 is substituted with an E; wherein K225 is substituted with either a R, or H; wherein S226 is substituted with either an A, G, M, or T; wherein V227 is substituted with either an A, I, or L; wherein D228 is substituted with an E; wherein F229 is substituted with either a W, or Y; wherein I230 is substituted with either an A, V, or L; wherein E231 is substituted with a D; wherein K232 is substituted with either a R, or H; wherein A233 is substituted with either a G, I, L, M, S, T, or V; wherein K234 is substituted with either a R, or H; wherein A235 is substituted with either a G, I, L, M, S, T, or V; wherein S236 is substituted with either an A, G, M, or T; wherein N237 is substituted with a Q; wherein G238 is substituted with either an A, M, S, or T; wherein C239 is a C; wherein V240 is substituted with either an A, I, or L; wherein L241 is substituted with either an A, I, or V; wherein V242 is substituted with either an A, I, or L; wherein H243 is substituted with either a K, or R; wherein C244 is a C; wherein L245 is substituted with either an

A, I, or V; wherein A246 is substituted with either a G, I, L, M, S, T, or V; wherein G247 is substituted with either an A, M, S, or T; wherein I248 is substituted with either an A, V, or L; wherein S249 is substituted with either an A, G, M, or T; wherein R250 is substituted with either a K, or H; wherein S251 is substituted with either an A, G, M, or T; wherein A252 is substituted with either a G, I, L, M, S, T, or V; wherein T253 is substituted with either an A, G, M, or S; wherein I254 is substituted with either an A, V, or L; wherein A255 is substituted with either a G, I, L, M, S, T, or V; wherein I256 is substituted with either an A, V, or L; wherein A257 is substituted with either a G, I, L, M, S, T, or V; wherein Y258 is either an F, or W; wherein I259 is substituted with either an A, V, or L; wherein M260 is substituted with either an A, G, S, or T; wherein K261 is substituted with either a R, or H; wherein R262 is substituted with either a K, or H; wherein M263 is substituted with either an A, G, S, or T; wherein D264 is substituted with an E; wherein M265 is substituted with either an A, G, S, or T; wherein S266 is substituted with either an A, G, M, or T; wherein L267 is substituted with either an A, I, or V; wherein D268 is substituted with an E; wherein E269 is substituted with a D; wherein A270 is substituted with either a G, I, L, M, S, T, or V; wherein Y271 is either an F, or W; wherein R272 is substituted with either a K, or H; wherein F273 is substituted with either a W, or Y; wherein V274 is substituted with either an A, I, or L; wherein K275 is substituted with either a R, or H; wherein E276 is substituted with a D; wherein K277 is substituted with either a R, or H; wherein R278 is substituted with either a K, or H; wherein P279 is a P; wherein T280 is substituted with either an A, G, M, or S; wherein I281 is substituted with either an A, V, or L; wherein S282 is substituted with either an A, G, M, or T; wherein P283 is a P; wherein N284 is substituted with a Q; wherein F285 is substituted with either a W, or Y; wherein N286 is substituted with a Q; wherein F287 is substituted with either a W, or Y; wherein L288 is substituted with either an A, I, or V; wherein G289 is substituted with either an A, M, S, or T; wherein Q290 is substituted with a N; wherein L291 is substituted with either an A, I, or V; wherein L292 is substituted with either an A, I, or V; wherein D293 is substituted with an E; wherein Y294 is either an F, or W; wherein E295 is substituted with a D; wherein K296 is substituted with either a R, or H; and/or wherein K297 is substituted with either a R, or H of SEQ ID NO:109 in addition to any combination

thereof. Other suitable substitutions within the human RET31 DSPc domain are encompassed by the present invention and are referenced elsewhere herein. The present invention also encompasses the use of these human RET31 DSPc domain conservative amino acid substituted polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In further confirmation of the human RET31 polypeptide representing a novel human phosphatase polypeptide, the RET31 polypeptide has been shown to comprise a tyrosine specific protein phosphatase active site domain according to the Motif algorithm (Genetics Computer Group, Inc.).

Tyrosine specific protein phosphatases (EC 3.1.3.48) (PTPase) are enzymes that catalyze the removal of a phosphate group attached to a tyrosine residue. These enzymes are very important in the control of cell growth, proliferation, differentiation and transformation. Multiple forms of PTPase have been characterized and can be classified into two categories: soluble PTPases and transmembrane receptor proteins that contain PTPase domain(s).

The currently known PTPases are listed below: Soluble PTPases, PTPN1 (PTP-1B), PTPN2 (T-cell PTPase; TC-PTP), PTPN3 (H1) and PTPN4 (MEG), enzymes that contain an N-terminal band 4.1-like domain and could act at junctions between the membrane and cytoskeleton, PTPN5 (STEP), PTPN6 (PTP-1C; HCP; SHP) and PTPN11 (PTP-2C; SH-PTP3; Syp), enzymes which contain two copies of the SH2 domain at its N-terminal extremity (e.g., the Drosophila protein corkscrew (gene csw) also belongs to this subgroup), PTPN7 (LC-PTP; Hematopoietic protein-tyrosine phosphatase; HePTP), PTPN8 (70Z-PEP), PTPN9 (MEG2), PTPN12 (PTP-G1; PTP-P19), Yeast PTP1, Yeast PTP2 which may be involved in the ubiquitin-mediated protein degradation pathway, Fission yeast pyp1 and pyp2 which play a role in inhibiting the onset of mitosis, Fission yeast pyp3 which contributes to the dephosphorylation of cdc2, Yeast CDC14 which may be involved in chromosome segregation, Yersinia virulence plasmid PTPAses (gene yopH), Autographa californica nuclear polyhedrosis virus 19 Kd PTPase, Dual specificity PTPases, DUSP1 (PTPN10; MAP kinase phosphatase-1; MKP-1); which dephosphorylates MAP kinase on both Thr-183 and Tyr-185, DUSP2 (PAC-1), a nuclear enzyme that dephosphorylates MAP kinases ERK1 and ERK2 on both Thr and Tyr residues,

DUSP3 (VHR), DUSP4 (HVH2), DUSP5 (HVH3), DUSP6 (Pyst1; MKP-3), DUSP7 (Pyst2; MKP-X), Yeast MSG5, a PTPase that dephosphorylates MAP kinase FUS3, Yeast YVH1, Vaccinia virus H1 PTPase - a dual specificity phosphatase,

Structurally, all known receptor PTPases, are made up of a variable length extracellular domain, followed by a transmembrane region and a C-terminal catalytic cytoplasmic domain. Some of the receptor PTPases contain fibronectin type III (FN-III) repeats, immunoglobulin-like domains, MAM domains or carbonic anhydrase-like domains in their extracellular region. The cytoplasmic region generally contains two copies of the PTPAse domain. The first seems to have enzymatic activity, while the second is inactive but seems to affect substrate specificity of the first. In these domains, the catalytic cysteine is generally conserved but some other, presumably important, residues are not.

PTPase domains consist of about 300 amino acids. There are two conserved cysteines, the second one has been shown to be absolutely required for activity. Furthermore, a number of conserved residues in its immediate vicinity have also been shown to be important.

A consensus sequence for tyrosine specific protein phophatases is provided as follows:

[LIVMF]-H-C-x(2)-G-x(3)-[STC]-[STAGP]-x-[LIVMFY], wherein C is the active site residue and "X" represents any amino acid.

Additional information related to tyrosine specific protein phosphatase domains and proteins may be found in reference to the following publications Fischer E.H., Charbonneau H., Tonks N.K., Science 253:401-406(1991); Charbonneau H., Tonks N.K., Annu. Rev. Cell Biol. 8:463-493(1992); Trowbridge I.S., J. Biol. Chem... 266:23517-23520(1991); Tonks N.K., Charbonneau H., Trends Biochem. Sci. 14:497-500(1989); and Hunter T., Cell 58:1013-1016(1989); which are hereby incorporated herein by reference in their entirety.

In preferred embodiments, the following tyrosine specific protein phosphatase active site domain polypeptide is encompassed by the present invention: NGCVLVHCLAGISRSATIAIAYI (SEQ ID NO:144). Polynucleotides encoding these polypeptides are also provided. The present invention also encompasses the use

of this tyrosine specific protein phosphatase active site domain polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

In addition to the human RET31 polynucleotide and polypeptide sequence, the present invention also relates to the isolated mouse ortholog of the RET31 polypeptide.

The polypeptide corresponding to the mouse RET31 gene provided as SEQ ID NO:113 (Figure 16A-C), encoded by the polynucleotide sequence according to SEQ ID NO:114 (Figure 16A-C), and/or encoded by the polynucleotide contained within the deposited clone, mRET31, has significant homology at the nucleotide and amino acid level to a number of phosphatases, which include, for example, the human RET31 protein of the present invention (SEQ ID NO:109); the human DUS8 (DUS8; Genbank Accession No:gi|U27193; SEQ ID NO:110); the human DUSP6 protein(DUSP6; Genbank Accession No:gi|AB013382; SEQ ID NO:111); and the human map kinase phosphatase MKP-5 protein (MKP-5; Genbank Accession No:gi|AB026436; SEQ ID NO:112) as determined by BLASTP. An alignment of the human phosphatase polypeptide with these proteins is provided in Figures 14A-C.

The determined nucleotide sequence of the mRET31 cDNA in Figures 16A-C (SEQ ID NO:114) contains an open reading frame encoding a protein of about 660 amino acid residues, with a deduced molecular weight of about 73kDa. The amino acid sequence of the predicted mRET31 polypeptide is shown in Figures 16A-C (SEQ ID NO:114). The mRET31 protein shown in Figures 16A-C was determined to share significant identity and similarity to several known phosphates, particularly, dual-specificity protein phosphatases. Specifically, the mRET31 protein shown in Figures 16A-C was determined to be about 90% identical and 92% similar to the human RET31 protein of the present invention (SEQ ID NO:109); to be about 48.5% identical and 55.7% similar to the human DUS8 (DUS8; Genbank Accession No:gi|U27193; SEQ ID NO:110); to be about 37.4% identical and 49.7% similar to the human DUSP6 protein(DUSP6; Genbank Accession No:gi|AB013382; SEQ ID NO:111); and to be about 35.2% identical and 46.9% similar to the human map kinase phosphatase MKP-5 protein (MKP-5; Genbank Accession No:gi|AB026436; SEQ ID NO:112), as shown in Figure 12.

The translational start nucleotide position of the mRET31 polynucleotide has been determined to begin at nucleotide 369 of SEQ ID NO:113 (Figures 16A-C), and the transational stop nucleotide position has been determined to be at nucleotide 2348 of SEQ ID NO:113 (Figures 16A-C).

In preferred embodiments, the following N-terminal mRET31 deletion polypeptides are encompassed by the present invention: M1-S660, A2-S660, H3-S660, E4-S660, M5-S660, I6-S660, G7-S660, T8-S660, Q9-S660, I10-S660, V11-S660, T12-S660, E13-S660, S14-S660, L15-S660, V16-S660, A17-S660, L18-S660, L19-S660, E20-S660, S21-S660, G22-S660, T23-S660, E24-S660, K25-S660, V26-S660, L27-S660, L28-S660, I29-S660, D30-S660, S31-S660, R32-S660, P33-S660, F34-S660, V35-S660, E36-S660, Y37-S660, N38-S660, T39-S660, S40-S660, H41-S660, I42-S660, L43-S660, E44-S660, A45-S660, I46-S660, N47-S660, I48-S660, N49-S660, C50-S660, S51-S660, K52-S660, L53-S660, M54-S660, K55-S660, R56-S660, R57-S660, L58-S660, Q59-S660, Q60-S660, D61-S660, K62-S660, V63-S660, L64-S660, I65-S660, T66-S660, E67-S660, L68-S660, I69-S660, H70-S660, Q71-S660, S72-S660, T73-S660, K74-S660, H75-S660, K76-S660, V77-S660, D78-S660, I79-S660, D80-S660, C81-S660, N82-S660, Q83-S660, R84-S660, V85-S660, V86-S660, V87-S660, Y88-S660, D89-S660, H90-S660, S91-S660, S92-S660, Q93-S660, D94-S660, V95-S660, G96-S660, S97-S660, L98-S660, S99-S660, S100-S660, D101-S660, C102-S660, F103-S660, L104-S660, T105-S660, V106-S660, L107-S660, L108-S660, G109-S660, K110-S660, L111-S660, E112-S660, R113-S660, S114-S660, F115-S660, N116-S660, S117-S660, V118-S660, H119-S660, L120-S660, L121-S660, A122-S660, G123-S660, G124-S660, F125-S660, A126-S660, E127-S660, F128-S660, S129-S660, R130-S660, C131-S660, F132-S660, P133-S660, G134-S660, L135-S660, C136-S660, E137-S660, G138-S660, K139-S660, S140-S660, T141-S660, L142-S660, V143-S660, P144-S660, T145-S660, C146-S660, I147-S660, S148-S660, Q149-S660, P150-S660, C151-S660, L152-S660, P153-S660, V154-S660, A155-S660, N156-S660, I157-S660, G158-S660, P159-S660, T160-S660, R161-S660, I162-S660, L163-S660, P164-S660, N165-S660, L166-S660, Y167-S660, L168-S660, G169-S660, C170-S660, Q171-S660, R172-S660, D173-S660, V174-S660, L175-S660, N176-S660, K177-S660, D178-S660, L179-S660, M180-S660, Q181-S660, Q182-S660, N183-S660, G184-S660, I185-

S660, G186-S660, Y187-S660, V188-S660, L189-S660, N190-S660, A191-S660, S192-S660, N193-S660, T194-S660, C195-S660, P196-S660, K197-S660, P198-S660, D199-S660, F200-S660, I201-S660, P202-S660, E203-S660, S204-S660, H205-S660, F206-S660, L207-S660, R208-S660, V209-S660, P210-S660, V211-S660, N212-S660, D213-S660, S214-S660, F215-S660, C216-S660, E217-S660, K218-S660, I219-S660, L220-S660, P221-S660, W222-S660, L223-S660, D224-S660, K225-S660, S226-S660, V227-S660, D228-S660, F229-S660, I230-S660, E231-S660, K232-S660, A233-S660, K234-S660, A235-S660, S236-S660, N237-S660, G238-S660, C239-S660, V240-S660, L241-S660, I242-S660, H243-S660, C244-S660, L245-S660, A246-S660, G247-S660, I248-S660, S249-S660, R250-S660, S251-S660, A252-S660, T253-S660, I254-S660, A255-S660, I256-S660, A257-S660, Y258-S660, I259-S660, M260-S660, K261-S660, R262-S660, M263-S660, D264-S660, M265-S660, S266-S660, L267-S660, D268-S660, E269-S660, A270-S660, Y271-S660, R272-S660, F273-S660, V274-S660, K275-S660, E276-S660, K277-S660, R278-S660, P279-S660, T280-S660, I281-S660, S282-S660, P283-S660, N284-S660, F285-S660, N286-S660, F287-S660, M288-S660, G289-S660, Q290-S660, L291-S660, M292-S660, D293-S660, Y294-S660, E295-S660, K296-S660, T297-S660, I298-S660, N299-S660, N300-S660, Q301-S660, T302-S660, G303-S660, M304-S660, S305-S660, G306-S660, P307-S660, K308-S660, S309-S660, K310-S660, L311-S660, K312-S660, L313-S660, L314-S660, H315-S660, L316-S660, D317-S660, K318-S660, P319-S660, S320-S660, E321-S660, P322-S660, V323-S660, P324-S660, A325-S660, A326-S660, S327-S660, E328-S660, G329-S660, G330-S660, W331-S660, K332-S660, S333-S660, A334-S660, L335-S660, S336-S660, L337-S660, S338-S660, P339-S660, P340-S660, C341-S660, A342-S660, N343-S660, S344-S660, T345-S660, S346-S660, E347-S660, A348-S660, S349-S660, G350-S660, Q351-S660, R352-S660, L353-S660, V354-S660, H355-S660, P356-S660, A357-S660, S358-S660, V359-S660, P360-S660, R361-S660, L362-S660, Q363-S660, P364-S660, S365-S660, L366-S660, L367-S660, E368-S660, D369-S660, S370-S660, P371-S660, L372-S660, V373-S660, Q374-S660, A375-S660, L376-S660, S377-S660, G378-S660, L379-S660, Q380-S660, L381-S660, S382-S660, S383-S660, E384-S660, K385-S660, L386-S660, E387-S660, D388-S660, S389-S660, T390-S660, K391-S660, L392-S660, K393-S660,

R394-S660, S395-S660, F396-S660, S397-S660, L398-S660, D399-S660, I400-S660, K401-S660, S402-S660, V403-S660, S404-S660, Y405-S660, S406-S660, A407-S660, S408-S660, M409-S660, A410-S660, A411-S660, S412-S660, L413-S660, H414-S660, G415-S660, F416-S660, S417-S660, S418-S660, E419-S660, E420-S660, A421-S660, L422-S660, D423-S660, Y424-S660, C425-S660, K426-S660, P427-S660, S428-S660, A429-S660, T430-S660, L431-S660, D432-S660, G433-S660, T434-S660, N435-S660, K436-S660, L437-S660, C438-S660, Q439-S660, F440-S660, S441-S660, P442-S660, V443-S660, Q444-S660, E445-S660, V446-S660, S447-S660, E448-S660, Q449-S660, S450-S660, P451-S660, E452-S660, T453-S660, S454-S660, P455-S660, D456-S660, K457-S660, E458-S660, E459-S660, A460-S660, H461-S660, I462-S660, P463-S660, K464-S660, Q465-S660, P466-S660, Q467-S660, P468-S660, P469-S660, R470-S660, P471-S660, S472-S660, E473-S660, S474-S660, Q475-S660, V476-S660, T477-S660, R478-S660, L479-S660, H480-S660, S481-S660, V482-S660, R483-S660, T484-S660, G485-S660, S486-S660, S487-S660, G488-S660, S489-S660, T490-S660, Q491-S660, R492-S660, P493-S660, F494-S660, F495-S660, S496-S660, P497-S660, L498-S660, H499-S660, R500-S660, S501-S660, G502-S660, S503-S660, V504-S660, E505-S660, D506-S660, N507-S660, Y508-S660, H509-S660, T510-S660, N511-S660, F512-S660, L513-S660, F514-S660, G515-S660, L516-S660, S517-S660, T518-S660, S519-S660, Q520-S660, Q521-S660, H522-S660, L523-S660, T524-S660, K525-S660, S526-S660, A527-S660, G528-S660, L529-S660, G530-S660, L531-S660, K532-S660, G533-S660, W534-S660, H535-S660, S536-S660, D537-S660, I538-S660, L539-S660, A540-S660, P541-S660, Q542-S660, S543-S660, S544-S660, A545-S660, P546-S660, S547-S660, L548-S660, T549-S660, S550-S660, S551-S660, W552-S660, Y553-S660, F554-S660, A555-S660, T556-S660, E557-S660, P558-S660, S559-S660, H560-S660, L561-S660, Y562-S660, S563-S660, A564-S660, S565-S660, A566-S660, I567-S660, Y568-S660, G569-S660, G570-S660, N571-S660, S572-S660, S573-S660, Y574-S660, S575-S660, A576-S660, Y577-S660, S578-S660, C579-S660, G580-S660, Q581-S660, L582-S660, P583-S660, T584-S660, C585-S660, S586-S660, D587-S660, Q588-S660, I589-S660, Y590-S660, S591-S660, V592-S660, R593-S660, R594-S660, R595-S660, Q596-S660, K597-S660, P598-S660, T599-S660, D600-S660, R601-S660, A602-S660, D603-

S660, S604-S660, R605-S660, R606-S660, S607-S660, W608-S660, H609-S660, E610-S660, E611-S660, S612-S660, P613-S660, F614-S660, E615-S660, K616-S660, Q617-S660, F618-S660, K619-S660, R620-S660, R621-S660, S622-S660, C623-S660, Q624-S660, M625-S660, E626-S660, F627-S660, G628-S660, E629-S660, S630-S660, I631-S660, M632-S660, S633-S660, E634-S660, N635-S660, R636-S660, S637-S660, R638-S660, E639-S660, E640-S660, L641-S660, G642-S660, K643-S660, V644-S660, G645-S660, S646-S660, Q647-S660, S648-S660, S649-S660, F650-S660, S651-S660, G652-S660, S653-S660, and/or M654-S660 of SEQ ID NO:114. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these N-terminal mRET31 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In preferred embodiments, the following C-terminal mRET31 deletion polypeptides are encompassed by the present invention: M1-S660, M1-V659, M1-E658, M1-I657, M1-I656, M1-E655, M1-M654, M1-S653, M1-G652, M1-S651, M1-F650, M1-S649, M1-S648, M1-Q647, M1-S646, M1-G645, M1-V644, M1-K643, M1-G642, M1-L641, M1-E640, M1-E639, M1-R638, M1-S637, M1-R636, M1-N635, M1-E634, M1-S633, M1-M632, M1-I631, M1-S630, M1-E629, M1-G628, M1-F627, M1-E626, M1-M625, M1-Q624, M1-C623, M1-S622, M1-R621, M1-R620, M1-K619, M1-F618, M1-Q617, M1-K616, M1-E615, M1-F614, M1-P613, M1-S612, M1-E611, M1-E610, M1-H609, M1-W608, M1-S607, M1-R606, M1-R605, M1-S604, M1-D603, M1-A602, M1-R601, M1-D600, M1-T599, M1-P598, M1-K597, M1-Q596, M1-R595, M1-R594, M1-R593, M1-V592, M1-S591, M1-Y590, M1-I589, M1-Q588, M1-D587, M1-S586, M1-C585, M1-T584, M1-P583, M1-L582, M1-Q581, M1-G580, M1-C579, M1-S578, M1-Y577, M1-A576, M1-S575, M1-Y574, M1-S573, M1-S572, M1-N571, M1-G570, M1-G569, M1-Y568, M1-I567, M1-A566, M1-S565, M1-A564, M1-S563, M1-Y562, M1-L561, M1-H560, M1-S559, M1-P558, M1-E557, M1-T556, M1-A555, M1-F554, M1-Y553, M1-W552, M1-S551, M1-S550, M1-T549, M1-L548, M1-S547, M1-P546, M1-A545, M1-S544, M1-S543, M1-Q542, M1-P541, M1-A540, M1-L539, M1-I538, M1-D537, M1-S536, M1-H535, M1-W534, M1-G533, M1-K532, M1-L531, M1-G530, M1-L529, M1-G528, M1-A527, M1-S526, M1-K525, M1-T524, M1-L523, M1-H522,

M1-Q521, M1-Q520, M1-S519, M1-T518, M1-S517, M1-L516, M1-G515, M1-F514, M1-L513, M1-F512, M1-N511, M1-T510, M1-H509, M1-Y508, M1-N507, M1-D506, M1-E505, M1-V504, M1-S503, M1-G502, M1-S501, M1-R500, M1-H499, M1-L498, M1-P497, M1-S496, M1-F495, M1-F494, M1-P493, M1-R492, M1-Q491, M1-T490, M1-S489, M1-G488, M1-S487, M1-S486, M1-G485, M1-T484, M1-R483, M1-V482, M1-S481, M1-H480, M1-L479, M1-R478, M1-T477, M1-V476, M1-Q475, M1-S474, M1-E473, M1-S472, M1-P471, M1-R470, M1-P469, M1-P468, M1-Q467, M1-P466, M1-Q465, M1-K464, M1-P463, M1-I462, M1-H461, M1-A460, M1-E459, M1-E458, M1-K457, M1-D456, M1-P455, M1-S454, M1-T453, M1-E452, M1-P451, M1-S450, M1-Q449, M1-E448, M1-S447, M1-V446, M1-E445, M1-Q444, M1-V443, M1-P442, M1-S441, M1-F440, M1-Q439, M1-C438, M1-L437, M1-K436, M1-N435, M1-T434, M1-G433, M1-D432, M1-L431, M1-T430, M1-A429, M1-S428, M1-P427, M1-K426, M1-C425, M1-Y424, M1-D423, M1-L422, M1-A421, M1-E420, M1-E419, M1-S418, M1-S417, M1-F416, M1-G415, M1-H414, M1-L413, M1-S412, M1-A411, M1-A410, M1-M409, M1-S408, M1-A407, M1-S406, M1-Y405, M1-S404, M1-V403, M1-S402, M1-K401, M1-I400, M1-D399, M1-L398, M1-S397, M1-F396, M1-S395, M1-R394, M1-K393, M1-L392, M1-K391, M1-T390, M1-S389, M1-D388, M1-E387, M1-L386, M1-K385, M1-E384, M1-S383, M1-S382, M1-L381, M1-Q380, M1-L379, M1-G378, M1-S377, M1-L376, M1-A375, M1-Q374, M1-V373, M1-L372, M1-P371, M1-S370, M1-D369, M1-E368, M1-L367, M1-L366, M1-S365, M1-P364, M1-Q363, M1-L362, M1-R361, M1-P360, M1-V359, M1-S358, M1-A357, M1-P356, M1-H355, M1-V354, M1-L353, M1-R352, M1-Q351, M1-G350, M1-S349, M1-A348, M1-E347, M1-S346, M1-T345, M1-S344, M1-N343, M1-A342, M1-C341, M1-P340, M1-P339, M1-S338, M1-L337, M1-S336, M1-L335, M1-A334, M1-S333, M1-K332, M1-W331, M1-G330, M1-G329, M1-E328, M1-S327, M1-A326, M1-A325, M1-P324, M1-V323, M1-P322, M1-E321, M1-S320, M1-P319, M1-K318, M1-D317, M1-L316, M1-H315, M1-L314, M1-L313, M1-K312, M1-L311, M1-K310, M1-S309, M1-K308, M1-P307, M1-G306, M1-S305, M1-M304, M1-G303, M1-T302, M1-Q301, M1-N300, M1-N299, M1-I298, M1-T297, M1-K296, M1-E295, M1-Y294, M1-D293, M1-M292, M1-L291, M1-Q290, M1-G289, M1-M288, M1-F287, M1-N286, M1-F285, M1-N284, M1-P283, M1-S282, M1-I281, M1-T280, M1-P279, M1-R278, M1-K277, M1-

E276, M1-K275, M1-V274, M1-F273, M1-R272, M1-Y271, M1-A270, M1-E269, M1-D268, M1-L267, M1-S266, M1-M265, M1-D264, M1-M263, M1-R262, M1-K261, M1-M260, M1-I259, M1-Y258, M1-A257, M1-I256, M1-A255, M1-I254, M1-T253, M1-A252, M1-S251, M1-R250, M1-S249, M1-I248, M1-G247, M1-A246, M1-L245, M1-C244, M1-H243, M1-I242, M1-L241, M1-V240, M1-C239, M1-G238, M1-N237, M1-S236, M1-A235, M1-K234, M1-A233, M1-K232, M1-E231, M1-I230, M1-F229, M1-D228, M1-V227, M1-S226, M1-K225, M1-D224, M1-L223, M1-W222, M1-P221, M1-L220, M1-I219, M1-K218, M1-E217, M1-C216, M1-F215, M1-S214, M1-D213, M1-N212, M1-V211, M1-P210, M1-V209, M1-R208, M1-L207, M1-F206, M1-H205, M1-S204, M1-E203, M1-P202, M1-I201, M1-F200, M1-D199, M1-P198, M1-K197, M1-P196, M1-C195, M1-T194, M1-N193, M1-S192, M1-A191, M1-N190, M1-L189, M1-V188, M1-Y187, M1-G186, M1-I185, M1-G184, M1-N183, M1-Q182, M1-Q181, M1-M180, M1-L179, M1-D178, M1-K177, M1-N176, M1-L175, M1-V174, M1-D173, M1-R172, M1-Q171, M1-C170, M1-G169, M1-L168, M1-Y167, M1-L166, M1-N165, M1-P164, M1-L163, M1-I162, M1-R161, M1-T160, M1-P159, M1-G158, M1-I157, M1-N156, M1-A155, M1-V154, M1-P153, M1-L152, M1-C151, M1-P150, M1-Q149, M1-S148, M1-I147, M1-C146, M1-T145, M1-P144, M1-V143, M1-L142, M1-T141, M1-S140, M1-K139, M1-G138, M1-E137, M1-C136, M1-L135, M1-G134, M1-P133, M1-F132, M1-C131, M1-R130, M1-S129, M1-F128, M1-E127, M1-A126, M1-F125, M1-G124, M1-G123, M1-A122, M1-L121, M1-L120, M1-H119, M1-V118, M1-S117, M1-N116, M1-F115, M1-S114, M1-R113, M1-E112, M1-L111, M1-K110, M1-G109, M1-L108, M1-L107, M1-V106, M1-T105, M1-L104, M1-F103, M1-C102, M1-D101, M1-S100, M1-S99, M1-L98, M1-S97, M1-G96, M1-V95, M1-D94, M1-Q93, M1-S92, M1-S91, M1-H90, M1-D89, M1-Y88, M1-V87, M1-V86, M1-V85, M1-R84, M1-Q83, M1-N82, M1-C81, M1-D80, M1-I79, M1-D78, M1-V77, M1-K76, M1-H75, M1-K74, M1-T73, M1-S72, M1-Q71, M1-H70, M1-I69, M1-L68, M1-E67, M1-T66, M1-I65, M1-L64, M1-V63, M1-K62, M1-D61, M1-Q60, M1-Q59, M1-L58, M1-R57, M1-R56, M1-K55, M1-M54, M1-L53, M1-K52, M1-S51, M1-C50, M1-N49, M1-I48, M1-N47, M1-I46, M1-A45, M1-E44, M1-L43, M1-I42, M1-H41, M1-S40, M1-T39, M1-N38, M1-Y37, M1-E36, M1-V35, M1-F34, M1-P33, M1-R32, M1-S31, M1-D30, M1-I29, M1-L28, M1-L27, M1-V26, M1-K25, M1-E24, M1-T23, M1-G22, M1-S21,

M1-E20, M1-L19, M1-L18, M1-A17, M1-V16, M1-L15, M1-S14, M1-E13, M1-T12, M1-V11, M1-I10, M1-Q9, M1-T8, and/or M1-G7 of SEQ ID NO:114. Polynucleotide sequences encoding these polypeptides are also provided. The present invention also encompasses the use of these C-terminal mRET31 deletion polypeptides as immunogenic and/or antigenic epitopes as described elsewhere herein.

In confirmation of the mouse RET31 representing a novel mouse phosphatase polypeptide, the mRET31 polypeptide has been shown to comprise a dual specificity phosphatase catalytic domain as identified by the BLAST2 algorithm using the DSPc PFAM HMM (PF00782) as a query sequence.

In preferred embodiments, the following mouse RET31 DSPc domain polypeptide is encompassed by the present invention: GPTRILPNLYLGCQRDVLNKDLMQQNGIGYVLNASNTCPKPDFIPESHFLRVP VNDSFCEKILPWLDKSVDFIEKAKASNGCVLIHCLAGISRSATIAIAYIMKRMD MSLDEAYRFVKEKRPTISPNFNFMGQLMDYEKT (SEQ ID NO:135). Polynucleotides encoding this polypeptide are also provided. The present invention also encompasses the use of this mouse RET31 DSPc domain polypeptide as an immunogenic and/or antigenic epitope as described elsewhere herein.

The present invention encompasses the use of RET31 inhibitors and/or activators of RET31 activity for the treatment, detectoin, amelioaration, or prevention of phosphatase associated disorders, including but not limited to metabolic diseases such as diabetes, in addition to neural and/or cardiovascular diseases and disorders. The present invention also encompasses the use of RET31 inhibitors and/or activators of RET31 activity as immunosuppressive agents, anti-inflammatory agents, and/or anti-tumor agents

The present invention encompasses the use of RET31 phosphatase inhibitors, including, antagonists such as antisense nucleic acids, in addition to other antagonists, as described herein, in a therapeutic regimen to diagnose, prognose, treat, ameliorate, and/or prevent diseases where a kinase activity is insufficient. One, non-limiting example of a disease which may occur due to insufficient kinase activity are certain types of diabetes, where one or more kinases involved in the insulin receptor signal pathway may have insufficient activity or insufficient expression, for example.

Moreover, the present invention encompasses the use of RET31 phosphatase activators, and/or the use of the RET31 phosphatase gene or protein in a gene therapy regimen, as described herein, for the diagnoses, prognoses, treatment, amelioration, and/or prevention of diseases and/or disorders where a kinase activity is overly high, such as a cancer where a kinase oncogene product has excessive activity or excessive expression.

The present invention also encompasses the use of catalytically inactive variants of RET31 proteins, including fragments thereof, such as a protein therapeutic, or the use of the encoding polynucleotide sequence or as gene therapy, for example, in the diagnoses, prognosis, treatment, amelioration, and/or prevention of diseases or disorders where phosphatase activity is overly high.

The present invention encompasses the use of antibodies directed against the RET31 polypeptides, including fragment and/or variants thereof, of the present invention in diagnostics, as a biomarkers, and/or as a therapeutic agents.

The present invention encompasses the use of an inactive, non-catalytic, mutant of the RET31 phosphatase as a substrate trapping mutant to bind cellular phosphoproteins or a library of phosphopeptides to identify substrates of the RET31 polypeptides.

The present invention encompasses the use of the RET31 polypeptides, to identify inhibitors or activators of the RET31 phosphatase activity using either in vitro or 'virtual' (in silico) screening methods.

One embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of the RET31 phosphatase comprising the steps of: i.) contacting a RET31 phosphatase inhibitor or activator labeled with an analytically detectable reagent with the RET31 phosphatase under conditions sufficient to form a complex with the inhibitor or activator; ii.) contacting said complex with a sample containing a compound to be identified; iii) and identifying the compound as an inhibitor or activator by detecting the ability of the test compound to alter the amount of labeled known RET31 phosphatase inhibitor or activator in the complex.

Another embodiment of the invention relates to a method for identifying a compound as an activator or inhibitor of a RET31 phosphatase comprising the steps

of: i.) contacting the RET31 phosphatase with a compound to be identified; and ii.) and measuring the ability of the RET31 phosphatase to remove phosphate from a substrate.

The present invention also encomposses a method for identifying a ligand for the RET31 phosphatase comprising the steps of: i.) contacting the RET31 phosphatase with a series of compounds under conditions to permit binding; and ii.) detecting the presence of any ligand-bound protein.

Preferably, the above referenced methods comprise the RET31 phosphatase in a form selected from the group consisting of whole cells, cytosolic cell fractions, membrane cell fractions, purified or partially purified forms. The invention also relates to recombinantly expressed RET31 phosphatase in a purified, substantially purified, or unpurified state. The invention further relates to RET31 phosphatase fused or conjugated to a protein, peptide, or other molecule or compound known in the art, or referenced herein.

The present invention also encompasses pharmaceutical composition of the RET31 phosphatase polypeptide comprising a compound identified by above referenced methods and a pharmaceutically acceptable carrier.

In preferred embodiments, the present invention encompasses a polynucleotide lacking the initiating start codon, in addition to, the resulting encoded polypeptide of RET31. Specifically, the present invention encompasses the polynucleotide corresponding to nucleotides 541 thru 2532 of SEQ ID NO:108, and the polypeptide corresponding to amino acids 2 thru 665 of SEQ ID NO:109. Also encompassed are recombinant vectors comprising said encoding sequence, and host cells comprising said vector.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO: 108 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides consisting of a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 5436 of SEQ ID NO:108,

b is an integer between 15 to 5450, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:108, and where b is greater than or equal to a+14.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO: 113 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides consisting of a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2742 of SEQ ID NO:113, b is an integer between 15 to 2756, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:113, and where b is greater than or equal to a+14.

Table I

Gene No.	CDNA CloneID	ATCC Deposit No. Z and Date	Vector	NT SEQ ID. No. X	Total NT Seq of Clone	5' NT of Start Codon of ORF	3' NT of ORF	AA Seq ID No. Y	Total AA of ORF
1.	BMY_HPP1_ FL	Xxxxxx Xx/xx/xx		149	4393	628	2448	150	607
1.	BMY_HPP1 – Fragment A	Xxxxxx Xx/xx/xx		1	144	1	144	2	48
1.	BMY_HPP1 – Fragment B	Xxxxxx Xx/xx/xx		3	33	1	33	4	11
2.	BMY_HPP2_ FL	Xxxxxx Xx/xx/xx		151	878	89	538	152	150
2.	BMY_HPP2_ partial	Xxxxxx Xx/xx/xx		5	746	2	745	6	248
3.	BMY_HPP3	Xxxxxx Xx/xx/xx		7	511	1	510	8	170
4.	BMY_HPP4	Xxxxxx Xx/xx/xx		9	1710	1	1710	10	570
5.	BMY_HPP5 (7IC-5-E2)	PTA-2966 01/24/01	pSport	41	5111	470	2464	42	665
6.	RET31 (also referred to as as 1hrTNF031, and/or Clone 31	PTA-3434 06/07/01	PTAdv	108	5450	538	2532	109	665

Table I summarizes the information corresponding to each "Gene No." described above. The nucleotide sequence identified as "NT SEQ ID NO:X" was assembled from partially homologous ("overlapping") sequences obtained from the "cDNA clone ID" identified in Table I and, in some cases, from additional related DNA clones. The overlapping sequences were assembled into a single contiguous sequence of high redundancy (usually several overlapping sequences at each nucleotide position), resulting in a final sequence identified as SEQ ID NO:X.

The cDNA Clone ID was deposited on the date and given the corresponding deposit number listed in "ATCC Deposit No:Z and Date." "Vector" refers to the type of vector contained in the cDNA Clone ID.

"Total NT Seq. Of Clone" refers to the total number of nucleotides in the clone contig identified by "Gene No." The deposited clone may contain all or most of the sequence of SEQ ID NO:X. The nucleotide position of SEQ ID NO:X of the putative start codon (methionine) is identified as "5' NT of Start Codon of ORF."

The translated amino acid sequence, beginning with the methionine, is identified as "AA SEQ ID NO:Y," although other reading frames can also be easily translated using known molecular biology techniques. The polypeptides produced by these alternative open reading frames are specifically contemplated by the present invention.

The total number of amino acids within the open reading frame of SEQ ID NO:Y is identified as "Total AA of ORF".

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing) are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further herein. For instance, SEQ ID NO:X is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used, for example, to generate antibodies which bind specifically to

proteins containing the polypeptides and the proteins encoded by the cDNA clones identified in Table I.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides may cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X and the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing a cDNA of the invention deposited with the ATCC, as set forth in Table I. The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited cDNA, collecting the protein, and determining its sequence.

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or the deposited clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are species homologs, allelic variants, and/or orthologs. The skilled artisan could, using procedures well-known in the art, obtain the polynucleotide sequence corresponding to full-length genes (including, but not limited to the full-length coding region), allelic variants, splice variants, orthologs,

and/or species homologues of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, or a deposited clone, relying on the sequence from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologues may be isolated and identified by making suitable probes or primers which correspond to the 5', 3', or internal regions of the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the protein, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using protocols described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the full-length form of the protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the sequence identified as SEQ ID NO:X, and/or a cDNA provided in ATCC Deposit No. Z:. The present invention also provides a polypeptide comprising, or alternatively consisting of, the sequence identified as SEQ ID NO:Y, and/or a polypeptide encoded by the cDNA provided in ATCC Deposit NO:Z. The present invention also provides polynucleotides encoding a polypeptide comprising, or

alternatively consisting of the polypeptide sequence of SEQ ID NO:Y, and/or a polypeptide sequence encoded by the cDNA contained in ATCC Deposit No:Z.

Preferably, the present invention is directed to a polynucleotide comprising, or alternatively consisting of, the sequence identified as SEQ ID NO:X, and/or a cDNA provided in ATCC Deposit No.: that is less than, or equal to, a polynucleotide sequence that is 5 mega basepairs, 1 mega basepairs, 0.5 mega basepairs, 0.1 mega basepairs, 50,000 basepairs, 20,000 basepairs, or 10,000 basepairs in length.

The present invention encompasses polynucleotides with sequences complementary to those of the polynucleotides of the present invention disclosed herein. Such sequences may be complementary to the sequence disclosed as SEQ ID NO:X, the sequence contained in a deposit, and/or the nucleic acid sequence encoding the sequence disclosed as SEQ ID NO:Y.

The present invention also encompasses polynucleotides capable of hybridizing, preferably under reduced stringency conditions, more preferably under stringent conditions, and most preferably under highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in Table II below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

TABLE II

Stringency Condition	Polynucleotide Hybrid±	Hybrid Length (bp) ‡	Hybridization Temperature and Buffer†	Wash Temperature and Buffer †
A	DNA:DNA	> or equal to 50	65°C; 1xSSC – or- 42°C; 1xSSC, 50% formamide	65°C; 0.3xSSC
B	DNA:DNA	< 50	Tb*; 1xSSC	Tb*; 1xSSC
C	DNA:RNA	> or equal to 50	67°C; 1xSSC – or- 45°C; 1xSSC, 50% formamide	67°C; 0.3xSSC
D	DNA:RNA	< 50	Td*; 1xSSC	Td*; 1xSSC
E	RNA:RNA	> or equal to 50	70°C; 1xSSC – or- 50°C; 1xSSC, 50% formamide	70°C; 0.3xSSC
F	RNA:RNA	< 50	Tf*; 1xSSC	Tf*; 1xSSC
G	DNA:DNA	> or equal to 50	65°C; 4xSSC – or- 45°C; 4xSSC, 50% formamide	65°C; 1xSSC
H	DNA:DNA	< 50	Th*; 4xSSC	Th*; 4xSSC
I	DNA:RNA	> or equal to 50	67°C; 4xSSC – or- 45°C; 4xSSC, 50% formamide	67°C; 1xSSC
J	DNA:RNA	< 50	Tj*; 4xSSC	Tj*; 4xSSC

Stringency Condition	Polynucleotide Hybrid±	Hybrid Length (bp) ‡	Hybridization Temperature and Buffer†	Wash Temperature and Buffer †
K	RNA:RNA	> or equal to 50	70°C; 4xSSC – or- 40°C; 6xSSC,, 50% formamide	67°C; 1xSSC
L	RNA:RNA	< 50	Tl*; 2xSSC	Tl*; 2xSSC
M	DNA:DNA	> or equal to 50	50°C; 4xSSC – or- 40°C 6xSSC, 50% formamide	50°C; 2xSSC
N	DNA:DNA	< 50	Tn*; 6xSSC	Tn*; 6xSSC
O	DNA:RNA	> or equal to 50	55°C; 4xSSC – or- 42°C; 6xSSC, 50% formamide	55°C; 2xSSC
P	DNA:RNA	< 50	Tp*; 6xSSC	Tp*; 6xSSC
Q	RNA:RNA	> or equal to 50	60°C; 4xSSC – or- 45°C; 6xSSC, 50% formamide	60°C; 2xSSC
R	RNA:RNA	< 50	Tr*; 4xSSC	Tr*; 4xSSC

‡: The "hybrid length" is the anticipated length for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide of unknown sequence, the hybrid is assumed to be that of the hybridizing polynucleotide of the present invention. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity. Methods of aligning two or more polynucleotide sequences and/or determining the percent

identity between two polynucleotide sequences are well known in the art (e.g., MegAlign program of the DNA*Star suite of programs, etc).

†: SSPE (1xSSPE is 0.15M NaCl, 10mM NaH2PO4, and 1.25mM EDTA, pH 7.4) can be substituted for SSC (1xSSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete. The hydridizations and washes may additionally include 5X Denhardt's reagent, .5-1.0% SDS, 100ug/ml denatured, fragmented salmon sperm DNA, 0.5% sodium pyrophosphate, and up to 50% formamide.

*Tb – Tr: The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5-10°C less than the melting temperature Tm of the hybrids there Tm is determined according to the following equations. For hybrids less than 18 base pairs in length, Tm(°C) = 2(# of A + T bases) + 4(# of G + C bases). For hybrids between 18 and 49 base pairs in length, Tm(°C) = 81.5 +16.6($\log_{10}$[Na+]) + 0.41(%G+C) – (600/N), where N is the number of bases in the hybrid, and [Na+] is the concentration of sodium ions in the hybridization buffer ([NA+] for 1xSSC = .165 M).

±: The present invention encompasses the substitution of any one, or more DNA or RNA hybrid partners with either a PNA, or a modified polynucleotide. Such modified polynucleotides are known in the art and are more particularly described elsewhere herein.

Additional examples of stringency conditions for polynucleotide hybridization are provided, for example, in Sambrook, J., E.F. Fritsch, and T.Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and Current Protocols in Molecular Biology, 1995, F.M., Ausubel et al., eds, John Wiley and Sons, Inc., sections 2.10 and 6.3-6.4, which are hereby incorporated by reference herein.

Preferably, such hybridizing polynucleotides have at least 70% sequence identity (more preferably, at least 80% identity; and most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which they hybridize, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity

while minimizing sequence gaps. The determination of identity is well known in the art, and discussed more specifically elsewhere herein.

The invention encompasses the application of PCR methodology to the polynucleotide sequences of the present invention, the clone deposited with the ATCC, and/or the cDNA encoding the polypeptides of the present invention. PCR techniques for the amplification of nucleic acids are described in US Patent No. 4, 683, 195 and Saiki et al., Science, 239:487-491 (1988). PCR, for example, may include the following steps, of denaturation of template nucleic acid (if double-stranded), annealing of primer to target, and polymerization. The nucleic acid probed or used as a template in the amplification reaction may be genomic DNA, cDNA, RNA, or a PNA. PCR may be used to amplify specific sequences from genomic DNA, specific RNA sequence, and/or cDNA transcribed from mRNA. References for the general use of PCR techniques, including specific method parameters, include Mullis et al., Cold Spring Harbor Symp. Quant. Biol., 51:263, (1987), Ehrlich (ed), PCR Technology, Stockton Press, NY, 1989; Ehrlich et al., Science, 252:1643-1650, (1991); and "PCR Protocols, A Guide to Methods and Applications", Eds., Innis et al., Academic Press, New York, (1990).

Signal Sequences

The present invention also encompasses mature forms of the polypeptide comprising, or alternatively consisting of, the polypeptide sequence of SEQ ID NO:Y, the polypeptide encoded by the polynucleotide described as SEQ ID NO:X, and/or the polypeptide sequence encoded by a cDNA in the deposited clone. The present invention also encompasses polynucleotides encoding mature forms of the present invention, such as, for example the polynucleotide sequence of SEQ ID NO:X, and/or the polynucleotide sequence provided in a cDNA of the deposited clone.

According to the signal hypothesis, proteins secreted by eukaryotic cells have a signal or secretary leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most eukaryotic cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known

that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, Virus Res. 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, Nucleic Acids Res. 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, supra.) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

The established method for identifying the location of signal sequences, in addition, to their cleavage sites has been the SignalP program (v1.1) developed by Henrik Nielsen et al., Protein Engineering 10:1-6 (1997). The program relies upon the algorithm developed by von Heinje, though provides additional parameters to increase the prediction accuracy.

More recently, a hidden Markov model has been developed (H. Neilson, et al., Ismb 1998;6:122-30), which has been incorporated into the more recent SignalP (v2.0). This new method increases the ability to identify the cleavage site by discriminating between signal peptides and uncleaved signal anchors. The present invention encompasses the application of the method disclosed therein to the prediction of the signal peptide location, including the cleavage site, to any of the polypeptide sequences of the present invention.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Accordingly, the polypeptide of the present invention may contain a signal sequence. Polypeptides of the invention which comprise a signal sequence have an N-terminus beginning within 5 residues (i.e., + or - 5 residues, or preferably at the –5, -4, -3, -2, -1, +1, +2, +3, +4, or +5 residue) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein

is not entirely uniform, resulting in more than one secreted species. These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein produced by expression of the polynucleotide sequence of SEQ ID NO:X and/or the polynucleotide sequence contained in the cDNA of a deposited clone, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

The present invention also encompasses variants (e.g., allelic variants, orthologs, etc.) of the polynucleotide sequence disclosed herein in SEQ ID NO:X, the complementary strand thereto, and/or the cDNA sequence contained in the deposited clone.

The present invention also encompasses variants of the polypeptide sequence, and/or fragments therein, disclosed in SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence in SEQ ID NO:X, and/or a polypeptide encoded by a cDNA in the deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a human phosphatase related polypeptide having an amino acid sequence as shown in the sequence listing and described in SEQ ID NO:X or the cDNA contained in ATCC

deposit No:Z; (b) a nucleotide sequence encoding a mature human phosphatase related polypeptide having the amino acid sequence as shown in the sequence listing and described in SEQ ID NO:X or the cDNA contained in ATCC deposit No:Z; (c) a nucleotide sequence encoding a biologically active fragment of a human phosphatase related polypeptide having an amino acid sequence shown in the sequence listing and described in SEQ ID NO:X or the cDNA contained in ATCC deposit No:Z; (d) a nucleotide sequence encoding an antigenic fragment of a human phosphatase related polypeptide having an amino acid sequence sown in the sequence listing and described in SEQ ID NO:X or the cDNA contained in ATCC deposit No:Z; (e) a nucleotide sequence encoding a human phosphatase related polypeptide comprising the complete amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA contained in ATCC deposit No:Z; (f) a nucleotide sequence encoding a mature human phosphatase related polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA contained in ATCC deposit No:Z; (g) a nucleotide sequence encoding a biologically active fragment of a human phosphatase related polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA contained in ATCC deposit No:Z; (h) a nucleotide sequence encoding an antigenic fragment of a human phosphatase related polypeptide having an amino acid sequence encoded by a human cDNA plasmid contained in SEQ ID NO:X or the cDNA contained in ATCC deposit No:Z; (I) a nucleotide sequence complimentary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h), above.

The present invention is also directed to polynucleotide sequences which comprise, or alternatively consist of, a polynucleotide sequence which is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, for example, any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h), above. Polynucleotides encoded by these nucleic acid molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecules which comprise, or alternatively, consist of a polynucleotide which hybridizes under stringent conditions, or alternatively, under lower stringency conditions, to a polynucleotide in (a), (b), (c), (d), (e), (f), (g), or (h), above. Polynucleotides which hybridize to the complement of these nucleic acid molecules under stringent

hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polypeptides.

Another aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively, consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a human phosphatase related polypeptide having an amino acid sequence as shown in the sequence listing and descried in Table I; (b) a nucleotide sequence encoding a mature human phosphatase related polypeptide having the amino acid sequence as shown in the sequence listing and descried in Table I; (c) a nucleotide sequence encoding a biologically active fragment of a human phosphatase related polypeptide having an amino acid sequence as shown in the sequence listing and descried in Table I; (d) a nucleotide sequence encoding an antigenic fragment of a human phosphatase related polypeptide having an amino acid sequence as shown in the sequence listing and described in Table I; (e) a nucleotide sequence encoding a human phosphatase related polypeptide comprising the complete amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table I; (f) a nucleotide sequence encoding a mature human phosphatase related polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table I: (g) a nucleotide sequence encoding a biologically active fragment of a human phosphatase related polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC Deposit and described in Table I; (h) a nucleotide sequence encoding an antigenic fragment of a human phosphatase related polypeptide having an amino acid sequence encoded by a human cDNA in a cDNA plasmid contained in the ATCC deposit and described in Table I; (i) a nucleotide sequence complimentary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h) above.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively, consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, for example, any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), or (h), above.

The present invention encompasses polypeptide sequences which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 98%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, the following non-limited examples, the polypeptide sequence identified as SEQ ID NO:Y, the polypeptide sequence encoded by a cDNA provided in the deposited clone, and/or polypeptide fragments of any of the polypeptides provided herein. Polynucleotides encoded by these nucleic acid molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecules which comprise, or alternatively, consist of a polynucleotide which hybridizes under stringent conditions, or alternatively, under lower stringency conditions, to a polynucleotide in (a), (b), (c), (d), (e), (f), (g), or (h), above. Polynucleotides which hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polypeptides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 98%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a polypeptide sequence encoded by the cDNA in cDNA plasmid:Z, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein). Polynucleotides which hybridize to the complement of the nucleic acid molecules encoding these polypeptides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompasses by the present invention, as are the polypeptides encoded by these polynucleotides.

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the

reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence referenced in Table I, the ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the CLUSTALW computer program (Thompson, J.D., et al., Nucleic Acids Research, 2(22):4673-4680, (1994)), which is based on the algorithm of Higgins, D.G., et al., Computer Applications in the Biosciences (CABIOS), 8(2):189-191, (1992). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. However, the CLUSTALW algorithm automatically converts U's to T's when comparing RNA sequences to DNA sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a CLUSTALW alignment of DNA sequences to calculate percent identity via pairwise alignments are: Matrix=IUB, k-tuple=1, Number of Top Diagonals=5, Gap Penalty=3, Gap Open Penalty 10, Gap Extension Penalty=0.1, Scoring Method=Percent, Window Size=5 or the length of the subject nucleotide sequence, whichever is shorter. For multiple alignments, the following CLUSTALW parameters are preferred: Gap Opening Penalty=10; Gap Extension Parameter=0.05; Gap Separation Penalty Range=8; End Gap Separation Penalty=Off; % Identity for Alignment Delay=40%; Residue Specific Gaps:Off; Hydrophilic Residue Gap=Off; and Transition Weighting=0. The pairwise and multple alignment parameters provided for CLUSTALW above represent the default parameters as provided with the AlignX software program (Vector NTI suite of programs, version 6.0).

The present invention encompasses the application of a manual correction to the percent identity results, in the instance where the subject sequence is shorter than

the query sequence because of 5' or 3' deletions, not because of internal deletions. If only the local pairwise percent identity is required, no manual correction is needed. However, a manual correction may be applied to determine the global percent identity from a global polynucleotide alignment. Percent identity calculations based upon global polynucleotide alignments are often preferred since they reflect the percent identity between the polynucleotide molecules as a whole (i.e., including any polynucleotide overhangs, not just overlapping regions), as opposed to, only local matching polynucleotides. Manual corrections for global percent identity determinations are required since the CLUSTALW program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the CLUSTALW sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above CLUSTALW program using the specified parameters, to arrive at a final percent identity score. This corrected score may be used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the CLUSTALW alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the CLUSTALW alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the CLUSTALW program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity

calculated by CLUSTALW is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are required for the purposes of the present invention.

In addition to the above method of aligning two or more polynucleotide or polypeptide sequences to arrive at a percent identity value for the aligned sequences, it may be desirable in some circumstances to use a modified version of the CLUSTALW algorithm which takes into account known structural features of the sequences to be aligned, such as for example, the SWISS-PROT designations for each sequence. The result of such a modifed CLUSTALW algorithm may provide a more accurate value of the percent identity for two polynucleotide or polypeptide sequences. Support for such a modified version of CLUSTALW is provided within the CLUSTALW algorithm and would be readily appreciated to one of skill in the art of bioinformatics.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the mRNA to those preferred by a bacterial host such as E. coli).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be

deleted from the N-terminus or C-terminus of the protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem... 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein (Dobeli et al., J. Biotechnology 7:199-216 (1988)).

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem.. 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the protein will likely be retained when less than the majority of the residues of the protein are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Alternatively, such N-terminus or C-terminus deletions of a polypeptide of the present invention may, in fact, result in a significant increase in one or more of the biological activities of the polypeptide(s). For example, biological activity of many polypeptides are governed by the presence of regulatory domains at either one or both termini. Such regulatory domains effectively inhibit the biological activity of such polypeptides in lieu of an activation event (e.g., binding to a cognate ligand or receptor, phosphorylation, proteolytic processing, etc.). Thus, by eliminating the

regulatory domain of a polypeptide, the polypeptide may effectively be rendered biologically active in the absence of an activation event.

Thus, the invention further includes polypeptide variants that show substantial biological activity. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity. For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved.

The invention encompasses polypeptides having a lower degree of identity but having sufficient similarity so as to perform one or more of the same functions performed by the polypeptide of the present invention. Similarity is determined by

conserved amino acid substitution. Such substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of like characteristics (e.g., chemical properties). According to Cunningham et al above, such conservative substitutions are likely to be phenotypically silent. Additional guidance concerning which amino acid changes are likely to be phenotypically silent are found in Bowie et al., Science 247:1306-1310 (1990).

Tolerated conservative amino acid substitutions of the present invention involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

In addition, the present invention also encompasses the conservative substitutions provided in Table VII below.

5

Table VII

For Amino Acid	Code	Replace with any of:
Alanine	A	D-Ala, Gly, beta-Ala, L-Cys, D-Cys
Arginine	R	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Aspartic Acid	D	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr
Glutamine	Q	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	E	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala, Pro, D-Pro, ß-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Met, D-Met
Lysine	K	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	F	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, cis-3,4, or 5-phenylproline
Proline	P	D-Pro, L-1-thioazolidine-4-carboxylic acid, D- or L-1-oxazolidine-4-carboxylic acid
Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Valine	V	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

Aside from the uses described above, such amino acid substitutions may also increase protein or peptide stability. The invention encompasses amino acid substitutions that contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the protein or peptide sequence. Also included are substitutions that include amino acid residues other than naturally occurring L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., ß or γ amino acids.

Both identity and similarity can be readily calculated by reference to the following publications: Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Informatics Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press,New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic

Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991.

In addition, the present invention also encompasses substitution of amino acids based upon the probability of an amino acid substitution resulting in conservation of function. Such probabilities are determined by aligning multiple genes with related function and assessing the relative penalty of each substitution to proper gene function. Such probabilities are often described in a matrix and are used by some algorithms (e.g., BLAST, CLUSTALW, GAP, etc.) in calculating percent similarity wherein similarity refers to the degree by which one amino acid may substitute for another amino acid without lose of function. An example of such a matrix is the PAM250 or BLOSUM62 matrix.

Aside from the canonical chemically conservative substitutions referenced above, the invention also encompasses substitutions which are typically not classified as conservative, but that may be chemically conservative under certain circumstances. Analysis of enzymatic catalysis for proteases, for example, has shown that certain amino acids within the active site of some enzymes may have highly perturbed pKa's due to the unique microenvironment of the active site. Such perturbed pKa's could enable some amino acids to substitute for other amino acids while conserving enzymatic structure and function. Examples of amino acids that are known to have amino acids with perturbed pKa's are the Glu-35 residue of Lysozyme, the Ile-16 residue of Chymotrypsin, the His-159 residue of Papain, etc. The conservation of function relates to either anomalous protonation or anomalous deprotonation of such amino acids, relative to their canonical, non-perturbed pKa. The pKa perturbation may enable these amino acids to actively participate in general acid-base catalysis due to the unique ionization environment within the enzyme active site. Thus, substituting an amino acid capable of serving as either a general acid or general base within the microenvironment of an enzyme active site or cavity, as may be the case, in the same or similar capacity as the wild-type amino acid, would effectively serve as a conservative amino substitution.

Besides conservative amino acid substitution, variants of the present invention include, but are not limited to, the following: (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or

may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

Moreover, the invention further includes polypeptide variants created through the application of molecular evolution ("DNA Shuffling") methodology to the polynucleotide disclosed as SEQ ID NO:X, the sequence of the clone submitted in a deposit, and/or the cDNA encoding the polypeptide disclosed as SEQ ID NO:Y. Such DNA Shuffling technology is known in the art and more particularly described elsewhere herein (e.g., WPC, Stemmer, PNAS, 91:10747, (1994)), and in the Examples provided herein).

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of the present invention having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a peptide or polypeptide to have an amino acid sequence which comprises the amino acid sequence of the present invention, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions,

substitutions, and/or deletions in the amino acid sequence of the present invention or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is directed to polynucleotide fragments of the polynucleotides of the invention, in addition to polypeptides encoded therein by said polynucleotides and/or fragments.

In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by the cDNA in a deposited clone; is a portion of that shown in SEQ ID NO:X or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:Y. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:X. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus, or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 2000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850,

1851-1900, 1901-1950, 1951-2000, or 2001 to the end of SEQ ID NO:X, or the complementary strand thereto, or the cDNA contained in a deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Also encompassed by the present invention are polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions, as are the polypeptides encoded by these polynucleotides.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments include the full-length protein. Further preferred polypeptide fragments include the full-length protein having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of the full-length polypeptide. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the full-length protein. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Also preferred are polypeptide and polynucleotide fragments characterized by structural or functional domains, such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Polypeptide fragments of SEQ ID NO:Y falling within conserved domains are specifically contemplated by the present invention. Moreover, polynucleotides encoding these domains are also contemplated.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

In a preferred embodiment, the functional activity displayed by a polypeptide encoded by a polynucleotide fragment of the invention may be one or more biological activities typically associated with the full-length polypeptide of the invention. Illustrative of these biological activities includes the fragments ability to bind to at least one of the same antibodies which bind to the full-length protein, the fragments ability to interact with at lease one of the same proteins which bind to the full-length, the fragments ability to elicit at least one of the same immune responses as the full-length protein (i.e., to cause the immune system to create antibodies specific to the same epitope, etc.), the fragments ability to bind to at least one of the same polynucleotides as the full-length protein, the fragments ability to bind to a receptor of the full-length protein, the fragments ability to bind to a ligand of the full-length protein, and the fragments ability to multimerize with the full-length protein. However, the skilled artisan would appreciate that some fragments may have biological activities which are desirable and directly inapposite to the biological activity of the full-length protein. The functional activity of polypeptides of the invention, including fragments, variants, derivatives, and analogs thereof can be

determined by numerous methods available to the skilled artisan, some of which are described elsewhere herein.

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC deposit No. Z or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:X or contained in ATCC deposit No. Z under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:1), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free

peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric

polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the

invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, monovalent, bispecific, heteroconjugate, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. Moreover, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules, as well as, antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation of the animal or plant, and may have less non-specific tissue binding than an intact antibody (Wahl et al., J. Nucl. Med.. 24:316-325 (1983)). Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies of the present invention include chimeric, single chain, and humanized antibodies.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody

fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homologue of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least

75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologues of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10-2 M, 10-2 M, 5 X 10-3 M, 10-3 M, 5 X 10-4 M, 10-4 M, 5 X 10-5 M, 10-5 M, 5 X 10-6 M, 10-6M, 5 X 10-7 M, 107 M, 5 X 10-8 M, 10-8 M, 5 X 10-9 M, 10-9 M, 5 X 10-10 M, 10-10 M, 5 X 10-11 M, 10-11 M, 5 X 10-12 M, 10-12 M, 5 X 10-13 M, 10-13 M, 5 X 10-14 M, 10-14 M, 5 X 10-15 M, or 10-15 M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind

an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem... 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al.,

Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionucleotides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art.

The antibodies of the present invention may comprise polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan (Harlow, et al., Antibodies: A Laboratory Manual, (Cold spring Harbor Laboratory Press, 2nd ed. (1988), which is hereby incorporated herein by reference in its entirety). For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. The administration of the polypeptides of the present invention may entail one or more injections of an immunizing agent and, if desired, an adjuvant. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art. For the purposes of the invention, "immunizing agent" may be defined as a polypeptide of the invention, including fragments, variants, and/or derivatives thereof, in addition to fusions with heterologous polypeptides and other forms of the polypeptides described herein.

Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections, though they may also be given intramuscularly, and/or through IV). The immunizing agent may include polypeptides of the present invention or a fusion protein or variants thereof. Depending upon the nature of the polypeptides (i.e., percent hydrophobicity, percent hydrophilicity, stability, net charge, isoelectric point etc.), it may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Such conjugation includes either chemical conjugation by derivitizing active chemical functional groups to both the polypeptide of the present invention and the immunogenic protein such that a covalent bond is formed, or through fusion-protein based methodology, or other methods known to the skilled artisan. Examples of such immunogenic proteins include, but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean

trypsin inhibitor. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and Corynebacterium parvum. Additional examples of adjuvants which may be employed includes the MPL-TDM adjuvant (monophosphoryl lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

The antibodies of the present invention may comprise monoclonal antibodies. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975) and U.S. Pat. No. 4,376,110, by Harlow, et al., Antibodies: A Laboratory Manual, (Cold spring Harbor Laboratory Press, 2nd ed. (1988), by Hammerling, et al., Monoclonal Antibodies and T-Cell Hybridomas (Elsevier, N.Y., (1981)), or other methods known to the artisan. Other examples of methods which may be employed for producing monoclonal antibodies includes, but are not limited to, the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today 4:72; Cole et al., 1983, Proc. Natl. Acad. Sci. USA 80:2026-2030), and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production.

In a hybridoma method, a mouse, a humanized mouse, a mouse with a human immune system, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized in vitro.

The immunizing agent will typically include polypeptides of the present invention or a fusion protein thereof. Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node

cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986), pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. As inferred throughout the specification, human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63).

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the polypeptides of the present invention. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoadsorbant assay (ELISA). Such techniques are known in the art and within the skill of the artisan. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollart, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, supra). Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640. Alternatively, the hybridoma cells may be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-sepharose, hydroxyapatite chromatography, gel exclusion chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The skilled artisan would acknowledge that a variety of methods exist in the art for the production of monoclonal antibodies and thus, the invention is not limited to their sole production in hydridomas. For example, the monoclonal antibodies may be made by recombinant DNA methods, such as those described in US patent No. 4, 816, 567. In this context, the term "monoclonal antibody" refers to an antibody derived from a single eukaryotic, phage, or prokaryotic clone. The DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies, or such chains from human, humanized, or other sources). The hydridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transformed into host cells such as Simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (US Patent No. 4, 816, 567; Morrison et al, supra) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the

variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for preparing monovalent antibodies are well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine techniques known in the art. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples herein. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention.

Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737;

WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988).

For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding

residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332). Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the methods of Winter and co-workers (Jones et al., Nature, 321:522-525 (1986); Reichmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (US Patent No. 4, 816, 567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possible some FR residues are substituted from analogous sites in rodent antibodies.

In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin

(Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature 332:323-329 (1988)l and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety. The techniques of cole et al., and Boerder et al., are also available for the preparation of human monoclonal antibodies (cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Riss, (1985); and Boerner et al., J. Immunol., 147(1):86-95, (1991)).

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of

this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA), Genpharm (San Jose, CA), and Medarex, Inc. (Princeton, NJ) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and creation of an antibody repertoire. This approach is described, for example, in US patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,106, and in the following scientific publications: Marks et al., Biotechnol., 10:779-783 (1992); Lonberg et al., Nature 368:856-859 (1994); Fishwild et al., Nature Biotechnol., 14:845-51 (1996); Neuberger, Nature Biotechnol., 14:826 (1996); Lonberg and Huszer, Intern. Rev. Immunol., 13:65-93 (1995).

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be

used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

The antibodies of the present invention may be bispecific antibodies. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present invention, one of the binding specificities may be directed towards a polypeptide of the present invention, the other may be for any other antigen, and preferably for a cell-surface protein, receptor, receptor subunit, tissue-specific antigen, virally derived protein, virally encoded envelope protein, bacterially derived protein, or bacterial surface protein, etc.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker et al., EMBO J., 10:3655-3659 (1991).

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transformed into a suitable

host organism. For further details of generating bispecific antibodies see, for example Suresh et al., Meth. In Enzym., 121:210 (1986).

Heteroconjugate antibodies are also contemplated by the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (US Patent No. 4, 676, 980), and for the treatment of HIV infection (WO 91/00360; WO 92/20373; and EP03089). It is contemplated that the antibodies may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioester bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in US Patent No. 4,676,980.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is

known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid

substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an

expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the

invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem... 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such

fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-

translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad.

Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal,

chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be

fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58

(1995); Johanson et al., J. Biol. Chem... 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99Tc.

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologues thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, a-interferon, ß-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi et al., Int. Immunol., 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti- angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Uses for Antibodies directed against polypeptides of the invention

The antibodies of the present invention have various utilities. For example, such antibodies may be used in diagnostic assays to detect the presence or quantification of the polypeptides of the invention in a sample. Such a diagnostic assay may be comprised of at least two steps. The first, subjecting a sample with the antibody, wherein the sample is a tissue (e.g., human, animal, etc.), biological fluid (e.g., blood, urine, sputum, semen, amniotic fluid, saliva, etc.), biological extract (e.g., tissue or cellular homogenate, etc.), a protein microchip (e.g., See Arenkov P, et al.,

Anal Biochem., 278(2):123-131 (2000)), or a chromatography column, etc. And a second step involving the quantification of antibody bound to the substrate. Alternatively, the method may additionally involve a first step of attaching the antibody, either covalently, electrostatically, or reversibly, to a solid support, and a second step of subjecting the bound antibody to the sample, as defined above and elsewhere herein.

Various diagnostic assay techniques are known in the art, such as competitive binding assays, direct or indirect sandwich assays and immunoprecipitation assays conducted in either heterogeneous or homogenous phases (Zola, Monoclonal Antibodies: A Manual of Techniques, CRC Press, Inc., (1987), pp147-158). The antibodies used in the diagnostic assays can be labeled with a detectable moiety. The detectable moiety should be capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety may be a radioisotope, such as 2H, 14C, 32P, or 125I, a florescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase, green fluorescent protein, or horseradish peroxidase. Any method known in the art for conjugating the antibody to the detectable moiety may be employed, including those methods described by Hunter et al., Nature, 144:945 (1962); Dafvid et al., Biochem., 13:1014 (1974); Pain et al., J. Immunol. Metho., 40:219(1981); and Nygren, J. Histochem. And Cytochem., 30:407 (1982).

Antibodies directed against the polypeptides of the present invention are useful for the affinity purification of such polypeptides from recombinant cell culture or natural sources. In this process, the antibodies against a particular polypeptide are immobilized on a suitable support, such as a Sephadex resin or filter paper, using methods well known in the art. The immobilized antibody then is contacted with a sample containing the polypeptides to be purified, and thereafter the support is washed with a suitable solvent that will remove substantially all the material in the sample except for the desired polypeptides, which are bound to the immobilized antibody. Finally, the support is washed with another suitable solvent that will release the desired polypeptide from the antibody.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison et al., Cell, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., 3H or 125I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., 3H or 125I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses Of Antibodies

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include,

but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10-2 M, 10-2 M, 5 X 10-3 M, 10-3 M, 5 X 10-4 M, 10-4 M, 5 X 10-5 M, 10-5 M, 5 X 10-6 M, 10-6 M, 5 X 10-7 M, 10-7 M, 5 X 10-8 M, 10-8 M, 5 X 10-9 M, 10-9 M, 5 X 10-10 M, 10-10 M, 5 X 10-11 M, 10-11 M, 5 X 10-12 M, 10-12 M, 5 X 10-13 M, 10- 13 M, 5 X 10-14 M, 10-14 M, 5 X 10-15 M, and 10-15 M.

Antibodies directed against polypeptides of the present invention are useful for inhibiting allergic reactions in animals. For example, by administering a therapeutically acceptable dose of an antibody, or antibodies, of the present invention, or a cocktail of the present antibodies, or in combination with other antibodies of varying sources, the animal may not elicit an allergic response to antigens.

Likewise, one could envision cloning the gene encoding an antibody directed against a polypeptide of the present invention, said polypeptide having the potential to elicit an allergic and/or immune response in an organism, and transforming the organism with said antibody gene such that it is expressed (e.g., constitutively, inducibly, etc.) in the organism. Thus, the organism would effectively become resistant to an allergic response resulting from the ingestion or presence of such an immune/allergic reactive polypeptide. Moreover, such a use of the antibodies of the present invention may have particular utility in preventing and/or ameliorating autoimmune diseases and/or disorders, as such conditions are typically a result of antibodies being directed against endogenous proteins. For example, in the instance where the polypeptide of the present invention is responsible for modulating the immune response to auto-antigens, transforming the organism and/or individual with a construct comprising any of the promoters disclosed herein or otherwise known in the art, in addition, to a polynucleotide encoding the antibody directed against the polypeptide of the present invention could effective inhibit the organisms immune

system from eliciting an immune response to the auto-antigen(s). Detailed descriptions of therapeutic and/or gene therapy applications of the present invention are provided elsewhere herein.

Alternatively, antibodies of the present invention could be produced in a plant (e.g., cloning the gene of the antibody directed against a polypeptide of the present invention, and transforming a plant with a suitable vector comprising said gene for constitutive expression of the antibody within the plant), and the plant subsequently ingested by an animal, thereby conferring temporary immunity to the animal for the specific antigen the antibody is directed towards (See, for example, US Patent Nos. 5,914,123 and 6,034,298).

In another embodiment, antibodies of the present invention, preferably polyclonal antibodies, more preferably monoclonal antibodies, and most preferably single-chain antibodies, can be used as a means of inhibiting gene expression of a particular gene, or genes, in a human, mammal, and/or other organism. See, for example, International Publication Number WO 00/05391, published 2/3/00, to Dow Agrosciences LLC. The application of such methods for the antibodies of the present invention are known in the art, and are more particularly described elsewhere herein.

In yet another embodiment, antibodies of the present invention may be useful for multimerizing the polypeptides of the present invention. For example, certain proteins may confer enhanced biological activity when present in a multimeric state (i.e., such enhanced activity may be due to the increased effective concentration of such proteins whereby more protein is available in a localized location).

Antibody-based Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according

to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue- specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing

them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem... 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143- 155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be

used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or

Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Compositions

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem... 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal,

intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and

Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid

carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those

formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging With Antibodies

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect,

diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal,

preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial

diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control

antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface- bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Fusion Proteins

Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because certain proteins target cellular locations based on trafficking signals, the polypeptides of the present invention can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. Similarly, peptide cleavage sites can be introduced in-between such

peptide moieties, which could additionally be subjected to protease activity to remove said peptide(s) from the protein of the present invention. The addition of peptide moieties, including peptide cleavage sites, to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, polypeptides of the present invention, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of the constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem... 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences (also referred to as "tags"). Due to the availability of antibodies specific to such "tags", purification of the fused polypeptide of the invention, and/or its

identification is significantly facilitated since antibodies specific to the polypeptides of the invention are not required. Such purification may be in the form of an affinity purification whereby an anti-tag antibody or another type of affinity matrix (e.g., anti-tag antibody attached to the matrix of a flow-thru column) that binds to the epitope tag is present. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984)).

The skilled artisan would acknowledge the existence of other "tags" which could be readily substituted for the tags referred to supra for purification and/or identification of polypeptides of the present invention (Jones C., et al., J Chromatogr A. 707(1):3-22 (1995)). For example, the c-myc tag and the 8F9, 3C7, 6E10, G4m B7 and 9E10 antibodies thereto (Evan et al., Molecular and Cellular Biology 5:3610-3616 (1985)); the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky et al., Protein Engineering, 3(6):547-553 (1990), the Flag–peptide – i.e., the octapeptide sequence DYKDDDDK (SEQ ID NO:75), (Hopp et al., Biotech. 6:1204-1210 (1988); the KT3 epitope peptide (Martin et al., Science, 255:192-194 (1992)); a-tubulin epitope peptide (Skinner et al., J. Biol. Chem..., 266:15136-15166, (1991)); the T7 gene 10 protein peptide tag (Lutz-Freyermuth et al., Proc. Natl. Sci. USA, 87:6363-6397 (1990)), the FITC epitope (Zymed, Inc.), the GFP epitope (Zymed, Inc.), and the Rhodamine epitope (Zymed, Inc.).

The present invention also encompasses the attachment of up to nine codons encoding a repeating series of up to nine arginine amino acids to the coding region of a polynucleotide of the present invention. The invention also encompasses chemically derivitizing a polypeptide of the present invention with a repeating series of up to nine arginine amino acids. Such a tag, when attached to a polypeptide, has recently been shown to serve as a universal pass, allowing compounds access to the interior of cells

without additional derivitization or manipulation (Wender, P., et al., unpublished data).

Protein fusions involving polypeptides of the present invention, including fragments and/or variants thereof, can be used for the following, non-limiting examples, subcellular localization of proteins, determination of protein-protein interactions via immunoprecipitation, purification of proteins via affinity chromatography, functional and/or structural characterization of protein. The present invention also encompasses the application of hapten specific antibodies for any of the uses referenced above for epitope fusion proteins. For example, the polypeptides of the present invention could be chemically derivatized to attach hapten molecules (e.g., DNP, (Zymed, Inc.)). Due to the availability of monoclonal antibodies specific to such haptens, the protein could be readily purified using immunoprecipation, for example.

Polypeptides of the present invention, including fragments and/or variants thereof, in addition to, antibodies directed against such polypeptides, fragments, and/or variants, may be fused to any of a number of known, and yet to be determined, toxins, such as ricin, saporin (Mashiba H, et al., Ann. N. Y. Acad. Sci. 1999;886:233-5), or HC toxin (Tonukari NJ, et al., Plant Cell. 2000 Feb;12(2):237-248), for example. Such fusions could be used to deliver the toxins to desired tissues for which a ligand or a protein capable of binding to the polypeptides of the invention exists.

The invention encompasses the fusion of antibodies directed against polypeptides of the present invention, including variants and fragments thereof, to said toxins for delivering the toxin to specific locations in a cell, to specific tissues, and/or to specific species. Such bifunctional antibodies are known in the art, though a review describing additional advantageous fusions, including citations for methods of production, can be found in P.J. Hudson, Curr. Opp. In. Imm. 11:548-557, (1999); this publication, in addition to the references cited therein, are hereby incorporated by reference in their entirety herein. In this context, the term "toxin" may be expanded to include any heterologous protein, a small molecule, radionucleotides, cytotoxic drugs, liposomes, adhesion molecules, glycoproteins, ligands, cell or tissue-specific ligands, enzymes, of bioactive agents, biological response modifiers, anti-fungal agents, hormones, steroids, vitamins, peptides, peptide analogs, anti-allergenic agents, anti-

tubercular agents, anti-viral agents, antibiotics, anti-protozoan agents, chelates, radioactive particles, radioactive ions, X-ray contrast agents, monoclonal antibodies, polyclonal antibodies and genetic material. In view of the present disclosure, one skilled in the art could determine whether any particular "toxin" could be used in the compounds of the present invention. Examples of suitable "toxins" listed above are exemplary only and are not intended to limit the "toxins" that may be used in the present invention.

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one

selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most

preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast Pichia pastoris is used to express the polypeptide of the present invention in a eukaryotic system. Pichia pastoris is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O2. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, Pichia pastoris must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O2. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (AOX1) is highly active. In the presence of methanol, alcohol oxidase produced from the AOX1 gene comprises up to approximately 30% of the total soluble protein in Pichia pastoris. See, Ellis, S.B., et al., Mol. Cell. Biol. 5:1111-21 (1985); Koutz, P.J, et al., Yeast 5:167-77 (1989); Tschopp, J.F., et al., Nucl. Acids Res. 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the AOX1

regulatory sequence is expressed at exceptionally high levels in Pichia yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a Pichea yeast system essentially as described in "Pichia Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a protein of the invention by virtue of the strong AOX1 promoter linked to the Pichia pastoris alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG, as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with the polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411,

published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., Nature, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide sequence of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH4; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and

addition or deletion of an N-terminal methionine residue as a result of prokaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein, the addition of epitope tagged peptide fragments (e.g., FLAG, HA, GST, thioredoxin, maltose binding protein, etc.), attachment of affinity tags such as biotin and/or streptavidin, the covalent attachment of chemical moieties to the amino acid backbone, N- or C-terminal processing of the polypeptides ends (e.g., proteolytic processing), deletion of the N-terminal methionine residue, etc.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent NO: 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The invention further encompasses chemical derivitization of the polypeptides of the present invention, preferably where the chemical is a hydrophilic polymer residue. Exemplary hydrophilic polymers, including derivatives, may be those that include polymers in which the repeating units contain one or more hydroxy groups (polyhydroxy polymers), including, for example, poly(vinyl alcohol); polymers in which the repeating units contain one or more amino groups (polyamine polymers), including, for example, peptides, polypeptides, proteins and lipoproteins, such as albumin and natural lipoproteins; polymers in which the repeating units contain one or more carboxy groups (polycarboxy polymers), including, for example, carboxymethylcellulose, alginic acid and salts thereof, such as sodium and calcium alginate, glycosaminoglycans and salts thereof, including salts of hyaluronic acid, phosphorylated and sulfonated derivatives of carbohydrates, genetic material, such as interleukin-2 and interferon, and phosphorothioate oligomers; and polymers in which the repeating units contain one or more saccharide moieties (polysaccharide polymers), including, for example, carbohydrates.

The molecular weight of the hydrophilic polymers may vary, and is generally about 50 to about 5,000,000, with polymers having a molecular weight of about 100 to about 50,000 being preferred. The polymers may be branched or unbranched. More preferred polymers have a molecular weight of about 150 to about 10,000, with molecular weights of 200 to about 8,000 being even more preferred.

For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

Additional preferred polymers which may be used to derivatize polypeptides of the invention, include, for example, poly(ethylene glycol) (PEG), poly(vinylpyrrolidine), polyoxomers, polysorbate and poly(vinyl alcohol), with PEG polymers being particularly preferred. Preferred among the PEG polymers are PEG polymers having a molecular weight of from about 100 to about 10,000. More preferably, the PEG polymers have a molecular weight of from about 200 to about 8,000, with PEG 2,000, PEG 5,000 and PEG 8,000, which have molecular weights of 2,000, 5,000 and 8,000, respectively, being even more preferred. Other suitable hydrophilic polymers, in addition to those exemplified above, will be readily apparent to one skilled in the art based on the present disclosure. Generally, the polymers used may include polymers that can be attached to the polypeptides of the invention via alkylation or acylation reactions.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free

amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminus) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As with the various polymers exemplified above, it is contemplated that the polymeric residues may contain functional groups in addition, for example, to those typically involved in linking the polymeric residues to the polypeptides of the present invention. Such functionalities include, for example, carboxyl, amine, hydroxy and thiol groups. These functional groups on the polymeric residues can be further reacted, if desired, with materials that are generally reactive with such functional groups and which can assist in targeting specific tissues in the body including, for example, diseased tissue. Exemplary materials which can be reacted with the

additional functional groups include, for example, proteins, including antibodies, carbohydrates, peptides, glycopeptides, glycolipids, lectins, and nucleosides.

In addition to residues of hydrophilic polymers, the chemical used to derivatize the polypeptides of the present invention can be a saccharide residue. Exemplary saccharides which can be derived include, for example, monosaccharides or sugar alcohols, such as erythrose, threose, ribose, arabinose, xylose, lyxose, fructose, sorbitol, mannitol and sedoheptulose, with preferred monosaccharides being fructose, mannose, xylose, arabinose, mannitol and sorbitol; and disaccharides, such as lactose, sucrose, maltose and cellobiose. Other saccharides include, for example, inositol and ganglioside head groups. Other suitable saccharides, in addition to those exemplified above, will be readily apparent to one skilled in the art based on the present disclosure. Generally, saccharides which may be used for derivitization include saccharides that can be attached to the polypeptides of the invention via alkylation or acylation reactions.

Moreover, the invention also encompasses derivitization of the polypeptides of the present invention, for example, with lipids (including cationic, anionic, polymerized, charged, synthetic, saturated, unsaturated, and any combination of the above, etc.). stabilizing agents.

The invention encompasses derivitization of the polypeptides of the present invention, for example, with compounds that may serve a stabilizing function (e.g., to increase the polypeptides half-life in solution, to make the polypeptides more water soluble, to increase the polypeptides hydrophilic or hydrophobic character, etc.). Polymers useful as stabilizing materials may be of natural, semi-synthetic (modified natural) or synthetic origin. Exemplary natural polymers include naturally occurring polysaccharides, such as, for example, arabinans, fructans, fucans, galactans, galacturonans, glucans, mannans, xylans (such as, for example, inulin), levan, fucoidan, carrageenan, galatocarolose, pectic acid, pectins, including amylose, pullulan, glycogen, amylopectin, cellulose, dextran, dextrin, dextrose, glucose, polyglucose, polydextrose, pustulan, chitin, agarose, keratin, chondroitin, dermatan, hyaluronic acid, alginic acid, xanthin gum, starch and various other natural homopolymer or heteropolymers, such as those containing one or more of the following aldoses, ketoses, acids or amines: erythose, threose, ribose, arabinose,

xylose, lyxose, allose, altrose, glucose, dextrose, mannose, gulose, idose, galactose, talose, erythrulose, ribulose, xylulose, psicose, fructose, sorbose, tagatose, mannitol, sorbitol, lactose, sucrose, trehalose, maltose, cellobiose, glycine, serine, threonine, cysteine, tyrosine, asparagine, glutamine, aspartic acid, glutamic acid, lysine, arginine, histidine, glucuronic acid, gluconic acid, glucaric acid, galacturonic acid, mannuronic acid, glucosamine, galactosamine, and neuraminic acid, and naturally occurring derivatives thereof Accordingly, suitable polymers include, for example, proteins, such as albumin, polyalginates, and polylactide-coglycolide polymers. Exemplary semi-synthetic polymers include carboxymethylcellulose, hydroxymethylcellulose, hydroxypropylmethylcellulose, methylcellulose, and methoxycellulose. Exemplary synthetic polymers include polyphosphazenes, hydroxyapatites, fluoroapatite polymers, polyethylenes (such as, for example, polyethylene glycol (including for example, the class of compounds referred to as Pluronics.RTM., commercially available from BASF, Parsippany, N.J.), polyoxyethylene, and polyethylene terephthlate), polypropylenes (such as, for example, polypropylene glycol), polyurethanes (such as, for example, polyvinyl alcohol (PVA), polyvinyl chloride and polyvinylpyrrolidone), polyamides including nylon, polystyrene, polylactic acids, fluorinated hydrocarbon polymers, fluorinated carbon polymers (such as, for example, polytetrafluoroethylene), acrylate, methacrylate, and polymethylmethacrylate, and derivatives thereof. Methods for the preparation of derivatized polypeptides of the invention which employ polymers as stabilizing compounds will be readily apparent to one skilled in the art, in view of the present disclosure, when coupled with information known in the art, such as that described and referred to in Unger, U.S. Pat. No. 5,205,290, the disclosure of which is hereby incorporated by reference herein in its entirety.

Moreover, the invention encompasses additional modifications of the polypeptides of the present invention. Such additional modifications are known in the art, and are specifically provided, in addition to methods of derivitization, etc., in US Patent No. 6,028,066, which is hereby incorporated in its entirety herein.

The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their

preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or encoded by the cDNA contained in a deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these polypeptides as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the

invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in the sequence listing, or contained in the polypeptide encoded by a deposited clone). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein of the invention.

In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in an Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins.

Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the

polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

In addition, the polynucleotide insert of the present invention could be operatively linked to "artificial" or chimeric promoters and transcription factors. Specifically, the artificial promoter could comprise, or alternatively consist, of any combination of cis-acting DNA sequence elements that are recognized by trans-acting transcription factors. Preferably, the cis acting DNA sequence elements and trans-acting transcription factors are operable in mammals. Further, the trans-acting transcription factors of such “artificial” promoters could also be "artificial" or

chimeric in design themselves and could act as activators or repressors to said "artificial" promoter.

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each polynucleotide of the present invention can be used as a chromosome marker.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. Disease mapping data are known in the art. Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the polynucleotide and the corresponding gene between affected and unaffected organisms can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected organisms, but not in normal organisms, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal organisms is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected organisms as compared to unaffected organisms can be assessed using polynucleotides of the present invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an organism and comparing the measured gene expression level with a standard level of polynucleotide expression

level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

By "measuring the expression level of a polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of organisms not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an organism, body fluids, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as the following non-limiting examples, sputum, amniotic fluid, urine, saliva, breast milk, secretions, interstitial fluid, blood, serum, spinal fluid, etc.) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from organisms are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may Preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying

disease loci for many disorders, including proliferative diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm, O. Buchardt, L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the stronger binding characteristics of PNA:DNA hybrids. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T.sub.m) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

In addition to the foregoing, a polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al.,

Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

The present invention encompasses the addition of a nuclear localization signal, operably linked to the 5' end, 3' end, or any location therein, to any of the oligonucleotides, antisense oligonucleotides, triple helix oligonucleotides, ribozymes, PNA oligonucleotides, and/or polynucleotides, of the present invention. See, for example, G. Cutrona, et al., Nat. Biotech., 18:300-303, (2000); which is hereby incorporated herein by reference.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell. In one example, polynucleotide sequences of the present invention may be used to construct chimeric RNA/DNA oligonucleotides corresponding to said sequences, specifically designed to induce host cell mismatch repair mechanisms in an organism upon systemic injection, for example (Bartlett, R.J., et al., Nat. Biotech, 18:615-622 (2000), which is hereby incorporated by reference herein in its entirety). Such RNA/DNA oligonucleotides could be designed to correct genetic defects in certain host strains, and/or to introduce desired

phenotypes in the host (e.g., introduction of a specific polymorphism within an endogenous gene corresponding to a polynucleotide of the present invention that may ameliorate and/or prevent a disease symptom and/or disorder, etc.). Alternatively, the polynucleotide sequence of the present invention may be used to construct duplex oligonucleotides corresponding to said sequence, specifically designed to correct genetic defects in certain host strains, and/or to introduce desired phenotypes into the host (e.g., introduction of a specific polymorphism within an endogenous gene corresponding to a polynucleotide of the present invention that may ameliorate and/or prevent a disease symptom and/or disorder, etc). Such methods of using duplex oligonucleotides are known in the art and are encompassed by the present invention (see EP1007712, which is hereby incorporated by reference herein in its entirety).

The polynucleotides are also useful for identifying organisms from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an organisms genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, organisms can be identified because each organism will have a unique set of DNA sequences. Once an unique ID database is established for an organism, positive identification of that organism, living or dead, can be made from extremely small tissue samples. Similarly, polynucleotides of the present invention can be used as polymorphic markers, in addition to, the identification of transformed or non-transformed cells and/or tissues.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, when presented with tissue of

unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination. Moreover, as mentioned above, such reagents can be used to screen and/or identify transformed and non-transformed cells and/or tissues.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

A polypeptide of the present invention can be used to assay protein levels in a biological sample using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. (Jalkanen, M., et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987).) Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99mTc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying protein levels in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable

radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, 131I, 112In, 99mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously, or intraperitoneally) into the mammal. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).)

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression of a polypeptide of the present invention in cells or body fluid of an individual; (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, polypeptides of the present invention can be used to treat, prevent, and/or diagnose disease. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the

polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat, prevent, and/or diagnose disease. For example, administration of an antibody directed to a polypeptide of the present invention can bind and reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of a polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the invention that operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the invention ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, see Belldegrun et al., J. Natl. Cancer Inst., 85:207-216 (1993); Ferrantini et al., Cancer Research, 53:107-1112 (1993); Ferrantini et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura et al., Cancer Research 50: 5102-5106 (1990); Santodonato, et al., Human Gene Therapy 7:1-10 (1996); Santodonato, et al., Gene Therapy 4:1246-1255 (1997); and Zhang, et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs of the invention used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and

pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of polynucleotide sequence of the invention. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotides of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct of the invention can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells

of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs of the invention are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA , 86:6077-6081 (1989), which is herein incorporated

by reference); and purified transcription factors (Debs et al., J. Biol. Chem..., 265:10189-10192 (1990), which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl. Acad. Sci. USA , 84:7413-7416 (1987), which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication NO: WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., Felgner et al., Proc. Natl. Acad. Sci. USA, 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an

inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology , 101:512-527 (1983), which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca2+-EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta, 394:483 (1975); Wilson et al., Cell , 17:77 (1979)); ether injection (Deamer et al., Biochim. Biophys. Acta, 443:629 (1976); Ostro et al., Biochem. Biophys. Res. Commun., 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA, 76:3348 (1979)); detergent dialysis (Enoch et al., Proc. Natl. Acad. Sci. USA , 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem..., 255:10431 (1980); Szoka et al., Proc. Natl. Acad. Sci. USA , 75:145 (1978); Schaefer-Ridder et al., Science, 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent NO: 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication NO: WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding polypeptides of the invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy , 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding polypeptides of the invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express polypeptides of the invention.

In certain other embodiments, cells are engineered, ex vivo or in vivo, with polynucleotides of the invention contained in an adenovirus vector. Adenovirus can

be manipulated such that it encodes and expresses polypeptides of the invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartzet al., Am. Rev. Respir. Dis., 109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld et al., Science, 252:431-434 (1991); Rosenfeld et al., Cell, 68:143-155 (1992)). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green et al. Proc. Natl. Acad. Sci. USA , 76:6606 (1979)).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel., 3:499-503 (1993); Rosenfeld et al., Cell , 68:143-155 (1992); Engelhardt et al., Human Genet. Ther., 4:759-769 (1993); Yang et al., Nature Genet., 7:362-369 (1994); Wilson et al., Nature , 365:691-692 (1993); and U.S. Patent NO: 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, Curr.

Topics in Microbiol. Immunol., 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct containing polynucleotides of the invention is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct of the invention. These viral particles are then used to transduce eukaryotic cells, either ex vivo or in vivo. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express the desired gene product.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding the polypeptide sequence of interest) via homologous recombination (see, e.g., U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently

complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5′ end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5′ and 3′ ends. Preferably, the 3′ end of the first targeting sequence contains the same restriction enzyme site as the 5′ end of the amplified promoter and the 5′ end of the second targeting sequence contains the same restriction site as the 3′ end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotides encoding polypeptides of the present invention may be administered along with other polynucleotides encoding angiogenic proteins. Angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2 (VEGF-C), VEGF-3 (VEGF-B), epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

Preferably, the polynucleotide encoding a polypeptide of the invention

contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5′ end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers. (Kaneda et al., Science, 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA , 189:11277-11281 (1992), which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian. Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

Biological Activities

The polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used in assays to test for one or more biological activities. If these polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides or polypeptides, or agonists or antagonists could be used to treat the associated disease.

Immune Activity

The polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer or some autoimmune diseases, disorders, and/or conditions, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating, preventing, and/or diagnosing diseases, disorders, and/or conditions of hematopoietic cells. A polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein diseases, disorders, and/or conditions (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, a polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to treat or prevent blood

coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies, arterial thrombosis, venous thrombosis, etc.), blood platelet diseases, disorders, and/or conditions (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. Polynucleotides or polypeptides, or agonists or antagonists of the present invention are may also be useful for the detection, prognosis, treatment, and/or prevention of heart attacks (infarction), strokes, scarring, fibrinolysis, uncontrolled bleeding, uncontrolled coagulation, uncontrolled complement fixation, and/or inflammation.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating, preventing, and/or diagnosing autoimmune diseases, disorders, and/or conditions. Many autoimmune diseases, disorders, and/or conditions result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune diseases, disorders, and/or conditions.

Examples of autoimmune diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed or detected by the present invention include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, and/or

diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

A polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat, prevent, and/or diagnose organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of a polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, the polypeptide or polynucleotide or agonists or antagonist may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat, prevent, and/or diagnose inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

Hyperproliferative Disorders

A polynucleotides or polypeptides, or agonists or antagonists of the invention can be used to treat, prevent, and/or diagnose hyperproliferative diseases, disorders, and/or conditions, including neoplasms. A polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, a polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative diseases, disorders, and/or conditions can be treated, prevented, and/or diagnosed. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating, preventing, and/or diagnosing hyperproliferative diseases, disorders, and/or conditions, such as a chemotherapeutic agent.

Examples of hyperproliferative diseases, disorders, and/or conditions that can be treated, prevented, and/or diagnosed by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative diseases, disorders, and/or conditions can also be treated, prevented, and/or diagnosed by a polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative diseases, disorders, and/or conditions include, but are not limited to: hypergammaglobulinemia, lymphoproliferative diseases, disorders, and/or conditions, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating or preventing cell proliferative diseases, disorders, and/or conditions by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating or preventing cell-proliferative diseases, disorders, and/or conditions in individuals

comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the polynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more Preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature

320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which

involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating, preventing, and/or diagnosing one or more of the described diseases, disorders, and/or conditions. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

In particular, the antibodies, fragments and derivatives of the present invention are useful for treating, preventing, and/or diagnosing a subject having or developing cell proliferative and/or differentiation diseases, disorders, and/or conditions as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors, for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of diseases, disorders, and/or conditions related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5X10-6M, 10-6M, 5X10-7M, 10-7M,

5X10-8M, 10-8M, 5X10-9M, 10-9M, 5X10-10M, 10-10M, 5X10-11M, 10-11M, 5X10-12M, 10-12M, 5X10-13M, 10-13M, 5X10-14M, 10-14M, 5X10-15M, and 10-15M.

Moreover, polypeptides of the present invention may be useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al., Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat. Res. 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem. Biol. Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int. J. Tissue React. 20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present

invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewhere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such therapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodies associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Cardiovascular Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the invention may be used to treat, prevent, and/or diagnose cardiovascular diseases, disorders, and/or conditions, including peripheral artery disease, such as limb ischemia.

Cardiovascular diseases, disorders, and/or conditions include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome,

levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

Cardiovascular diseases, disorders, and/or conditions also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis,

pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular diseases, disorders, and/or conditions, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms, false aneurysms, infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular diseases, disorders, and/or conditions include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases, cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subaraxhnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia,

vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromoboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides of the invention may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides of the invention are described in more detail herein.

Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad et al., Cell 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally

delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of eye diseases, disorders, and/or conditions, and psoriasis. See, e.g., reviews by Moses et al., Biotech. 9:630-634 (1991); Folkman et al., N. Engl. J. Med., 333:1757-1763 (1995); Auerbach et al., J. Microvasc. Res. 29:401-411 (1985); Folkman, Advances in Cancer Research, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, Am. J. Opthalmol. 94:715-743 (1982); and Folkman et al., Science 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, Science 235:442-447 (1987).

The present invention provides for treatment of diseases, disorders, and/or conditions associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman et al., Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)).Thus, the present invention provides a method of treating, preventing, and/or diagnosing an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists may be utilized in a variety of additional methods in order to therapeutically treat or prevent a cancer or tumor. Cancers which may be treated, prevented, and/or diagnosed with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder,

thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non- small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat or prevent cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating, preventing, and/or diagnosing other diseases, disorders, and/or conditions, besides cancers, which involve angiogenesis. These diseases, disorders, and/or conditions include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating, preventing, and/or diagnosing hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating, preventing, and/or diagnosing neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular diseases, disorders, and/or conditions associated with neovascularization which can be treated, prevented, and/or diagnosed with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman et al., Am. J. Ophthal. 85:704-710 (1978) and Gartner et al., Surv. Ophthal. 22:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating or preventing neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of diseases, disorders, and/or conditions can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis

and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for

treating or preventing neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat or prevent early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating or preventing proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating or preventing retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreous injection and/or via intraocular implants.

Additionally, diseases, disorders, and/or conditions which can be treated, prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, diseases, disorders, and/or conditions and/or states, which can be treated, prevented, and/or diagnosed with the polynucleotides, polypeptides, agonists

and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uvietis, delayed wound healing, endometriosis, vascluogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (Helicobacter pylori), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g.,

in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d

group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate;

4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem... 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides and/or antagonists or agonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated, prevented or diagnosed by the polynucleotides or polypeptides, or

agonists or antagonists of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, and/or diagnosed by the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, include AIDS; neurodegenerative diseases, disorders, and/or conditions (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune diseases, disorders, and/or conditions (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g.,

hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote dermal reestablishment subsequent to dermal loss

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are a non-exhaustive list of grafts that polynucleotides or polypeptides, agonists or antagonists of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepidermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, can be used to promote skin strength and to improve the

appearance of aged skin.

It is believed that the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intestine, and large intestine. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may have a cytoprotective effect on the small intestine mucosa. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could also be used to treat gastric and doudenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflamamatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large

intestine, respectively. Thus, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to treat diseases associate with the under expression of the polynucleotides of the invention.

Moreover, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to prevent and heal damage to the lungs due to various pathological states. A growth factor such as the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated, prevented, and/or diagnosed using the polynucleotides or polypeptides, and/or agonists or antagonists of the invention. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

The polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetraholoride and other hepatotoxins known in the art).

In addition, the polynucleotides or polypeptides, and/or agonists or antagonists

of the invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, the polynucleotides or polypeptides, and/or agonists or antagonists of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

Neurological Diseases

Nervous system diseases, disorders, and/or conditions, which can be treated, prevented, and/or diagnosed with the compositions of the invention (e.g., polypeptides, polynucleotides, and/or agonists or antagonists), include, but are not limited to, nervous system injuries, and diseases, disorders, and/or conditions which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated, prevented, and/or diagnosed in a patient (including human and non-human mammalian patients) according to the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6)

lesions associated with nutritional diseases, disorders, and/or conditions, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In a preferred embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral hypoxia. In one aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral ischemia. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with cerebral infarction. In another aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose or prevent neural cell injury associated with a stroke. In a further aspect of this embodiment, the polypeptides, polynucleotides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may

be useful according to the invention: (1) increased survival time of neurons in culture; (2) increased sprouting of neurons in culture or in vivo; (3) increased production of a neuron-associated molecule in culture or in vivo, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction in vivo. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, the method set forth in Arakawa et al. (J. Neurosci. 10:3507-3515 (1990)); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk et al. (Exp. Neurol. 70:65-82 (1980)) or Brown et al. (Ann. Rev. Neurosci. 4:17-42 (1981)); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron diseases, disorders, and/or conditions that may be treated, prevented, and/or diagnosed according to the invention include, but are not limited to, diseases, disorders, and/or conditions such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as diseases, disorders, and/or conditions that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

Infectious Disease

A polypeptide or polynucleotide and/or agonist or antagonist of the present invention can be used to treat, prevent, and/or diagnose infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated, prevented,

and/or diagnosed. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polypeptide or polynucleotide and/or agonist or antagonist of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available

hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi), Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis (e.g., mengitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used

to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, agonists or antagonists of the invention are used to treat, prevent, and/or diagnose: tetanus, Diptheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparium, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used totreat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat, prevent, and/or diagnose malaria.

Preferably, treatment or prevention using a polypeptide or polynucleotide and/or agonist or antagonist of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects,

trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteocarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated, prevented, and/or diagnosed include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using a polynucleotide or polypeptide and/or agonist or antagonist of the present invention to proliferate and differentiate nerve cells. Diseases that could be treated, prevented, and/or diagnosed using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic diseases, disorders, and/or conditions (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stoke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated, prevented, and/or diagnosed using the polynucleotide or polypeptide and/or agonist or antagonist of the present invention.

Chemotaxis

A polynucleotide or polypeptide and/or agonist or antagonist of the present

invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat, prevent, and/or diagnose inflammation, infection, hyperproliferative diseases, disorders, and/or conditions, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat, prevent, and/or diagnose wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat, prevent, and/or diagnose wounds.

It is also contemplated that a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may inhibit chemotactic activity. These molecules could also be used to treat, prevent, and/or diagnose diseases, disorders, and/or conditions. Thus, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors),or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable

of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or E. coli. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which a polypeptide of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the

polypeptide of the present invention, after they have been labeled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of polypeptides of the invention thereby effectively generating agonists and antagonists of polypeptides of the invention. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, S. Trends Biotechnol. 16(2):76-82 (1998); Hansson, L. O., et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides of the invention may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired polynucleotide sequence of the invention molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides of the invention may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments,

etc., of the polypeptides of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptides of the invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and 3[H] thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of 3[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of 3[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured.

Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat, prevent, and/or diagnose disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues. Therefore, the invention includes a method of identifying compounds which bind to the polypeptides of the invention comprising the steps of: (a) incubating a candidate binding compound with the polypeptide; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with the polypeptide, (b) assaying a biological activity, and (b) determining if a biological activity of the polypeptide has been altered.

Also, one could identify molecules bind a polypeptide of the invention experimentally by using the beta-pleated sheet regions contained in the polypeptide sequence of the protein. Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of each beta pleated sheet regions in a disclosed polypeptide sequence. Additional embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, any combination or all of contained in the polypeptide sequences of the invention. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the amino acid sequence of each of the beta pleated sheet regions in one of the polypeptide sequences of the invention. Additional embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, any combination or all of the beta pleated sheet regions in one

of the polypeptide sequences of the invention.

Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, Pseudomonas exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into

a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

The human phosphatase polypeptides and/or peptides of the present invention, or immunogenic fragments or oligopeptides thereof, can be used for screening therapeutic drugs or compounds in a variety of drug screening techniques. The fragment employed in such a screening assay may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The reduction or abolition of activity of the formation of binding complexes between the ion channel protein and the agent being tested can be measured. Thus, the present invention provides a method for screening or assessing a plurality of compounds for their specific binding affinity with a phosphatase polypeptide, or a bindable peptide fragment, of this invention, comprising providing a plurality of compounds, combining the phosphatase polypeptide, or a bindable peptide fragment, with each of a plurality of compounds for a time sufficient to allow binding under suitable

conditions and detecting binding of the phosphatase polypeptide or peptide to each of the plurality of test compounds, thereby identifying the compounds that specifically bind to the phosphatase polypeptide or peptide.

Methods of identifying compounds that modulate the activity of the novel human phosphatase polypeptides and/or peptides are provided by the present invention and comprise combining a potential or candidate compound or drug modulator of phosphatase activity with a phosphatase polypeptide or peptide, for example, the phosphatase amino acid sequence as set forth in SEQ ID NO:42, 109, 150, or 152, and measuring an effect of the candidate compound or drug modulator on the biological activity of the phosphatase polypeptide or peptide. Such measurable effects include, for example, physical binding interaction; the ability to phosphorylate a suitable calpain substrate; effects on native and cloned phosphatase-expressing cell line; and effects of modulators or other phosphatase-mediated physiological measures.

Another method of identifying compounds that modulate the biological activity of the novel phosphatase polypeptides of the present invention comprises combining a potential or candidate compound or drug modulator of a phosphatase activity with a host cell that expresses the phosphatase polypeptide and measuring an effect of the candidate compound or drug modulator on the biological activity of the phosphatase polypeptide. The host cell can also be capable of being induced to express the phosphatase polypeptide, e.g., via inducible expression. Physiological effects of a given modulator candidate on the phosphatase polypeptide can also be measured. Thus, cellular assays for particular phosphatase modulators may be either direct measurement or quantification of the physical biological activity of the phosphatase polypeptide, or they may be measurement or quantification of a physiological effect. Such methods preferably employ a phosphatase polypeptide as described herein, or an overexpressed recombinant phosphatase polypeptide in suitable host cells containing an expression vector as described herein, wherein the phosphatase polypeptide is expressed, overexpressed, or undergoes upregulated expression.

Another aspect of the present invention embraces a method of screening for a compound that is capable of modulating the biological activity of a phosphatase

polypeptide, comprising providing a host cell containing an expression vector harboring a nucleic acid sequence encoding a phosphatase polypeptide, or a functional peptide or portion thereof (e.g., SEQ ID NO:42, 109, 150, or 152); determining the biological activity of the expressed phosphatase polypeptide in the absence of a modulator compound; contacting the cell with the modulator compound and determining the biological activity of the expressed phosphatase polypeptide in the presence of the modulator compound. In such a method, a difference between the activity of the phosphatase polypeptide in the presence of the modulator compound and in the absence of the modulator compound indicates a modulating effect of the compound.

Essentially any chemical compound can be employed as a potential modulator or ligand in the assays according to the present invention. Compounds tested as phosphatase modulators can be any small chemical compound, or biological entity (e.g., protein, sugar, nucleic acid, lipid). Test compounds will typically be small chemical molecules and peptides. Generally, the compounds used as potential modulators can be dissolved in aqueous or organic (e.g., DMSO-based) solutions. The assays are designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source. Assays are typically run in parallel, for example, in microtiter formats on microtiter plates in robotic assays. There are many suppliers of chemical compounds, including Sigma (St. Louis, MO), Aldrich (St. Louis, MO), Sigma-Aldrich (St. Louis, MO), Fluka Chemika-Biochemica Analytika (Buchs, Switzerland), for example. Also, compounds may be synthesized by methods known in the art.

High throughput screening methodologies are particularly envisioned for the detection of modulators of the novel phosphatase polynucleotides and polypeptides described herein. Such high throughput screening methods typically involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic compounds (e.g., ligand or modulator compounds). Such combinatorial chemical libraries or ligand libraries are then screened in one or more assays to identify those library members (e.g., particular chemical species or subclasses) that display a desired characteristic activity. The compounds so identified can serve as

conventional lead compounds, or can themselves be used as potential or actual therapeutics.

A combinatorial chemical library is a collection of diverse chemical compounds generated either by chemical synthesis or biological synthesis, by combining a number of chemical building blocks (i.e., reagents such as amino acids). As an example, a linear combinatorial library, e.g., a polypeptide or peptide library, is formed by combining a set of chemical building blocks in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide or peptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

The preparation and screening of combinatorial chemical libraries is well known to those having skill in the pertinent art. Combinatorial libraries include, without limitation, peptide libraries (e.g. U.S. Patent No. 5,010,175; Furka, 1991, *Int. J. Pept. Prot. Res.*, 37:487-493; and Houghton et al., 1991, *Nature*, 354:84-88). Other chemistries for generating chemical diversity libraries can also be used. Nonlimiting examples of chemical diversity library chemistries include, peptides (PCT Publication No. WO 91/019735), encoded peptides (PCT Publication No. WO 93/20242), random bio-oligomers (PCT Publication No. WO 92/00091), benzodiazepines (U.S. Patent No. 5,288,514), diversomers such as hydantoins, benzodiazepines and dipeptides (Hobbs et al., 1993, *Proc. Natl. Acad. Sci. USA*, 90:6909-6913), vinylogous polypeptides (Hagihara et al., 1992, *J. Amer. Chem. Soc.*, 114:6568), nonpeptidal peptidomimetics with glucose scaffolding (Hirschmann et al., 1992, *J. Amer. Chem. Soc.*, 114:9217-9218), analogous organic synthesis of small compound libraries (Chen et al., 1994, *J. Amer. Chem. Soc.*, 116:2661), oligocarbamates (Cho et al., 1993, *Science*, 261:1303), and/or peptidyl phosphonates (Campbell et al., 1994, *J. Org. Chem.*, 59:658), nucleic acid libraries (see Ausubel, Berger and Sambrook, all supra), peptide nucleic acid libraries (U.S. Patent No. 5,539,083), antibody libraries (e.g., Vaughn et al., 1996, *Nature Biotechnology*, 14(3):309-314) and PCT/US96/10287), carbohydrate libraries (e.g., Liang et al., 1996, *Science*, 274-1520-1522) and U.S. Patent No. 5,593,853), small organic molecule libraries (e.g., benzodiazepines, Baum C&EN, Jan. 18, 1993, page 33; and U.S. Patent No. 5,288,514; isoprenoids, U.S. Patent No. 5,569,588; thiazolidinones and metathiazanones, U.S. Patent No.

5,549,974; pyrrolidines, U.S. Patent Nos. 5,525,735 and 5,519,134; morpholino compounds, U.S. Patent No. 5,506,337; and the like).

Devices for the preparation of combinatorial libraries are commercially available (e.g., 357 MPS, 390 MPS, Advanced Chem Tech, Louisville KY; Symphony, Rainin, Woburn, MA; 433A Applied Biosystems, Foster City, CA; 9050 Plus, Millipore, Bedford, MA). In addition, a large number of combinatorial libraries are commercially available (e.g., ComGenex, Princeton, NJ; Asinex, Moscow, Russia; Tripos, Inc., St. Louis, MO; ChemStar, Ltd., Moscow, Russia; 3D Pharmaceuticals, Exton, PA; Martek Biosciences, Columbia, MD, and the like).

In one embodiment, the invention provides solid phase based *in vitro* assays in a high throughput format, where the cell or tissue expressing an ion channel is attached to a solid phase substrate. In such high throughput assays, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well of a microtiter plate can be used to perform a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 96 modulators. If 1536 well plates are used, then a single plate can easily assay from about 100 to about 1500 different compounds. It is possible to assay several different plates per day; thus, for example, assay screens for up to about 6,000-20,000 different compounds are possible using the described integrated systems.

In another of its aspects, the present invention encompasses screening and small molecule (e.g., drug) detection assays which involve the detection or identification of small molecules that can bind to a given protein, i.e., a phosphatase polypeptide or peptide. Particularly preferred are assays suitable for high throughput screening methodologies.

In such binding-based detection, identification, or screening assays, a functional assay is not typically required. All that is needed is a target protein, preferably substantially purified, and a library or panel of compounds (e.g., ligands, drugs, small molecules) or biological entities to be screened or assayed for binding to the protein target. Preferably, most small molecules that bind to the target protein will

modulate activity in some manner, due to preferential, higher affinity binding to functional areas or sites on the protein.

An example of such an assay is the fluorescence based thermal shift assay (3-Dimensional Pharmaceuticals, Inc., 3DP, Exton, PA) as described in U.S. Patent Nos. 6,020,141 and 6,036,920 to Pantoliano et al.; see also, J. Zimmerman, 2000, *Gen. Eng. News*, 20(8)). The assay allows the detection of small molecules (e.g., drugs, ligands) that bind to expressed, and preferably purified, ion channel polypeptide based on affinity of binding determinations by analyzing thermal unfolding curves of protein-drug or ligand complexes. The drugs or binding molecules determined by this technique can be further assayed, if desired, by methods, such as those described herein, to determine if the molecules affect or modulate function or activity of the target protein.

To purify a phosphatase polypeptide or peptide to measure a biological binding or ligand binding activity, the source may be a whole cell lysate that can be prepared by successive freeze-thaw cycles (e.g., one to three) in the presence of standard protease inhibitors. The phosphatase polypeptide may be partially or completely purified by standard protein purification methods, e.g., affinity chromatography using specific antibody described *infra*, or by ligands specific for an epitope tag engineered into the recombinant phosphatase polypeptide molecule, also as described herein. Binding activity can then be measured as described.

Compounds which are identified according to the methods provided herein, and which modulate or regulate the biological activity or physiology of the phosphatase polypeptides according to the present invention are a preferred embodiment of this invention. It is contemplated that such modulatory compounds may be employed in treatment and therapeutic methods for treating a condition that is mediated by the novel phosphatase polypeptides by administering to an individual in need of such treatment a therapeutically effective amount of the compound identified by the methods described herein.

In addition, the present invention provides methods for treating an individual in need of such treatment for a disease, disorder, or condition that is mediated by the phosphatase polypeptides of the invention, comprising administering to the individual

a therapeutically effective amount of the phosphatase-modulating compound identified by a method provided herein.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained a deposited clone. In one embodiment, antisense sequence is generated internally by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, Neurochem., 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research, 6:3073 (1979); Cooney et al., Science, 241:456 (1988); and Dervan et al., Science, 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5 end and a HindIII site on the 3 end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl2, 10MM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is

designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the invention. Such a vector would contain a sequence encoding the antisense nucleic acid of the invention. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding a polypeptide of the invention, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature, 29:304-310 (1981), the promoter contained in the 3′ long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell, 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A., 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster et al., Nature, 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of interest. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids of the invention, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA sequence of the invention it may contain and still form a stable duplex (or triplex as the case may be).

One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5′ end of the message, e.g., the 5′ untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3′ untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., Nature, 372:333-335 (1994). Thus, oligonucleotides complementary to either the 5′ - or 3′ - non-translated, non-coding regions of a polynucleotide sequence of the invention could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5′ untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5′ -, 3′ - or coding region of mRNA, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556 (1989); Lemaitre et al., Proc. Natl. Acad. Sci., 84:648-652 (1987); PCT Publication NO: WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication NO: WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., BioTechniques, 6:958-976 (1988)) or intercalating agents. (See, e.g., Zon, Pharm. Res., 5:539-549 (1988)). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent,

etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5′-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., Nucl. Acids Res., 15:6625-6641 (1987)). The oligonucleotide is a 2-0-methylribonucleotide (Inoue et al., Nucl. Acids Res., 15:6131-6148 (1987)), or a chimeric RNA-DNA analogue (Inoue et al., FEBS Lett. 215:327-330 (1987)).

Polynucleotides of the invention may be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are

commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (Nucl. Acids Res., 16:3209 (1988)), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., Proc. Natl. Acad. Sci. U.S.A., 85:7448-7451 (1988)), etc.

While antisense nucleotides complementary to the coding region sequence of the invention could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science, 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs corresponding to the polynucleotides of the invention, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5′-UG-3′. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature, 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within each nucleotide sequence disclosed in the sequence listing. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5′ end of the mRNA corresponding to the polynucleotides of the invention; i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the polynucleotides of the invention in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to

destroy endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat, prevent, and/or diagnose the diseases described herein.

Thus, the invention provides a method of treating or preventing diseases, disorders, and/or conditions, including but not limited to the diseases, disorders, and/or conditions listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

Biotic Associations

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase the organisms ability, either directly or indirectly, to initiate and/or maintain biotic associations with other organisms. Such associations may be symbiotic, nonsymbiotic, endosymbiotic, macrosymbiotic, and/or microsymbiotic in nature. In general, a polynucleotide or polypeptide and/or agonist or antagonist of the

present invention may increase the organisms ability to form biotic associations with any member of the fungal, bacterial, lichen, mycorrhizal, cyanobacterial, dinoflaggellate, and/or algal, kingdom, phylums, families, classes, genuses, and/or species.

The mechanism by which a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase the host organisms ability, either directly or indirectly, to initiate and/or maintain biotic associations is variable, though may include, modulating osmolarity to desirable levels for the symbiont, modulating pH to desirable levels for the symbiont, modulating secretions of organic acids, modulating the secretion of specific proteins, phenolic compounds, nutrients, or the increased expression of a protein required for host-biotic organisms interactions (e.g., a receptor, ligand, etc.). Additional mechanisms are known in the art and are encompassed by the invention (see, for example, "Microbial Signalling and Communication", eds., R. England, G. Hobbs, N. Bainton, and D. McL. Roberts, Cambridge University Press, Cambridge, (1999); which is hereby incorporated herein by reference).

In an alternative embodiment, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may decrease the host organisms ability to form biotic associations with another organism, either directly or indirectly. The mechanism by which a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may decrease the host organisms ability, either directly or indirectly, to initiate and/or maintain biotic associations with another organism is variable, though may include, modulating osmolarity to undesirable levels, modulating pH to undesirable levels, modulating secretions of organic acids, modulating the secretion of specific proteins, phenolic compounds, nutrients, or the decreased expression of a protein required for host-biotic organisms interactions (e.g., a receptor, ligand, etc.). Additional mechanisms are known in the art and are encompassed by the invention (see, for example, "Microbial Signalling and Communication", eds., R. England, G. Hobbs, N. Bainton, and D. McL. Roberts, Cambridge University Press, Cambridge, (1999); which is hereby incorporated herein by reference).

The hosts ability to maintain biotic associations with a particular pathogen has significant implications for the overall health and fitness of the host. For example, human hosts have symbiosis with enteric bacteria in their gastrointestinal tracts, particularly in the small and large intestine. In fact, bacteria counts in feces of the distal colon often approach 10^{12} per milliliter of feces. Examples of bowel flora in the gastrointestinal tract are members of the Enterobacteriaceae, Bacteriodes, in addition to a-hemolytic streptococci, E. coli, Bifobacteria, Anaerobic cocci, Eubacteria, Costridia, lactobacilli, and yeasts. Such bacteria, among other things, assist the host in the assimilation of nutrients by breaking down food stuffs not typically broken down by the hosts digestive system, particularly in the hosts bowel. Therefore, increasing the hosts ability to maintain such a biotic association would help assure proper nutrition for the host.

Aberrations in the enteric bacterial population of mammals, particularly humans, has been associated with the following disorders: diarrhea, ileus, chronic inflammatory disease, bowel obstruction, duodenal diverticula, biliary calculous disease, and malnutrition. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention are useful for treating, detecting, diagnosing, prognosing, and/or ameliorating, either directly or indirectly, and of the above mentioned diseases and/or disorders associated with aberrant enteric flora population.

The composition of the intestinal flora, for example, is based upon a variety of factors, which include, but are not limited to, the age, race, diet, malnutrition, gastric acidity, bile salt excretion, gut motility, and immune mechanisms. As a result, the polynucleotides and polypeptides, including agonists, antagonists, and fragments thereof, may modulate the ability of a host to form biotic associations by affecting, directly or indirectly, at least one or more of these factors.

Although the predominate intestinal flora comprises anaerobic organisms, an underlying percentage represents aerobes (e.g., E. coli). This is significant as such aerobes rapidly become the predominate organisms in intraabdominal infections – effectively becoming opportunistic early in infection pathogenesis. As a result, there is an intrinsic need to control aerobe populations, particularly for immune compromised individuals.

In a preferred embodiment, a polynucleotides and polypeptides, including agonists, antagonists, and fragments thereof, are useful for inhibiting biotic associations with specific enteric symbiont organisms in an effort to control the population of such organisms.

Biotic associations occur not only in the gastrointestinal tract, but also on an in the integument. As opposed to the gastrointestinal flora, the cutaneous flora is comprised almost equally with aerobic and anaerobic organisms. Examples of cutaneous flora are members of the gram-positive cocci (e.g., S. aureus, coagulase-negative staphylococci, micrococcus, M.sedentarius), gram-positive bacilli (e.g., Corynebacterium species, C. minutissimum, Brevibacterium species, Propoionibacterium species, P.acnes), gram-negative bacilli (e.g., Acinebacter species), and fungi (Pityrosporum orbiculare). The relatively low number of flora associated with the integument is based upon the inability of many organisms to adhere to the skin. The organisms referenced above have acquired this unique ability. Therefore, the polynucleotides and polypeptides of the present invention may have uses which include modulating the population of the cutaneous flora, either directly or indirectly.

Aberrations in the cutaneous flora are associated with a number of significant diseases and/or disorders, which include, but are not limited to the following: impetigo, ecthyma, blistering distal dactulitis, pustules, folliculitis, cutaneous abscesses, pitted keratolysis, trichomycosis axcillaris, dermatophytosis complex, axillary odor, erthyrasma, cheesy foot odor, acne, tinea versicolor, seborrheic dermititis, and Pityrosporum folliculitis, to name a few. A polynucleotide or polypeptide and/or agonist or antagonist of the present invention are useful for treating, detecting, diagnosing, prognosing, and/or ameliorating, either directly or indirectly, and of the above mentioned diseases and/or disorders associated with aberrant cutaneous flora population.

Additional biotic associations, including diseases and disorders associated with the aberrant growth of such associations, are known in the art and are encompassed by the invention. See, for example, "Infectious Disease", Second Edition, Eds., S.L., Gorbach, J.G., Bartlett, and N.R., Blacklow, W.B. Saunders Company, Philadelphia, (1998); which is hereby incorporated herein by reference).

Pheromones

In another embodiment, a polynucleotide or polypeptide and/or agonist or antagonist of the present invention may increase the organisms ability to synthesize and/or release a pheromone. Such a pheromone may, for example, alter the organisms behavior and/or metabolism.

A polynucleotide or polypeptide and/or agonist or antagonist of the present invention may modulate the biosynthesis and/or release of pheromones, the organisms ability to respond to pheromones (e.g., behaviorally, and/or metabolically), and/or the organisms ability to detect pheromones. Preferably, any of the pheromones, and/or volatiles released from the organism, or induced, by a polynucleotide or polypeptide and/or agonist or antagonist of the invention have behavioral effects the organism.

Other Activities

The polypeptide of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. These polypeptide may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

The polypeptide may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

The polypeptide of the present invention may also be employed stimulate neuronal growth and to treat, prevent, and/or diagnose neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. The polypeptide of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

The polypeptide of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

The polypeptide of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, the polypeptides of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

The polypeptide of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues.

The polypeptide of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

The polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, polypeptides or polynucleotides and/or agonist or antagonists of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, caricadic rhythms, depression (including depressive diseases, disorders, and/or conditions), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to increase the efficacy of a pharmaceutical composition, either directly or indirectly. Such a use may be administered in simultaneous

conjunction with said pharmaceutical, or separately through either the same or different route of administration (e.g., intravenous for the polynucleotide or polypeptide of the present invention, and orally for the pharmaceutical, among others described herein.).

Polypeptide or polynucleotides and/or agonist or antagonists of the present invention may also be used to prepare individuals for extraterrestrial travel, low gravity environments, prolonged exposure to extraterrestrial radiation levels, low oxygen levels, reduction of metabolic activity, exposure to extraterrestrial pathogens, etc. Such a use may be administered either prior to an extraterrestrial event, during an extraterrestrial event, or both. Moreover, such a use may result in a number of beneficial changes in the recipient, such as, for example, any one of the following, non-limiting, effects: an increased level of hematopoietic cells, particularly red blood cells which would aid the recipient in coping with low oxygen levels; an increased level of B-cells, T-cells, antigen presenting cells, and/or macrophages, which would aid the recipient in coping with exposure to extraterrestrial pathogens, for example; a temporary (i.e., reversible) inhibition of hematopoietic cell production which would aid the recipient in coping with exposure to extraterrestrial radiation levels; increase and/or stability of bone mass which would aid the recipient in coping with low gravity environments; and/or decreased metabolism which would effectively facilitate the recipients ability to prolong their extraterrestrial travel by any one of the following, non-limiting means: (i) aid the recipient by decreasing their basal daily energy requirements; (ii) effectively lower the level of oxidative and/or metabolic stress in recipient (i.e., to enable recipient to cope with increased extraterrestial radiation levels by decreasing the level of internal oxidative/metabolic damage acquired during normal basal energy requirements; and/or (iii) enabling recipient to subsist at a lower metabolic temperature (i.e., cryogenic, and/or sub-cryogenic environment).

Other Preferred Embodiments

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table I.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions beginning with the nucleotide at about the position of the "5′ NT of Start Codon of ORF" and ending with the nucleotide at about the position of the "3′ NT of ORF" as defined for SEQ ID NO:X in Table I.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X beginning with the nucleotide at about the position of the "5′ NT of ORF" and ending with the nucleotide at about the position of the "3′ NT of ORF" as defined for SEQ ID NO:X in Table I.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule, wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a cDNA clone identified by a cDNA Clone Identifier in Table I, which DNA molecule is contained in the material deposited with the American Type Culture Collection and given the ATCC Deposit Number shown in Table I for said cDNA Clone Identifier.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of a cDNA clone identified by a cDNA Clone

Identifier in Table I, which DNA molecule is contained in the deposit given the ATCC Deposit Number shown in Table I.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of the complete open reading frame sequence encoded by said cDNA clone.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by said cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by said cDNA clone.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by said cDNA clone.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table I; and a nucleotide sequence encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected

from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table I; and a nucleotide sequence encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

The method for identifying the species, tissue or cell type of a biological sample can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a gene encoding a protein identified in Table I, which method comprises a step of detecting in a biological sample obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table I; and a nucleotide sequence encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

The method for diagnosing a pathological condition can comprise a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise

a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X wherein X is any integer as defined in Table I; and a nucleotide sequence encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table I.

Also preferred is a polypeptide, wherein said sequence of contiguous amino acids is included in the amino acid sequence of SEQ ID NO:Y in the range of positions "Total AA of the Open Reading Frame (ORF)" as set forth for SEQ ID NO:Y in Table I.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of the protein encoded by a cDNA clone

identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of the protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of the protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of the protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table I; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table I; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and

determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table I; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table I; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing a pathological condition associated with an organism with abnormal structure or expression of a gene encoding a protein

identified in Table I, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table I; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table I; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is any integer as defined in Table I; and a complete amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule(s) into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a

method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a protein comprising an amino acid sequence selected from the group consisting of: an amino acid sequence of SEQ ID NO:Y wherein Y is an integer set forth in Table I and said position of the "Total AA of ORF" of SEQ ID NO:Y is defined in Table I; and an amino acid sequence of a protein encoded by a cDNA clone identified by a cDNA Clone Identifier in Table I and contained in the deposit with the ATCC Deposit Number shown for said cDNA clone in Table I. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a protein activity, which method comprises administering to such an individual a pharmaceutical composition comprising an amount of an isolated polypeptide, polynucleotide, or antibody of the claimed invention effective to increase the level of said protein activity in said individual.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

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Examples

Description of the Preferred Embodiments

Example 1 – Method Of Identifying The Novel BMY_HPP Human Phosphatases Of The Present Invention.

Polynucleotide sequences encoding the novel BMY_HPP phosphoprotein phosphatases of the present invention were identified by a combination of the following methods:

Homology-based searches using the TBLASTN program [Altschul, 1997] to compare known phosphoprotein phosphatases with human genomic (gDNA) and EST sequences. EST or gDNA sequences having significant homology to one or more of the known phosphatases listed in Table III (expect score less than or equal to $1x10^{-3}$) were retained for further analysis.

Hidden Markov Model (HMM) searches using PFAM motifs (listed in Table IV) [Bateman, 2000 #9; Sonnhammer, 1997] were used to search human genomic sequence using the Genewise program. EST or gDNA sequences having a significant score (greater than or equal to 10) with any of the following motifs were retained for further analysis.

HMM searches using PFAM motifs (listed in Table IV) were used to search predicted protein sequences identified by GENSCAN analysis of human genomic sequence [Burge, 1997 #10]. gDNA sequences having a significant score (greater than or equal to 10) with any of the following motifs were retained for further analysis.

Table IV: PFAM motifs used to identify phopsphoprotein phosphatases

Motif Name	PFAM Accession No.	Description
DSPc	PF00782	Dual specificity phosphatase, catalytic domain
ST_phosphatase	PF00149	Ser/Thr protein phosphatase
Y_phosphatase	PF00102	Protein-tyrosine phosphatase

Once a bacterial artificial chromosomes (BACs) encoding a novel phosphoprotein phosphatase was identified by any one of the methods above, additional potential exons were identified using GENSCAN analysis of all nearby BACs (identified by the Golden Path tiling map, UCSC). Intron/exon boundaries,

transcript cDNA sequence and protein sequence were determined using GENSCAN. The predicted protein sequence were used to identify the most closely related known phosphatase using the BLASTP program as described in herein.

In the case of BMY_HPP5, BMY_HPP5 was identified as an Incyte EST (ID 4155374) with homology to known protein phosphatases and significant expression in the central nervous system. The Incyte clone sequence was used to design oligonucleotides for isolation of additional cDNAs. Such cDNAs have been recovered and sequenced and compared to a full-length Incyte template (assembly of EST sequences) (ID1026659.7). The BMY_HPP5 cDNA has significant identity to Incyte 1026659.7 but diverges at the five-prime and three-prime ends, suggesting that it may be an alternatively spliced product of the same gene.

Example 2 - Cloning of the Novel Human BMY_HPP Phosphatases Of The Present Invention.

A variety of methods known in the art may be used for cloning the novel BMY_HPP phosphatases of the present invention. Breifly, using the predicted or observed cDNA sequences for the BMY-HPP genes of the present invention, antisense oligonucleotides with biotin on the 5' end could be designed (the sequences of these oligos are provided in Table VI). These oligos will be used to isolate cDNA clones according to the following procedure:

One microliter (one hundred and fifty nanograms) of a biotinylated oligo is added to six microliters (six micrograms) of a mixture of single-stranded covalently closed circular cDNA libraries (such libraries are commercially available from Life Technologies, Rockville, Maryland, or may be created using routine methods known in the art) and seven microliters of 100% formamide in a 0.5 ml PCR tube. The cDNA libraries used for specific BMY_HPP genes will be determined by the results of the expression patterns as described herein.

The mixture is heated in a thermal cycler to 95° C for 2 mins.

Fourteen microliters of 2X hybridization buffer (50% formamide, 1.5 M NaCl, 0.04 M $NaPO_4$, pH 7.2, 5 mM EDTA, 0.2% SDS) wis added to the heated probe/cDNA library mixture and incubated at 42° C for 26 hours.

Hybrids between the biotinylated oligo and the circular cDNA are isolated by diluting the hybridization mixture to 220 microliters in a solution containing 1 M NaCl, 10 mM Tris-HCl pH 7.5, 1mM EDTA, pH 8.0 and adding 125 microliters of streptavidin magnetic beads. This solution is incubated at 42° C for 60 mins, mixing every 5 mins to resuspend the beads.

The beads are separated from the solution with a magnet and the beads washed three times in 200 microliters of 0.1 X SSPE, 0.1% SDS at 45° C.

The single stranded cDNAs are released from the biotinlyated oligo/streptavidin magnetic bead complex by adding 50 microliters of 0.1 N NaOH and incubating at room temperature for 10 mins.

The cDNAs are precipitated by adding six microliters of 3 M Sodium Acetate , 5 micrograms of glycogen and 120 microliters of 100% ethanol followed by centrifugation.

The cDNAs are resuspended in 12 microliters of TE (10 mM Tris-HCl, pH 8.0), 1mM EDTA, pH 8.0).

The single stranded cDNAs are converted into double stranded molecules in a thermal cycler by mixing 5 microliters of the captured DNA with 1.5 microliters of a standard SP6 primer (homologous to a sequence on the cDNA cloning vector) at 10 micromolar concentration and 1.5 microliters of 10 X PCR buffer. The mixture is heated to 95° C for 20 seconds, then ramped down to 59 ° C. At this time 15 microliters of a repair mix preheated to 70° C is added (repair mix contains 4 microliters of 5 mM dNTPs (1.25 mM each), 1.5 microliters of 10X PCR buffer, 9.25 microliters of water, and 0.25 microliters of Taq polymerase). The solution is ramped back to 73° C and incubated for 23 mins.

The repaired DNA was precipitated as described above and resuspended in 10 microliters of TE.

Two microliters of double-stranded cDNA are used to transform E. coli DH12S cells by electroporation.

The resulting colonies are screened by PCR, using a primer pair designed to identify the proper cDNAs (primer sequences, as provided in Table VI, may be used).

Those cDNA clones that are positive by PCR are then assessed to determine the inserts size. Two clones for each BMY_HPP gene are chosen for DNA sequencing using standard methods known in the art and described herein.

The polynucleotide(s) of the present invention, the polynucleotide encoding the polypeptide of the present invention, or the polypeptide encoded by the deposited clone may represent partial, or incomplete versions of the complete coding region (i.e., full-length gene). Several methods are known in the art for the identification of the 5′ or 3′ non-coding and/or coding portions of a gene which may not be present in the deposited clone. The methods that follow are exemplary and should not be construed as limiting the scope of the invention. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5′ and 3′ "RACE" protocols that are well known in the art. For instance, a method similar to 5′ RACE is available for generating the missing 5′ end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993)).

Briefly, a specific RNA oligonucleotide is ligated to the 5′ ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5′ portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full-length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5′ phosphate groups on degraded or damaged RNA that may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5′ ends of messenger RNAs. This reaction leaves a 5′ phosphate group at the 5′ end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5′ end using a primer specific

to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5′ end sequence belongs to the desired gene. Moreover, it may be advantageous to optimize the RACE protocol to increase the probability of isolating additional 5' or 3' coding or non-coding sequences. Various methods of optimizing a RACE protocol are known in the art, though a detailed description summarizing these methods can be found in B.C. Schaefer, Anal. Biochem., 227:255-273, (1995).

An alternative method for carrying out 5' or 3' RACE for the identification of coding or non-coding sequences is provided by Frohman, M.A., et al., Proc.Nat'l.Acad.Sci.USA, 85:8998-9002 (1988). Briefly, a cDNA clone missing either the 5' or 3' end can be reconstructed to include the absent base pairs extending to the translational start or stop codon, respectively. In some cases, cDNAs are missing the start of translation, therefor. The following briefly describes a modification of this original 5' RACE procedure. Poly A+ or total RNAs reverse transcribed with Superscript II (Gibco/BRL) and an antisense or I complementary primer specific to the cDNA sequence. The primer is removed from the reaction with a Microcon Concentrator (Amicon). The first-strand cDNA is then tailed with dATP and terminal deoxynucleotide transferase (Gibco/BRL). Thus, an anchor sequence is produced which is needed for PCR amplification. The second strand is synthesized from the dA-tail in PCR buffer, Taq DNA polymerase (Perkin-Elmer Cetus), an oligo-dT primer containing three adjacent restriction sites (XhoIJ Sail and ClaI) at the 5' end and a primer containing just these restriction sites. This double-stranded cDNA is PCR amplified for 40 cycles with the same primers as well as a nested cDNA-specific antisense primer. The PCR products are size-separated on an ethidium bromide-agarose gel and the region of gel containing cDNA products the predicted size of missing protein-coding DNA is removed. cDNA is purified from the agarose with the Magic PCR Prep kit (Promega), restriction digested with XhoI or SalI, and ligated to a plasmid such as pBluescript SKII (Stratagene) at XhoI and EcoRV sites. This DNA is transformed into bacteria and the plasmid clones sequenced to identify the correct protein-coding inserts. Correct 5' ends are confirmed by comparing this sequence with the putatively identified homologue and overlap with the partial cDNA clone. Similar

methods known in the art and/or commercial kits are used to amplify and recover 3' ends.

Several quality-controlled kits are commercially available for purchase. Similar reagents and methods to those above are supplied in kit form from Gibco/BRL for both 5' and 3' RACE for recovery of full length genes. A second kit is available from Clontech which is a modification of a related technique, SLIC (single-stranded ligation to single-stranded cDNA), developed by Dumas et al., Nucleic Acids Res., 19:5227-32(1991). The major differences in procedure are that the RNA is alkaline hydrolyzed after reverse transcription and RNA ligase is used to join a restriction site-containing anchor primer to the first-strand cDNA. This obviates the necessity for the dA-tailing reaction which results in a polyT stretch that is difficult to sequence past.

An alternative to generating 5' or 3' cDNA from RNA is to use cDNA library double- stranded DNA. An asymmetric PCR-amplified antisense cDNA strand is synthesized with an antisense cDNA-specific primer and a plasmid-anchored primer. These primers are removed and a symmetric PCR reaction is performed with a nested cDNA-specific antisense primer and the plasmid-anchored primer.

RNA Ligase Protocol For Generating The 5' or 3' End Sequences To Obtain Full Length Genes

Once a gene of interest is identified, several methods are available for the identification of the 5' or 3' portions of the gene which may not be present in the original cDNA plasmid. These methods include, but are not limited to, filter probing, clone enrichment using specific probes and protocols similar and identical to 5' and 3'RACE. While the full-length gene may be present in the library and can be identified by probing, a useful method for generating the 5' or 3' end is to use the existing sequence information from the original cDNA to generate the missing information. A method similar to 5'RACE is available for generating the missing 5' end of a desired full-length gene. (This method was published by Fromont-Racine et al., Nucleic Acids Res., 21(7): 1683-1684 (1993)). Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably 30 containing full-length gene RNA transcript and a primer set containing a primer

specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest, is used to PCR amplify the 5' portion of the desired full length gene which may then be sequenced and used to generate the full length gene. This method starts with total RNA isolated from the desired source, poly A RNA may be used but is not a prerequisite for this procedure. The RNA preparation may then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase if used is then inactivated and the RNA is treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase. This modified RNA preparation can then be used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction can then be used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the relevant family.

Representative primers for cloning any one of the human phosphatases of the present invention are provided in Table VI herein as 'Left Cloning Primer', 'Right Cloning Primer', 'Internal RevComp Cloning Primer', and/or 'Internal Cloning Primer'. Other primers could be subsitituted for any of the above as would be appreciated by one skilled in the art.

In the case of the full-length BMY_HPP1, BMY_HPP1 was cloned using the polynucleotide sequences of the identified BMY_HPP1 fragments BMY_HPP1_A (SEQ ID NO:1) and BMY_HPP1_B (SEQ ID NO:3) to design the following antisense 80 bp oligo with biotin on the 5' end:

Name	Sequence
Phos4-80b	5'bTGACAATGGATAGCTACTTTTCCTTCCTGTAAGGCAAATGTCATC ACCTTCACCATATCTAGGATAGTAGTAAGAGACGC -3 (SEQ ID NO:45)

One microliter (one hundred and fifty nanograms) of the gel-purified biotinylated PCR fragment was added to six microliters (six micrograms) of a single-stranded covalently closed circular brain, fetal brain, bone marrow, prostate, spleen, testis, and thymus cDNA libraries and seven microliters of 100% formamide in a 0.5 ml PCR tube. The mixture was heated in a thermal cycler to 95° C for 2 mins. Fourteen microliters of 2X hybridization buffer (50% formamide, 1.5 M NaCl, 0.04 M $NaPO_4$, pH 7.2, 5 mM EDTA, 0.2% SDS) was added to the heated probe/cDNA library mixture and incubated at 42° C for 26 hours. Hybrids between the biotinylated oligo and the circular cDNA were isolated by diluting the hybridization mixture to 220 microliters in a solution containing 1 M NaCl, 10 mM Tris-HCl pH 7.5, 1mM EDTA, pH 8.0 and adding 125 microliters of streptavidin magnetic beads. This solution was incubated at 42° C for 60 mins, mixing every 5 mins to resuspend the beads. The beads were separated from the solution with a magnet and the beads washed three times in 200 microliters of 0.1 X SSPE, 0.1% SDS at 45° C.

The single stranded cDNAs were released from the biotinlyated probe/streptavidin magnetic bead complex by adding 50 microliters of 0.1 N NaOH and incubating at room temperature for 10 mins. Six microliters of 3 M Sodium Acetate was added along with 15 micrograms of glycogen and the solution ethanol precipitated with 120 microliters of 100% ethanol. The DNA was resuspend in 12 microliters of TE (10 mM Tris-HCl, pH 8.0), 1mM EDTA, pH 8.0). The single stranded cDNA was converted into double strands in a thermal cycler by mixing 5 microliters of the captured DNA with 1.5 microliters 10 micromolar standard SP6 primer (homologous to a sequence on the cDNA cloning vector) and 1.5 microliters of 10 X PCR buffer. The mixture was heated to 95° C for 20 seconds, then ramped down to 59 ° C. At this time 15 microliters of a repair mix, that was preheated to 70° C (Repair mix contains 4 microliters of 5 mM dNTPs (1.25 mM each), 1.5 microliters of 10X PCR buffer, 9.25 microliters of water, and 0.25 microliters of Taq polymerase). The solution was ramped back to 73° C and incubated for 23 mins. The repaired DNA was ethanol precipitated and resuspended in 10 microliters of TE. Two microliters were electroporated in E. coli DH12S cells and resulting colonies were screened by PCR, using the following primer pair number:

Name	Sequence
Phos2-2s	TACAATTTCGGATGGAAGGATTAT (SEQ ID NO:154)
Phos2-2a	GCATGACAATGGATAGCTACTTT (SEQ ID NO:155)

The sequence of the BMY_HPP1 polynucleotide was sequenced and is provided in Figures 20A-D (SEQ ID NO:149).

In the case of the full-length BMY_HPP2, BMY_HPP1 was cloned using the polynucleotide sequences of the identified BMY_HPP2 fragment (SEQ ID NO:5) to design the following antisense 80 bp oligo with biotin on the 5' end:

Name	Sequence
Phos2-80b	5'bGTGCCGCACGCCCAGGTCCAACAGGAACTGGTAGTGGGCGGGG AGCCGCGGCAGCGCCAGTCCCGCCAGCCGGCCCGGA -3 (SEQ ID NO:51)

One microliter (one hundred and fifty nanograms) of the gel-purified biotinylated PCR fragment was added to six microliters (six micrograms) of a single-stranded covalently closed circular brain, fetal brain, bone marrow, prostate, spleen, testis, and thymus cDNA libraries and seven microliters of 100% formamide in a 0.5 ml PCR tube. The mixture was heated in a thermal cycler to 95° C for 2 mins. Fourteen microliters of 2X hybridization buffer (50% formamide, 1.5 M NaCl, 0.04 M $NaPO_4$, pH 7.2, 5 mM EDTA, 0.2% SDS) was added to the heated probe/cDNA library mixture and incubated at 42° C for 26 hours. Hybrids between the biotinylated oligo and the circular cDNA were isolated by diluting the hybridization mixture to 220 microliters in a solution containing 1 M NaCl, 10 mM Tris-HCl pH 7.5, 1mM EDTA, pH 8.0 and adding 125 microliters of streptavidin magnetic beads. This solution was incubated at 42° C for 60 mins, mixing every 5 mins to resuspend the beads. The beads were separated from the solution with a magnet and the beads washed three times in 200 microliters of 0.1 X SSPE, 0.1% SDS at 45° C.

The single stranded cDNAs were released from the biotinlyated probe/streptavidin magnetic bead complex by adding 50 microliters of 0.1 N NaOH and incubating at room temperature for 10 mins. Six microliters of 3 M Sodium Acetate was added along with 15 micrograms of glycogen and the solution ethanol

precipitated with 120 microliters of 100% ethanol. The DNA was resuspend in 12 microliters of TE (10 mM Tris-HCl, pH 8.0), 1mM EDTA, pH 8.0). The single stranded cDNA was converted into double strands in a thermal cycler by mixing 5 microliters of the captured DNA with 1.5 microliters 10 micromolar standard SP6 primer (homologous to a sequence on the cDNA cloning vector) and 1.5 microliters of 10 X PCR buffer. The mixture was heated to 95° C for 20 seconds, then ramped down to 59 ° C. At this time 15 microliters of a repair mix, that was preheated to 70° C (Repair mix contains 4 microliters of 5 mM dNTPs (1.25 mM each), 1.5 microliters of 10X PCR buffer, 9.25 microliters of water, and 0.25 microliters of Taq polymerase). The solution was ramped back to 73° C and incubated for 23 mins. The repaired DNA was ethanol precipitated and resuspended in 10 microliters of TE. Two microliters were electroporated in E. coli DH12S cells and resulting colonies were screened by PCR, using the following primer pair number:

Name	Sequence
Phos2-2s	GAGAAAGCAGTCTTCCAGTTCTAC (SEQ ID NO:156)
Phos2-2a	ATGGGAGCTAGAGGGTTTAATACT (SEQ ID NO:157)

The sequence of the BMY_HPP2 polynucleotide was sequenced and is provided in Figure 21 (SEQ ID NO:151).

In the case of BMY_HPP5, BMY_HPP5 was cloned using the sequence of Incyte clone 4155374 to design the following PCR oligos:

Oligo number	Name	Sequence
686	4155374-C3.s	5'-GGCCAAAGAGCAAACTCAAG-3 (SEQ ID NO:69)
687	4155374-C3.Ba	5'-bGCATAGCTTGTTGGTCCCAT-3 (SEQ ID NO:70)

A biotinylated nucleotide was included on the 5' end of oligo 687. Using the PCR primer pair, a 414bp biotinylated fragment was amplified using the Incyte clone as the template. The fragment was gel purified by agarose electrophoresis and stored at 4° C. The same PCR primer pair was used to screen cDNA libraries for the presence of homologous sequences. Positive PCR results were obtained in our HPLC-size fractionated brain and testis libraries. One microliter (one hundred and fifty nanograms) of the gel-purified biotinylated PCR fragment was added to six

microliters (six micrograms) of a single-stranded covalently closed circular testis cDNA library and seven microliters of 100% formamide in a 0.5 ml PCR tube. The mixture was heated in a thermal cycler to 95° C for 2 mins. Fourteen microliters of 2X hybridization buffer (50% formamide, 1.5 M NaCl, 0.04 M $NaPO_4$, pH 7.2, 5 mM EDTA, 0.2% SDS) was added to the heated probe/cDNA library mixture and incubated at 42° C for 26 hours. Hybrids between the biotinylated oligo and the circular cDNA were isolated by diluting the hybridization mixture to 220 microliters in a solution containing 1 M NaCl, 10 mM Tris-HCl pH 7.5, 1mM EDTA, pH 8.0 and adding 125 microliters of streptavidin magnetic beads. This solution was incubated at 42° C for 60 mins, mixing every 5 mins to resuspend the beads. The beads were separated from the solution with a magnet and the beads washed three times in 200 microliters of 0.1 X SSPE, 0.1% SDS at 45° C.

The single stranded cDNAs were released from the biotinlyated probe/streptavidin magnetic bead complex by adding 50 microliters of 0.1 N NaOH and incubating at room temperature for 10 mins. Six microliters of 3 M Sodium Acetate was added along with 15 micrograms of glycogen and the solution ethanol precipitated with 120 microliters of 100% ethanol. The DNA was resuspend in 12 microliters of TE (10 mM Tris-HCl, pH 8.0), 1mM EDTA, pH 8.0). The single stranded cDNA was converted into double strands in a thermal cycler by mixing 5 microliters of the captured DNA with 1.5 microliters 10 micromolar standard SP6 primer (homologous to a sequence on the cDNA cloning vector) and 1.5 microliters of 10 X PCR buffer. The mixture was heated to 95° C for 20 seconds, then ramped down to 59 ° C. At this time 15 microliters of a repair mix, that was preheated to 70° C (Repair mix contains 4 microliters of 5 mM dNTPs (1.25 mM each), 1.5 microliters of 10X PCR buffer, 9.25 microliters of water, and 0.25 microliters of Taq polymerase). The solution was ramped back to 73° C and incubated for 23 mins. The repaired DNA was ethanol precipitated and resuspended in 10 microliters of TE. Two microliters were electroporated in E. coli DH12S cells and resulting colonies were screened by PCR, using the primer pair number 686/687. The sequence of the BMY_HPP5 polynucleotide was sequenced and is provided in Figures 5A-E (SEQ ID NO:41).

Example 3 – Expression Profiling Of The Novel Human BMY_HPP Phosphatase Polypeptides Of The Present Invention.

PCR primers designed from the predicted or observed cDNA sequences (described elsewhere herein) will be used in a real-time PCR assay to determine relative steady state mRNA expression levels of BMY_HPP1, BMY_HPP2, BMY_HPP3, and BMY_HPP4 across a panel of human tissues according to the following protocol.

First strand cDNA may be synthesized from commercially available mRNA (Clontech) and subjected to real time quantitative PCR using a PE 5700 instrument (Applied Biosystems, Foster City, CA) using the manufacturers recommended protocol. This instrument detects the amount of DNA amplified during each cycle by the fluorescent output of SYBR green, a DNA binding dye specific for double strands. The specificity of the primer pair for its target may be verified by performing a thermal denaturation profile at the end of the run which provides an indication of the number of different DNA sequences present by determining melting Tm. Only primer pairs giving a single PCR product are considered. Contributions of contaminating genomic DNA to the assessment of tissue abundance may be controlled by performing the PCR with first strand made with and without reverse transcriptase. Only samples where the contribution of material amplified in the no reverse transcriptase controls was negligible are considered.

Small variations in the amount of cDNA used in each tube can be determined by performing a parallel experiment using a primer pair for the cyclophilin gene, which is expressed in equal amounts in all tissues. The data is then used to normalize the data obtained with each primer pair. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested.

Representative primers for expression profiling analysis for each gene are provided in Table VI herein as 'EP Sense' and 'EP Anti-sense Primer', though may also include one or more of the following: 'Left Cloning Primer', 'Right Cloning Primer', 'Internal RevComp Cloning Primer', and/or 'Internal Cloning Primer'. Other primers could be subsitituted for any of the above as would be appreciated by one skilled in the art.

In the case of BMY_HPP1, the following PCR primer pair was used to measure the steady state levels of BMY_HPP1 mRNA by quantitative PCR:

Sense: 5'- TACAATTTCGGATGGAAGGATTAT -3' (SEQ ID NO:154)
Antisense: 5'- GCATGACAATGGATAGCTACTTT -3' (SEQ ID NO:155)

Briefly, first strand cDNA was made from commercially available mRNA. The relative amount of cDNA used in each assay was determined by performing a parallel experiment using a primer pair for a gene expressed in equal amounts in all tissues, cyclophilin. The cyclophilin primer pair detected small variations in the amount of cDNA in each sample and these data were used for normalization of the data obtained with the primer pair for the novel BMY_HPP1. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested and the data is presented in Figure 22. Transcripts corresponding to BMY_HPP1 were expressed highly in testis; to a significant extent, in the spinal cord, and to a lesser extent, in pancreas, brain, pituitary, heart, and lung.

In the case of BMY_HPP2, the following PCR primer pair was used to measure the steady state levels of BMY_HPP2 mRNA by quantitative PCR:

Sense: 5'- GAGAAAGCAGTCTTCCAGTTCTAC -3' (SEQ ID NO:156)
Antisense: 5'- ATGGGAGCTAGAGGGTTTAATACT -3' (SEQ ID NO:157)

Briefly, first strand cDNA was made from commercially available mRNA. The relative amount of cDNA used in each assay was determined by performing a parallel experiment using a primer pair for a gene expressed in equal amounts in all tissues, cyclophilin. The cyclophilin primer pair detected small variations in the amount of cDNA in each sample and these data were used for normalization of the data obtained with the primer pair for the novel BMY_HPP2. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested and the data is presented in Figure 23. Transcripts corresponding to BMY_HPP2 were expressed highly in liver and kidney; to a significant extent, in the spleen, and to a lesser extent, in lung, testis, heart, intestine, pancreas, lymph node, spinal cord, and prostate.

In the case of BMY_HPP5, the following PCR primer pair was used to measure the steady state levels of BMY_HPP5 mRNA by quantitative PCR:

Sense: 5'- ATGGGACCAACAAGCTATGC -3' (SEQ ID NO:67)
Antisense: 5'- TTATCAGGACTGGTTTCGGG -3' (SEQ ID NO:68)

Briefly, first strand cDNA was made from commercially available mRNA. The relative amount of cDNA used in each assay was determined by performing a parallel experiment using a primer pair for a gene expressed in equal amounts in all tissues, cyclophilin. The cyclophilin primer pair detected small variations in the amount of cDNA in each sample and these data were used for normalization of the data obtained with the primer pair for the novel BMY_HPP5. The PCR data was converted into a relative assessment of the difference in transcript abundance amongst the tissues tested and the data is presented in Figure 11. Transcripts corresponding to BMY_HPP5 were expressed highly in the testis, spinal cord, an to a lesser extent in bone marrow, brain, thymus, and liver.

Example 4 – Method of Assaying The Phosphatase Activity of the BMY_HPP Polypeptides of the present invention.

The Phosphatase Activity of the BMY_HPP Polypeptides of the present invention may be assessed through the application of various biochemical assays known in the art and described herein.

Hydrolysis of para-nitrophenyl phosphate

The phosphatase activity for BMY_HPP proteins may be measured by assaying the ability of the proteins to hydrolize para-nitrophenyl phosphate, a compound known to be a substrate for phosphatases, as described in Krejsa, C. et al., J. Biol. Chem... Vol. 272, p.11541-11549, 1997 (which is hereby incorporated in its entirety herein). The proteins are incubated in 3 mg/ml para-nitrophenyl phosphate in a solution containing 60 mM MES, pH 6.0, 5% glycerol, 5 mM dithiothreitol, and 0.1% Triton X-100 for 15 min, or such other time as may be desired. The pH of the reaction may be varied to provide an optimal pH for each individual BMY_HPP

protein by those with ordinary skill in the art of enzyme assays. The phosphatase reaction is stopped by the addition of 3 N NaOH to give a final NaOH concentration of 0.7 M. The product of the reaction is measured by reading the absorbance of the solution at 405 nm.

Two dimensional gel electrophoresis

The BMY_HPP polynucleotides of the present invention may be subcloned into appropriate vectors for expression in host cells. Representative vectors are known in the art and described herein. 2-D gel electrophoresis (IEF followed by SDS-PAGE) will be used to assay BMY_HPP-dependent dephosphorylation of host cell proteins. These proteins can be recovered from the gel and identified by mass spectrometric or other protein sequencing techniques known in the art.

Briefly, Methods for 2-dimensional gel analysis and labeling cells with proteins are well known in the art. Cells would be labeled with 32P orthophosphate, cellular proteins would be resolved on 2D gels and their positions determined by autoradiography. Proteins of interest would be identified by excising the spots and analyzing their sequence by mass spectroscopy. The following paper and the references therein describe the methods of labeling cells, analyzing the proteins on 2D gels and mass spec identification: Gerner, C. et al., J. Biol. Chem..., Vol. 275, p.39018 - 39026, 2000. Substrates affected by the phosphatase would be identified by comparing wild type cells to cells where expression of the phosphatase is inhibited by deletion, anti-sense, or other means. Proteins whose phosphorylation increased would be either direct substrates or indirectly regulated by the phosphatase. Conversely, in cells where the active phosphatase was overexpressed, proteins whose phosphorylation decreased would either be direct substrates or indirectly regulated by the phosphatase.

Example 5 – Method of Identifying The Substrate of the BMY_HPP Phosphatase Polypeptides of the present invention.

Substrate identification

Protein substrates for BMY_HPP polypeptides of the present invention may be identified by recovery of proteins dephosphorylated in the 2-D gel electrophoesis assay described above. Phosphopeptide substrates may also be identified as proteins whose phosphorylation increases when the activity or expression of a BMY_HPP protein is decreased (for example, by an antibody, antisense or double-stranded inhibitory RNA or by a small moloecule inhibitor of BMY_HPP activity). In either case, mass spectrometry can be used to identify the recovered proteins.

Phosphopeptide substrates for BMY-HPP polypeptides may also be identified by incubation of a phosphopeptide library with a catalytically inactive version of the protein, recovery of the complex, and peptide sequencing by standard methods such as Edman degradation or mass spectrometry.

Phosphopeptide substrates can also be identified by expressing a substrate trapping mutant phosphatase (one that is catalytically inactive due to active site mutation) and isolating the proteins that bind preferentially to the substrate trapping phosphatase relative to the wild type phosphatase.

Example 6 – Method of Identifying RET31 of the Present Invention.

In an effort to identify gene products involved in regulatory events, the RNA expressed in TNF-α-stimulated human lung microvascular endothelial cells was analyzed. Resting cells were stimulated for 1 h with TNF-α, and the RNA was isolated from the cells. Complementary DNA (cDNA) was created from the isolated RNA using methods known in the art. The cDNA that were upregulated in response to TNFα were identified using subtractive hybridization. A clone corresponding to a portion of the RET31 polynucleotide was identified and used to identify the full-length (SEQ ID NO:115). Additional methods are provided below.

HMVEC Cell culture

Primary cultures of human lung microvascular endothelial cells (HMVEC), from a single donor, were obtained from Clonetics (Walkersville, MD). The cells were grown in the endothelial cell growth medium-2 kit (CC-3202) with 5% Fetal Bovine Serum (Hyclone). Initially, the cells were seeded into a T-25 flask and, after reaching approximately 90% confluence, they were trypsinized and transferred into T-225 flasks at 1.2 x 10^6/flask in 80 mls of medium. For normal growth conditions, the medium was changed each 48 h. When the cells reached approximately 90% confluence, they were passaged again and seeded into T-225 flasks at 1.8 x 10^6/ml in 80 mls of medium.

HMVEC Cell Treatment for RNA isolation

Subconfluent (90% confluent) T-225 flasks of HMVEC were adjusted to 40 ml of medium per flask by removing excess medium. HMVEC were either left untreated (time 0) or treated with 10 ng/ml TNF-α for 1, 6 or 24 h. The medium was not changed at the time of TNF-α addition.

RNA isolation

At the designated time points, The flasks of HMVEC were trypsinized briefly to remove cells from the flasks and trypsinization was terminated by the addition of fetal calf serum. The cells were removed from the flasks and the flasks rinsed with PBS. The cells were pelleted, rinsed once in PBS and re-pelleted. The supernatant was removed and the cell pellet used for RNA isolation. Poly A+RNA was isolated directly using Fast Track 2.0™ (Invitrogen, Carlsbad, CA).

Construction of the Subtraction Library

The PCR-select cDNA subtraction kit™ (Clontech, Palo Alto, CA) was used to generate a subtraction library from untreated HMVEC poly A+ RNA (tester) and 1 h TNF-α-treated HMVEC poly A+ RNA (driver), according to the manufacturer's protocols. Ten secondary PCR reactions were combined and run on a 2% agarose gel. Fragments ranging from approximately 0.3kb – 10 kb were gel purified using the QIAgen gel extraction kit (QIAgen Inc., Valencia, CA) and inserted into the TA cloning vector, pCR2.1 (Invitrogen). TOP10F' competent *E. coli* (Invitrogen) were

transformed and plated on LB plates containing 50 micrograms/ml ampicillin. Clones were isolated and grown in LB broth containing similar concentrations of ampicillin. Plasmids were sequenced using methods known in the art or described elsewhere herein.

As referenced above, the methods utilized for constructing the subtraction library followed the PCR-Select cDNA Subtraction Kit (Clonetech; Protocol # PT1117-1; Version # PR85431) which is hereby incorporated herein by reference in its entirety. Additional references to this method may be found in Diatchenko, L., et al., PNAS 93:6025-6030 (1996), which is hereby incorporated herein by reference in its entirety.

Example7 – Method of Cloning RET31 of the Present Invention.

A clone containing the predicted coding sequence of RET31 was isolated from human microvascular endothelial cells (HMVECs) treated with tumor necrosis factor alpha (TNFα) for 6 hours using reverse transcription/polymerase chain reaction (RT/PCR). RNA was purified from the TNFa stimulated HMVEC cells according to methods known in the art. A primer set (each at 400 nM final concentration) was used to amplify a 3 kb sequence using the following primers and conditions:

primer JNF388: CACACCACCATTACATCATCGTGGC (SEQ ID NO:145)
primer JNF525: TGCTGCTCTGCTACCAACCC (SEQ ID NO:146)

with 200 μM dNTP's, 1X Advantage 2 polymerase, and 2.0 μl DNA in 25.0 μl reaction. The experiment was cycled 35 times through the following sequence: : 94°C for 30 sec, 68°C for 30 sec. then 72°C for 3.5 min. At the completion of the reaction, 6.0μl of loading dye was added and the entire reaction was separated by gel electrophoresis in a 1.2% agarose gel containing ethidium bromide. An ~3 kb size band was excised from the gel and purified using the QIAgen extraction kit (QIAgen, Valencia, CA). This fragment was ligated into the pTAdv cloning vector (Clontech, Palo Alto, CA) and sequenced using standard methods. The RET31 clone (SEQ ID NO:108; Figures 13A-F) contains about a 3 kb sequence corresponding to nucleotides 472 to 3513 of the predicted RET31 coding sequence (SEQ ID NO:147). The

predicted RET31 coding sequence (SEQ ID NO:147) was derived from Incyte gene cluster 1026659.7.

A nucleic acid sequence corresponding to the nucleic acid sequence encoding the RET31 polypeptide was first identified in a subtraction library from TNF-α stimulated human lung microvascular endiothelial cells (HMVEC). This subtraction clone sequence encoded a 408bp partial cDNA sequence, as shown:

RET31 subtraction clone

ACAATGGAGTGGCTGAGCCTTTGAGCACACCACCATTACATCATCGTGGCAAATTAAAGAAGGAGGTGG
GAAAAGAGGACTTATTGTTGTCATGGCCCATGAGATGATTGGAACTCAAATTGTTACTGAGAGGTTGGT
GGCTCTGCTGGAAAGTGGAACGGAAAAAGTGCTGCTAATTGATAGCCGGCCATTTGTGGAATACAATAC
ATCCCACATTTTGGAAGCCATTAATATCAACTGCTCCAAGCTTATGAAGCGAAGGTTGCAACAGGACAA
AGTGTTAATTACAGAGCTCATCCAGCATTCAGCGAAACATAAGGTTGACATTGATTGCAGTCAGAAGGT
TGTAGTTTACGATCAAAGCTCCCAAGATGTTGCCTCTCTCTCTTCAGACTGTTTTCTCACTGT (SEQ ID NO:115)

Example 8 – Method of Determining the mRNA Expression Profile of RET31 Using Northern Hybidization.

Multiple tissue northern blots (MTN) were purchased from Clontech Laboratories (Palo Alto, CA) and hybridized with P^{32}-labeled RET31. Briefly, a 408 bp RET31 fragment (RET31/RsaI) was isolated from subtraction clone 1hrTNF031 (SEQ ID NO:115) using RsaI restriction endonuclease, run on a 2.0% agarose gel, and purified using the QIAgen Gel Extraction Kit (QIAgen, Valencia, CA). Approximately 30 ng of RET31/RsaI was radiolabeled (6000Ci/mmol P^{32}-dCTP) using the Random Primed DNA Labeling Kit (Roche, Indianapolis, IN). Unincorporated nucleotides were removed using NucTrap Probe Purification Columns (Stratgene, La Jolla, CA). Radiolabeled RET31/RsaI probe was added at a specific activity of $1.5x10^6$ cpm/ml of ExpressHyb hybridization solution (Clontech) and incubated overnight at 65°C. Blots were washed to 2.0XSSC/0.05%SDS at 50°C and exposed to film for 24 and 48 h. The MTN's used were human MTN (#7760-1), human MTN II (#7759-1), human MTN III (#7767-1), and human cancer cell line MTN (#7757-1).

The results show the RET31 polypeptide was expressed predominately in adrenal gland, testis, and skeletal muscle; to a significant extent, in the liver, prostate ovary, and to a lesser extent, in placenta, pancreas, thymus, small intestine, thyroid, heart, kidney and liver (see Figure 15).

Example 9 – Method of Assessing The Affect of TNF-alpha on RET31 mRNA Expression.

In an effort to confirm the the TNF-alpha dependent regulation of RET31 expression, HMVEC cells were treated with TNF-alpha over several time periods and the mRNA subsequently harvested and probed by northern hybridization. Briefly, untreated HMVEC, 1h TNF-α stimulated HMVEC, 6 h TNF-α stimulated HMVEC, 24h TNF-α stimulated HMVEC poly A+RNA (2 μg each) were run on a 1.2% agarose gel containing 3.0% formaldehyde and transferred to Hybond N+ nucleic acid transfer membrane (Amersham, Piscataway, NJ) using standard blotting techniques (see Maniatis et al. referenced herein). Membranes were auto cross-linked using Stratalinker (Stratagene) and prehybridized in ExpressHyb hybridization solution for 1 h and probed in parallel with the multiple tissue northern blots.

After hybridization, membranes were washed by continuous shaking for 30 minutes with low stringency solution (2XSSC/0.05%SDS) at room temperature with 2 changes of solution. Membranes were then washed for 30 minutes with high stringency solution (0.1XSSC/0.1%SDS) at 50°C with 1 change of solution. The membranes were exposed with intensifying screens to X-ray film at -70°C for 10 days.

The endothelial cell blot was reprobed for E-selectin and GAPDH.

The results confirmed RET31 is up-regulated by TNF-α, reaching a peak of expression at 6 h by northern blot analysis (see Figure 18).

Example 6 – Method Of Assessing The Physiological Function Of The human phosphatase Polypeptide At The Cellular Level.

The physiological function of the human phosphatase polypeptide may be assessed by expressing the sequences encoding human phosphatase at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a

mammalian expression vector containing a strong promoter that drives high levels of cDNA expression (examples are provided elsewhere herein). Vectors of choice include pCMV SPORT (Life Technologies) and pCR3.1 (Invitrogen, Carlsbad CA), both of which contain the cytomegalovirus promoter. 5-10, ug of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2ug of an additional plasmid containing sequences encoding a marker protein are cotransfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP; Clontech), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP and to evaluate the apoptotic state of the cells and other cellular properties. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) Flow Cytometry, Oxford, New York NY.

The influence of human phosphatase polypeptides on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding human phosphatase and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding

human phosphatase polypeptides and other genes of interest can be analyzed by northern analysis or microarray techniques.

Example 7 – Method Of Screening For Compounds That Interact With The human phosphatase Polypeptide.

The following assays are designed to identify compounds that bind to the human phosphatase polypeptide, bind to other cellular proteins that interact with the human phosphatase polypeptide, and to compounds that interfere with the interaction of the human phosphatase polypeptide with other cellular proteins.

Such compounds can include, but are not limited to, other cellular proteins. Specifically, such compounds can include, but are not limited to, peptides, such as, for example, soluble peptides, including, but not limited to Ig-tailed fusion peptides, comprising extracellular portions of human phosphatase polypeptide transmembrane receptors, and members of random peptide libraries (see, e.g., Lam, K. S. et al., 1991, Nature 354:82-84; Houghton, R. et al., 1991, Nature 354:84-86), made of D-and/or L-configuration amino acids, phosphopeptides (including, but not limited to, members of random or partially degenerate phosphopeptide libraries; see, e.g., Songyang, Z., et al., 1993, Cell 72:767-778), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab').sub.2 and FAb expression libary fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

Compounds identified via assays such as those described herein can be useful, for example, in elaborating the biological function of the human phosphatase polypeptide, and for ameliorating symptoms of tumor progression, for example. In instances, for example, whereby a tumor progression state or disorder results from a lower overall level of human phosphatase expression, human phosphatase polypeptide, and/or human phosphatase polypeptide activity in a cell involved in the tumor progression state or disorder, compounds that interact with the human phosphatase polypeptide can include ones which accentuate or amplify the activity of the bound human phosphatase polypeptide. Such compounds would bring about an effective increase in the level of human phosphatase polypeptide activity, thus ameliorating symptoms of the tumor progression disorder or state. In instances

whereby mutations within the human phosphatase polypeptide cause aberrant human phosphatase polypeptides to be made which have a deleterious effect that leads to tumor progression, compounds that bind human phosphatase polypeptide can be identified that inhibit the activity of the bound human phosphatase polypeptide. Assays for testing the effectiveness of such compounds are known in the art and discussed, elsewhere herein.

Example 8 – Method Of Screening, In Vitro, Compounds That Bind To The Human Phosphatase Polypeptide.

In vitro systems can be designed to identify compounds capable of binding the human phosphatase polypeptide of the invention. Compounds identified can be useful, for example, in modulating the activity of wild type and/or mutant human phosphatase polypeptide, preferably mutant human phosphatase polypeptide, can be useful in elaborating the biological function of the human phosphatase polypeptide, can be utilized in screens for identifying compounds that disrupt normal human phosphatase polypeptide interactions, or can in themselves disrupt such interactions.

The principle of the assays used to identify compounds that bind to the human phosphatase polypeptide involves preparing a reaction mixture of the human phosphatase polypeptide and the test compound under conditions and for a time sufficient to allow the two components to interact and bind, thus forming a complex which can be removed and/or detected in the reaction mixture. These assays can be conducted in a variety of ways. For example, one method to conduct such an assay would involve anchoring human phosphatase polypeptide or the test substance onto a solid phase and detecting human phosphatase polypeptide /test compound complexes anchored on the solid phase at the end of the reaction. In one embodiment of such a method, the human phosphatase polypeptide can be anchored onto a solid surface, and the test compound, which is not anchored, can be labeled, either directly or indirectly.

In practice, microtitre plates can conveniently be utilized as the solid phase. The anchored component can be immobilized by non-covalent or covalent attachments. Non-covalent attachment can be accomplished by simply coating the solid surface with a solution of the protein and drying. Alternatively, an immobilized antibody, preferably a monoclonal antibody, specific for the protein to be

immobilized can be used to anchor the protein to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the nonimmobilized component is added to the coated surface containing the anchored component. After the reaction is complete, unreacted components are removed (e.g., by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the previously immobilized component is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the previously nonimmobilized component is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the immobilized component (the antibody, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig antibody).

Alternatively, a reaction can be conducted in a liquid phase, the reaction products separated from unreacted components, and complexes detected; e.g., using an immobilized antibody specific for human phosphatase polypeptide or the test compound to anchor any complexes formed in solution, and a labeled antibody specific for the other component of the possible complex to detect anchored complexes.

Example 9 – Method Of Identifying Compounds That Interfere With Human Phosphatase Polypeptide/Cellular Product Interaction.

The human phosphatase polypeptide of the invention can, in vivo, interact with one or more cellular or extracellular macromolecules, such as proteins. Such macromolecules include, but are not limited to, polypeptides, particularly ligands, and those products identified via screening methods described, elsewhere herein. For the purposes of this discussion, such cellular and extracellular macromolecules are referred to herein as "binding partner(s)". For the purpose of the present invention, "binding partner" may also encompass polypeptides, small molecule compounds, polysaccarides, lipids, and any other molecule or molecule type referenced herein. Compounds that disrupt such interactions can be useful in regulating the activity of the human phosphatase polypeptide, especially mutant human phosphatase

polypeptide. Such compounds can include, but are not limited to molecules such as antibodies, peptides, and the like described in elsewhere herein.

The basic principle of the assay systems used to identify compounds that interfere with the interaction between the human phosphatase polypeptide and its cellular or extracellular binding partner or partners involves preparing a reaction mixture containing the human phosphatase polypeptide, and the binding partner under conditions and for a time sufficient to allow the two products to interact and bind, thus forming a complex. In order to test a compound for inhibitory activity, the reaction mixture is prepared in the presence and absence of the test compound. The test compound can be initially included in the reaction mixture, or can be added at a time subsequent to the addition of human phosphatase polypeptide and its cellular or extracellular binding partner. Control reaction mixtures are incubated without the test compound or with a placebo. The formation of any complexes between the human phosphatase polypeptide and the cellular or extracellular binding partner is then detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the test compound, indicates that the compound interferes with the interaction of the human phosphatase polypeptide and the interactive binding partner. Additionally, complex formation within reaction mixtures containing the test compound and normal human phosphatase polypeptide can also be compared to complex formation within reaction mixtures containing the test compound and mutant human phosphatase polypeptide. This comparison can be important in those cases wherein it is desirable to identify compounds that disrupt interactions of mutant but not normal human phosphatase polypeptide.

The assay for compounds that interfere with the interaction of the human phosphatase polypeptide and binding partners can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the human phosphatase polypeptide or the binding partner onto a solid phase and detecting complexes anchored on the solid phase at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. In either approach, the order of addition of reactants can be varied to obtain different information about the compounds being tested. For example, test compounds that interfere with the interaction between the human phosphatase polypeptide and the binding partners, e.g.,

by competition, can be identified by conducting the reaction in the presence of the test substance; i.e., by adding the test substance to the reaction mixture prior to or simultaneously with the human phosphatase polypeptide and interactive cellular or extracellular binding partner. Alternatively, test compounds that disrupt preformed complexes, e.g. compounds with higher binding constants that displace one of the components from the complex, can be tested by adding the test compound to the reaction mixture after complexes have been formed. The various formats are described briefly below.

In a heterogeneous assay system, either the human phosphatase polypeptide or the interactive cellular or extracellular binding partner, is anchored onto a solid surface, while the non-anchored species is labeled, either directly or indirectly. In practice, microtitre plates are conveniently utilized. The anchored species can be immobilized by non-covalent or covalent attachments. Non-covalent attachment can be accomplished simply by coating the solid surface with a solution of the human phosphatase polypeptide or binding partner and drying. Alternatively, an immobilized antibody specific for the species to be anchored can be used to anchor the species to the solid surface. The surfaces can be prepared in advance and stored.

In order to conduct the assay, the partner of the immobilized species is exposed to the coated surface with or without the test compound. After the reaction is complete, unreacted components are removed (e.g., by washing) and any complexes formed will remain immobilized on the solid surface. The detection of complexes anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized species is pre-labeled, the detection of label immobilized on the surface indicates that complexes were formed. Where the non-immobilized species is not pre-labeled, an indirect label can be used to detect complexes anchored on the surface; e.g., using a labeled antibody specific for the initially non-immobilized species (the antibody, in turn, can be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, test compounds which inhibit complex formation or which disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the test compound, the reaction products separated from unreacted

components, and complexes detected; e.g., using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect anchored complexes. Again, depending upon the order of addition of reactants to the liquid phase, test compounds which inhibit complex or which disrupt preformed complexes can be identified.

In an alternate embodiment of the invention, a homogeneous assay can be used. In this approach, a preformed complex of the human phosphatase polypeptide and the interactive cellular or extracellular binding partner product is prepared in which either the human phosphatase polypeptide or their binding partners are labeled, but the signal generated by the label is quenched due to complex formation (see, e.g., U.S. Pat. No. 4,109,496 by Rubenstein which utilizes this approach for immunoassays). The addition of a test substance that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. In this way, test substances which disrupt human phosphatase polypeptide -cellular or extracellular binding partner interaction can be identified.

In a particular embodiment, the human phosphatase polypeptide can be prepared for immobilization using recombinant DNA techniques known in the art. For example, the human phosphatase polypeptide coding region can be fused to a glutathione-S-transferase (GST) gene using a fusion vector such as pGEX-5X-1, in such a manner that its binding activity is maintained in the resulting fusion product. The interactive cellular or extracellular product can be purified and used to raise a monoclonal antibody, using methods routinely practiced in the art and described above. This antibody can be labeled with the radioactive isotope .sup.125 I, for example, by methods routinely practiced in the art. In a heterogeneous assay, e.g., the GST- human phosphatase polypeptide fusion product can be anchored to glutathione-agarose beads. The interactive cellular or extracellular binding partner product can then be added in the presence or absence of the test compound in a manner that allows interaction and binding to occur. At the end of the reaction period, unbound material can be washed away, and the labeled monoclonal antibody can be added to the system and allowed to bind to the complexed components. The interaction between the human phosphatase polypeptide and the interactive cellular or extracellular binding partner can be detected by measuring the amount of radioactivity that remains

associated with the glutathione-agarose beads. A successful inhibition of the interaction by the test compound will result in a decrease in measured radioactivity.

Alternatively, the GST- human phosphatase polypeptide fusion product and the interactive cellular or extracellular binding partner product can be mixed together in liquid in the absence of the solid glutathione-agarose beads. The test compound can be added either during or after the binding partners are allowed to interact. This mixture can then be added to the glutathione-agarose beads and unbound material is washed away. Again the extent of inhibition of the binding partner interaction can be detected by adding the labeled antibody and measuring the radioactivity associated with the beads.

In another embodiment of the invention, these same techniques can be employed using peptide fragments that correspond to the binding domains of the human phosphatase polypeptide product and the interactive cellular or extracellular binding partner (in case where the binding partner is a product), in place of one or both of the full length products.

Any number of methods routinely practiced in the art can be used to identify and isolate the protein's binding site. These methods include, but are not limited to, mutagenesis of one of the genes encoding one of the products and screening for disruption of binding in a co-immunoprecipitation assay. Compensating mutations in the gene encoding the second species in the complex can be selected. Sequence analysis of the genes encoding the respective products will reveal the mutations that correspond to the region of the product involved in interactive binding. Alternatively, one product can be anchored to a solid surface using methods described in this Section above, and allowed to interact with and bind to its labeled binding partner, which has been treated with a proteolytic enzyme, such as trypsin. After washing, a short, labeled peptide comprising the binding domain can remain associated with the solid material, which can be isolated and identified by amino acid sequencing. Also, once the gene coding for the cellular or extracellular binding partner product is obtained, short gene segments can be engineered to express peptide fragments of the product, which can then be tested for binding activity and purified or synthesized.

Example 10 - Isolation Of A Specific Clone From The Deposited Sample.

The deposited material in the sample assigned the ATCC Deposit Number cited in Table I for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone identified in Table I. Typically, each ATCC deposit sample cited in Table I comprises a mixture of approximately equal amounts (by weight) of about 1-10 plasmid DNAs, each containing a different cDNA clone and/or partial cDNA clone; but such a deposit sample may include plasmids for more or less than 2 cDNA clones.

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNA(s) cited for that clone in Table I. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with 32P-(-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO:X (i.e., within the region of SEQ ID NO:X bounded by the 5′ NT and the 3′ NT of the clone defined in Table I) are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain

reaction is carried out under routine conditions, for instance, in 25 ul of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM MgCl2, 0.01% (w/v) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for 1 min; annealing at 55 degree C for 1 min; elongation at 72 degree C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Example 11 - Tissue Distribution Of Polypeptide.

Tissue distribution of mRNA expression of polynucleotides of the present invention is determined using protocols for Northern blot analysis, described by, among others, Sambrook et al. For example, a cDNA probe produced by the method described in Example 10 is labeled with p32 using the rediprime ™ DNA labeling system (Amersham Life Science), according to manufacturer's instructions. After labeling, the probe is purified using CHROMA SPIN0-100 column (Clontech Laboratories, Inc.) according to manufacturer's protocol number PT1200-1. The purified labeled probe is then used to examine various tissues for mRNA expression.

Tissue Northern blots containing the bound mRNA of various tissues are examined with the labeled probe using ExpressHybtm hybridization solution (Clonetech according to manufacturers protocol number PT1190-1. Northern blots can be produced using various protocols well known in the art (e.g., Sambrook et al). Following hybridization and washing, the blots are mounted and exposed to film at – 70C overnight, and the films developed according to standard procedures.

Example 12 - Chromosomal Mapping Of The Polynucleotides.

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions: 30 seconds,95 degree C; 1 minute, 56 degree C; 1 minute, 70 degree C. This cycle is repeated 32 times followed by one 5 minute cycle at 70 degree C.

Mammalian DNA, preferably human DNA, is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions are analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

Example 13 - Bacterial Expression Of A Polypeptide.

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 10, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Ampr), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, that expresses the lacI repressor and also confers kanamycin resistance (Kanr). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.600) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1

mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., supra). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., supra).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4 degree C or frozen at -80 degree C.

Example 14 - Purification Of A Polypeptide From An Inclusion Body.

The following alternative method can be used to purify a polypeptide expressed in E coli when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10 degree C.

Upon completion of the production phase of the E. coli fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield

of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfluidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 um membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perceptive Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perceptive Biosystems) and weak anion (Poros CM-20, Perceptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a

10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A280 monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Coomassie blue stained 16% SDS-PAGE gel when 5 ug of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 15 - Cloning And Expression Of A Polypeptide In A Baculovirus Expression System.

In this example, the plasmid shuttle vector pAc373 is used to insert a polynucleotide into a baculovirus to express a polypeptide. A typical baculovirus expression vector contains the strong polyhedrin promoter of the Autographa californica nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites, which may include, for example BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is often used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from E. coli under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pVL941 and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-39 (1989).

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA

sequence, as outlined in Example 10, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites at the 5' end of the primers in order to clone the amplified product into the expression vector. Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon and the naturally associated leader sequence identified elsewhere herein (if applicable), is amplified using the PCR protocol described in Example 10. If the naturally occurring signal sequence is used to produce the protein, the vector used does not need a second signal peptide. Alternatively, the vector can be modified to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. E. coli HB101 or other suitable E. coli hosts such as XL-1 Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transformed with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGoldtm baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One ug of BaculoGoldtm virus DNA and 5ug of the plasmid are mixed in a sterile well of a

microtiter plate containing 50ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, supra. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 uCi of 35S-methionine and 5 uCi 35S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 16 - Expression Of A Polypeptide In Mammalian Cells.

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSport 2.0, and pCMVSport 3.0. Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transformation with a selectable marker such as dhfr, gpt, neomycin, hygromycin allows the identification and isolation of the transformed cells.

The transformed gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem... 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is

the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

A polynucleotide of the present invention is amplified according to the protocol outlined in herein. If the naturally occurring signal sequence is used to produce the protein, the vector does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.) The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. E. coli HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transformation. Five μg of an expression plasmid is cotransformed with 0.5 ug of the plasmid pSVneo using lipofectin (Felgner et al., supra). The plasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 uM, 2 uM, 5 uM, 10 mM, 20 mM). The same

procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 uM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 17 - Protein Fusions.

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example described herein; see also EP A 394,827; Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the half-life time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will not be produced.

The naturally occurring signal sequence may be used to produce the protein (if applicable). Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891 and/or US Patent No. 6,066,781, supra.)

Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACC
GTGCCCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCC

AAAACCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCG
TGGTGGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGC
AGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAG
GACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCT
CCCAACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAG
AACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAAC
CAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGC
CGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACG
CCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACC
GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGAT
GCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTC
CGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:76)

Example 18 - Production Of An Antibody From A Polypeptide.

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing a polypeptide of the present invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of the protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Köhler et al., Nature 256:495 (1975); Köhler et al., Eur. J. Immunol. 6:511 (1976); Köhler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with polypeptide or, more preferably, with a polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture medium; however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56 degrees

C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981).) The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide.

Alternatively, additional antibodies capable of binding to the polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody that binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones that produce an antibody whose ability to bind to the protein-specific antibody can be blocked by the polypeptide. Such antibodies comprise anti-idiotypic antibodies to the protein-specific antibody and can be used to immunize an animal to induce formation of further protein-specific antibodies.

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214

(1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

Moreover, in another preferred method, the antibodies directed against the polypeptides of the present invention may be produced in plants. Specific methods are disclosed in US Patent Nos. 5,959,177, and 6,080,560, which are hereby incorporated in their entirety herein. The methods not only describe methods of expressing antibodies, but also the means of assembling foreign multimeric proteins in plants (i.e., antibodies, etc,), and the subsequent secretion of such antibodies from the plant.

Example 19 - Regulation of Protein Expression Via Controlled Aggregation In The Endoplasmic Reticulum.

As described more particularly herein, proteins regulate diverse cellular processes in higher organisms, ranging from rapid metabolic changes to growth and differentiation. Increased production of specific proteins could be used to prevent certain diseases and/or disease states. Thus, the ability to modulate the expression of specific proteins in an organism would provide significant benefits.

Numerous methods have been developed to date for introducing foreign genes, either under the control of an inducible, constitutively active, or endogenous promoter, into organisms. Of particular interest are the inducible promoters (see, M. Gossen, et al., Proc. Natl. Acad. Sci. USA., 89:5547 (1992); Y. Wang, et al., Proc. Natl. Acad. Sci. USA, 91:8180 (1994), D. No., et al., Proc. Natl. Acad. Sci. USA, 93:3346 (1996); and V.M. Rivera, et al., Nature Med, 2:1028 (1996); in addition to additional examples disclosed elsewhere herein). In one example, the gene for erthropoietin (Epo) was transferred into mice and primates under the control of a small molecule inducer for expression (e.g., tetracycline or rapamycin) (see, D. Bohl, et al., Blood, 92:1512, (1998); K.G. Rendahl, et al., Nat. Biotech, 16:757, (1998); V.M. Rivera, et al., Proc. Natl. Acad. Sci. USA, 96:8657 (1999); and X.Ye et al., Science, 283:88 (1999). Although such systems enable efficient induction of the gene of interest in the organism upon addition of the inducing agent (i.e., tetracycline, rapamycin, etc,.), the levels of expression tend to peak at 24 hours and trail off to

background levels after 4 to 14 days. Thus, controlled transient expression is virtually impossible using these systems, though such control would be desirable.

A new alternative method of controlling gene expression levels of a protein from a transgene (i.e., includes stable and transient transformants) has recently been elucidated (V.M. Rivera., et al., Science, 287:826-830, (2000)). This method does not control gene expression at the level of the mRNA like the aforementioned systems. Rather, the system controls the level of protein in an active secreted form. In the absence of the inducing agent, the protein aggregates in the ER and is not secreted. However, addition of the inducing agent results in dis-aggregation of the protein and the subsequent secretion from the ER. Such a system affords low basal secretion, rapid, high level secretion in the presence of the inducing agent, and rapid cessation of secretion upon removal of the inducing agent. In fact, protein secretion reached a maximum level within 30 minutes of induction, and a rapid cessation of secretion within 1 hour of removing the inducing agent. The method is also applicable for controlling the level of production for membrane proteins.

Detailed methods are presented in V.M. Rivera., et al., Science, 287:826-830, (2000)), briefly:

Fusion protein constructs are created using polynucleotide sequences of the present invention with one or more copies (preferably at least 2, 3, 4, or more) of a conditional aggregation domain (CAD) a domain that interacts with itself in a ligand-reversible manner (i.e., in the presence of an inducing agent) using molecular biology methods known in the art and discussed elsewhere herein. The CAD domain may be the mutant domain isolated from the human FKBP12 (Phe^{36} to Met) protein (as disclosed in V.M. Rivera., et al., Science, 287:826-830, (2000), or alternatively other proteins having domains with similar ligand-reversible, self-aggregation properties. As a principle of design the fusion protein vector would contain a furin cleavage sequence operably linked between the polynucleotides of the present invention and the CAD domains. Such a cleavage site would enable the proteolytic cleavage of the CAD domains from the polypeptide of the present invention subsequent to secretion from the ER and upon entry into the trans-Golgi (J.B. Denault, et al., FEBS Lett., 379:113, (1996)). Alternatively, the skilled artisan would recognize that any proteolytic cleavage sequence could be substituted for the furin sequence provided the

substituted sequence is cleavable either endogenously (e.g., the furin sequence) or exogenously (e.g., post secretion, post purification, post production, etc.). The preferred sequence of each feature of the fusion protein construct, from the 5' to 3' direction with each feature being operably linked to the other, would be a promoter, signal sequence, "X" number of (CAD)x domains, the furin sequence (or other proteolytic sequence), and the coding sequence of the polypeptide of the present invention. The artisan would appreciate that the promotor and signal sequence, independent from the other, could be either the endogenous promotor or signal sequence of a polypeptide of the present invention, or alternatively, could be a heterologous signal sequence and promotor.

The specific methods described herein for controlling protein secretion levels through controlled ER aggregation are not meant to be limiting are would be generally applicable to any of the polynucleotides and polypeptides of the present invention, including variants, homologues, orthologs, and fragments therein.

Example 20 - Alteration Of Protein Glycosylation Sites to Enhance Characteristics Of Polypeptides Of The Invention.

Many eukaryotic cell surface and proteins are post-translationally processed to incorporate N-linked and O-linked carbohydrates (Kornfeld and Kornfeld (1985) Annu. Rev. Biochem. 54:631-64; Rademacher et al., (1988) Annu. Rev. Biochem. 57:785-838). Protein glycosylation is thought to serve a variety of functions including: augmentation of protein folding, inhibition of protein aggregation, regulation of intracellular trafficking to organelles, increasing resistance to proteolysis, modulation of protein antigenicity, and mediation of intercellular adhesion (Fieldler and Simons (1995) Cell, 81:309-312; Helenius (1994) Mol. Biol. Of the Cell 5:253-265; Olden et al., (1978) Cell, 13:461-473; Caton et al., (1982) Cell, 37:417-427; Alexamnder and Elder (1984), Science, 226:1328-1330; and Flack et al., (1994), J. Biol. Chem..., 269:14015-14020). In higher organisms, the nature and extent of glycosylation can markedly affect the circulating half-life and bio-availability of proteins by mechanisms involving receptor mediated uptake and clearance (Ashwell and Morrell, (1974), Adv. Enzymol., 41:99-128; Ashwell and Harford (1982), Ann. Rev. Biochem., 51:531-54). Receptor systems have been

identified that are thought to play a major role in the clearance of serum proteins through recognition of various carbohydrate structures on the glycoproteins (Stockert (1995), Physiol. Rev., 75:591-609; Kery et al., (1992), Arch. Biochem. Biophys., 298:49-55). Thus, production strategies resulting in incomplete attachment of terminal sialic acid residues might provide a means of shortening the bioavailability and half-life of glycoproteins. Conversely, expression strategies resulting in saturation of terminal sialic acid attachment sites might lengthen protein bioavailability and half-life.

In the development of recombinant glycoproteins for use as pharmaceutical products, for example, it has been speculated that the pharmacodynamics of recombinant proteins can be modulated by the addition or deletion of glycosylation sites from a glycoproteins primary structure (Berman and Lasky (1985a) Trends in Biotechnol., 3:51-53). However, studies have reported that the deletion of N-linked glycosylation sites often impairs intracellular transport and results in the intracellular accumulation of glycosylation site variants (Machamer and Rose (1988), J. Biol Chem., 263:5955-5960; Gallagher et al., (1992), J. Virology., 66:7136-7145; Collier et al., (1993), Biochem., 32:7818-7823; Claffey et al., (1995) Biochemica et Biophysica Acta, 1246:1-9; Dube et al., (1988), J. Biol. Chem... 263:17516-17521). While glycosylation site variants of proteins can be expressed intracellularly, it has proved difficult to recover useful quantities from growth conditioned cell culture medium.

Moreover, it is unclear to what extent a glycosylation site in one species will be recognized by another species glycosylation machinery. Due to the importance of glycosylation in protein metabolism, particularly the secretion and/or expression of the protein, whether a glycosylation signal is recognized may profoundly determine a proteins ability to be expressed, either endogenously or recombinately, in another organism (i.e., expressing a human protein in E.coli, yeast, or viral organisms; or an E.coli, yeast, or viral protein in human, etc.). Thus, it may be desirable to add, delete, or modify a glycosylation site, and possibly add a glycosylation site of one species to a protein of another species to improve the proteins functional, bioprocess purification, and/or structural characteristics (e.g., a polypeptide of the present invention).

A number of methods may be employed to identify the location of glycosylation sites within a protein. One preferred method is to run the translated protein sequence through the PROSITE computer program (Swiss Institute of Bioinformatics). Once identified, the sites could be systematically deleted, or impaired, at the level of the DNA using mutagenesis methodology known in the art and available to the skilled artisan, Preferably using PCR-directed mutagenesis (See Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982)). Similarly, glycosylation sites could be added, or modified at the level of the DNA using similar methods, preferably PCR methods (See, Maniatis, supra). The results of modifying the glycosylation sites for a particular protein (e.g., solubility, secretion potential, activity, aggregation, proteolytic resistance, etc.) could then be analyzed using methods know in the art.

The skilled artisan would acknowledge the existence of other computer algorithms capable of predicting the location of glycosylation sites within a protein. For example, the Motif computer program (Genetics Computer Group suite of programs) provides this function, as well.

Example 21 - Method Of Enhancing The Biological Activity/Functional Characteristics Of Invention Through Molecular Evolution.

Although many of the most biologically active proteins known are highly effective for their specified function in an organism, they often possess characteristics that make them undesirable for transgenic, therapeutic, and/or industrial applications. Among these traits, a short physiological half-life is the most prominent problem, and is present either at the level of the protein, or the level of the proteins mRNA. The ability to extend the half-life, for example, would be particularly important for a proteins use in gene therapy, transgenic animal production, the bioprocess production and purification of the protein, and use of the protein as a chemical modulator among others. Therefore, there is a need to identify novel variants of isolated proteins possessing characteristics which enhance their application as a therapeutic for treating diseases of animal origin, in addition to the proteins applicability to common industrial and pharmaceutical applications.

Thus, one aspect of the present invention relates to the ability to enhance specific characteristics of invention through directed molecular evolution. Such an enhancement may, in a non-limiting example, benefit the inventions utility as an essential component in a kit, the inventions physical attributes such as its solubility, structure, or codon optimization, the inventions specific biological activity, including any associated enzymatic activity, the proteins enzyme kinetics, the proteins Ki, Kcat, Km, Vmax, Kd, protein-protein activity, protein-DNA binding activity, antagonist/inhibitory activity (including direct or indirect interaction), agonist activity (including direct or indirect interaction), the proteins antigenicity (e.g., where it would be desirable to either increase or decrease the antigenic potential of the protein), the immunogenicity of the protein, the ability of the protein to form dimers, trimers, or multimers with either itself or other proteins, the antigenic efficacy of the invention, including its subsequent use a preventative treatment for disease or disease states, or as an effector for targeting diseased genes. Moreover, the ability to enhance specific characteristics of a protein may also be applicable to changing the characterized activity of an enzyme to an activity completely unrelated to its initially characterized activity. Other desirable enhancements of the invention would be specific to each individual protein, and would thus be well known in the art and contemplated by the present invention.

For example, an engineered phosphatase may be constitutively active. Alternatively, an engineered phosphatase may be constitutively active in the absence of ligand binding. In yet another example, an engineered phosphatase may be capable of being activated with less than all of the regulatory factors and/or conditions typically required for phosphatase activation (e.g., ligand binding, phosphorylation, conformational changes, etc.). Alternatively, an engineered phosphatase may have altered substrate specificity. Such phosphatases would be useful in screens to identify phosphatase modulators, among other uses described herein.

Directed evolution is comprised of several steps. The first step is to establish a library of variants for the gene or protein of interest. The most important step is to then select for those variants that entail the activity you wish to identify. The design of the screen is essential since your screen should be selective enough to eliminate non-useful variants, but not so stringent as to eliminate all variants. The last step is

then to repeat the above steps using the best variant from the previous screen. Each successive cycle, can then be tailored as necessary, such as increasing the stringency of the screen, for example.

Over the years, there have been a number of methods developed to introduce mutations into macromolecules. Some of these methods include, random mutagenesis, "error-prone" PCR, chemical mutagenesis, site-directed mutagenesis, and other methods well known in the art (for a comprehensive listing of current mutagenesis methods, see Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982)). Typically, such methods have been used, for example, as tools for identifying the core functional region(s) of a protein or the function of specific domains of a protein (if a multi-domain protein). However, such methods have more recently been applied to the identification of macromolecule variants with specific or enhanced characteristics.

Random mutagenesis has been the most widely recognized method to date. Typically, this has been carried out either through the use of "error-prone" PCR (as described in Moore, J., et al, Nature Biotechnology 14:458, (1996), or through the application of randomized synthetic oligonucleotides corresponding to specific regions of interest (as described by Derbyshire, K.M. et al, Gene, 46:145-152, (1986), and Hill, DE, et al, Methods Enzymol., 55:559-568, (1987). Both approaches have limits to the level of mutagenesis that can be obtained. However, either approach enables the investigator to effectively control the rate of mutagenesis. This is particularly important considering the fact that mutations beneficial to the activity of the enzyme are fairly rare. In fact, using too high a level of mutagenesis may counter or inhibit the desired benefit of a useful mutation.

While both of the aforementioned methods are effective for creating randomized pools of macromolecule variants, a third method, termed "DNA Shuffling", or "sexual PCR" (WPC, Stemmer, PNAS, 91:10747, (1994)) has recently been elucidated. DNA shuffling has also been referred to as "directed molecular evolution", "exon-shuffling", "directed enzyme evolution", "in vitro evolution", and "artificial evolution". Such reference terms are known in the art and are encompassed by the invention. This new, preferred, method apparently overcomes the limitations of

the previous methods in that it not only propagates positive traits, but simultaneously eliminates negative traits in the resulting progeny.

DNA shuffling accomplishes this task by combining the principal of in vitro recombination, along with the method of "error-prone" PCR. In effect, you begin with a randomly digested pool of small fragments of your gene, created by Dnase I digestion, and then introduce said random fragments into an "error-prone" PCR assembly reaction. During the PCR reaction, the randomly sized DNA fragments not only hybridize to their cognate strand, but also may hybridize to other DNA fragments corresponding to different regions of the polynucleotide of interest – regions not typically accessible via hybridization of the entire polynucleotide. Moreover, since the PCR assembly reaction utilizes "error-prone" PCR reaction conditions, random mutations are introduced during the DNA synthesis step of the PCR reaction for all of the fragments -further diversifying the potential hybridization sites during the annealing step of the reaction.

A variety of reaction conditions could be utilized to carry-out the DNA shuffling reaction. However, specific reaction conditions for DNA shuffling are provided, for example, in PNAS, 91:10747, (1994). Briefly:

Prepare the DNA substrate to be subjected to the DNA shuffling reaction. Preparation may be in the form of simply purifying the DNA from contaminating cellular material, chemicals, buffers, oligonucleotide primers, deoxynucleotides, RNAs, etc., and may entail the use of DNA purification kits as those provided by Qiagen, Inc., or by the Promega, Corp., for example.

Once the DNA substrate has been purified, it would be subjected to Dnase I digestion. About 2-4ug of the DNA substrate(s) would be digested with .0015 units of Dnase I (Sigma) per ul in 100ul of 50mM Tris-HCL, pH 7.4/1mM MgCl2 for 10-20 min. at room temperature. The resulting fragments of 10-50bp could then be purified by running them through a 2% low-melting point agarose gel by electrophoresis onto DE81 ion-exchange paper (Whatmann) or could be purified using Microcon concentrators (Amicon) of the appropriate molecular weight cutoff, or could use oligonucleotide purification columns (Qiagen), in addition to other methods known in the art. If using DE81 ion-exchange paper, the 10-50bp fragments could be eluted from said paper using 1M NaCl, followed by ethanol precipitation.

The resulting purified fragments would then be subjected to a PCR assembly reaction by re-suspension in a PCR mixture containing: 2mM of each dNTP, 2.2mM MgCl2, 50 mM KCl, 10mM Tris•HCL, pH 9.0, and 0.1% Triton X-100, at a final fragment concentration of 10-30ng/ul. No primers are added at this point. *Taq* DNA polymerase (Promega) would be used at 2.5 units per 100ul of reaction mixture. A PCR program of 94 C for 60s; 94 C for 30s, 50-55 C for 30s, and 72 C for 30s using 30-45 cycles, followed by 72 C for 5min using an MJ Research (Cambridge, MA) PTC-150 thermocycler. After the assembly reaction is completed, a 1:40 dilution of the resulting primerless product would then be introduced into a PCR mixture (using the same buffer mixture used for the assembly reaction) containing 0.8um of each primer and subjecting this mixture to 15 cycles of PCR (using 94 C for 30s, 50 C for 30s, and 72 C for 30s). The referred primers would be primers corresponding to the nucleic acid sequences of the polynucleotide(s) utilized in the shuffling reaction. Said primers could consist of modified nucleic acid base pairs using methods known in the art and referred to else where herein, or could contain additional sequences (i.e., for adding restriction sites, mutating specific base-pairs, etc.).

The resulting shuffled, assembled, and amplified product can be purified using methods well known in the art (e.g., Qiagen PCR purification kits) and then subsequently cloned using appropriate restriction enzymes.

Although a number of variations of DNA shuffling have been published to date, such variations would be obvious to the skilled artisan and are encompassed by the invention. The DNA shuffling method can also be tailored to the desired level of mutagenesis using the methods described by Zhao, et al. (Nucl Acid Res., 25(6):1307-1308, (1997).

As described above, once the randomized pool has been created, it can then be subjected to a specific screen to identify the variant possessing the desired characteristic(s). Once the variant has been identified, DNA corresponding to the variant could then be used as the DNA substrate for initiating another round of DNA shuffling. This cycle of shuffling, selecting the optimized variant of interest, and then re-shuffling, can be repeated until the ultimate variant is obtained. Examples of model screens applied to identify variants created using DNA shuffling technology may be found in the following publications: J. C., Moore, et al., J. Mol. Biol., 272:336-347,

(1997), F.R., Cross, et al., Mol. Cell. Biol., 18:2923-2931, (1998), and A. Crameri., et al., Nat. Biotech., 15:436-438, (1997).

DNA shuffling has several advantages. First, it makes use of beneficial mutations. When combined with screening, DNA shuffling allows the discovery of the best mutational combinations and does not assume that the best combination contains all the mutations in a population. Secondly, recombination occurs simultaneously with point mutagenesis. An effect of forcing DNA polymerase to synthesize full-length genes from the small fragment DNA pool is a background mutagenesis rate. In combination with a stringent selection method, enzymatic activity has been evolved up to 16000 fold increase over the wild-type form of the enzyme. In essence, the background mutagenesis yielded the genetic variability on which recombination acted to enhance the activity.

A third feature of recombination is that it can be used to remove deleterious mutations. As discussed above, during the process of the randomization, for every one beneficial mutation, there may be at least one or more neutral or inhibitory mutations. Such mutations can be removed by including in the assembly reaction an excess of the wild-type random-size fragments, in addition to the random-size fragments of the selected mutant from the previous selection. During the next selection, some of the most active variants of the polynucleotide/polypeptide/enzyme, should have lost the inhibitory mutations.

Finally, recombination enables parallel processing. This represents a significant advantage since there are likely multiple characteristics that would make a protein more desirable (e.g. solubility, activity, etc.). Since it is increasingly difficult to screen for more than one desirable trait at a time, other methods of molecular evolution tend to be inhibitory. However, using recombination, it would be possible to combine the randomized fragments of the best representative variants for the various traits, and then select for multiple properties at once.

DNA shuffling can also be applied to the polynucleotides and polypeptides of the present invention to decrease their immunogenicity in a specified host. For example, a particular variant of the present invention may be created and isolated using DNA shuffling technology. Such a variant may have all of the desired characteristics, though may be highly immunogenic in a host due to its novel intrinsic

structure. Specifically, the desired characteristic may cause the polypeptide to have a non-native structure which could no longer be recognized as a "self" molecule, but rather as a "foreign", and thus activate a host immune response directed against the novel variant. Such a limitation can be overcome, for example, by including a copy of the gene sequence for a xenobiotic ortholog of the native protein in with the gene sequence of the novel variant gene in one or more cycles of DNA shuffling. The molar ratio of the ortholog and novel variant DNAs could be varied accordingly. Ideally, the resulting hybrid variant identified would contain at least some of the coding sequence which enabled the xenobiotic protein to evade the host immune system, and additionally, the coding sequence of the original novel variant that provided the desired characteristics.

Likewise, the invention encompasses the application of DNA shuffling technology to the evolution of polynucleotides and polypeptides of the invention, wherein one or more cycles of DNA shuffling include, in addition to the gene template DNA, oligonucleotides coding for known allelic sequences, optimized codon sequences, known variant sequences, known polynucleotide polymorphism sequences, known ortholog sequences, known homologue sequences, additional homologous sequences, additional non-homologous sequences, sequences from another species, and any number and combination of the above.

In addition to the described methods above, there are a number of related methods that may also be applicable, or desirable in certain cases. Representative among these are the methods discussed in PCT applications WO 98/31700, and WO 98/32845, which are hereby incorporated by reference. Furthermore, related methods can also be applied to the polynucleotide sequences of the present invention in order to evolve invention for creating ideal variants for use in gene therapy, protein engineering, evolution of whole cells containing the variant, or in the evolution of entire enzyme pathways containing polynucleotides of the invention as described in PCT applications WO 98/13485, WO 98/13487, WO 98/27230, WO 98/31837, and Crameri, A., et al., Nat. Biotech., 15:436-438, (1997), respectively.

Additional methods of applying "DNA Shuffling" technology to the polynucleotides and polypeptides of the present invention, including their proposed applications, may be found in US Patent No. 5,605,793; PCT Application No. WO

95/22625; PCT Application No. WO 97/20078; PCT Application No. WO 97/35966; and PCT Application No. WO 98/42832; PCT Application No. WO 00/09727 specifically provides methods for applying DNA shuffling to the identification of herbicide selective crops which could be applied to the polynucleotides and polypeptides of the present invention; additionally, PCT Application No. WO 00/12680 provides methods and compositions for generating, modifying, adapting, and optimizing polynucleotide sequences that confer detectable phenotypic properties on plant species; each of the above are hereby incorporated in their entirety herein for all purposes.

Example 22 - Method Of Determining Alterations In A Gene Corresponding To A Polynucleotide.

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Manheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal

fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

Example 23 - Method Of Detecting Abnormal Levels Of A Polypeptide In A Biological Sample.

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described elsewhere herein. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale).

Interpolate the concentration of the polypeptide in the sample using the standard curve.

Example 24 – Formulation.

The invention also provides methods of treatment and/or prevention diseases, disorders, and/or conditions (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto, in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating

material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention may also be suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (see, generally, Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos.

4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or

arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic will typically be formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the

invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diptheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include,

but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892),TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR((zidovudine/AZT), VIDEX((didanosine/ddI), HIVID((zalcitabine/ddC), ZERIT((stavudine/d4T), EPIVIR((lamivudine/3TC), and COMBIVIR((zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, VIRAMUNE((nevirapine), RESCRIPTOR((delavirdine), and SUSTIVA((efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN((indinavir), NORVIR((ritonavir), INVIRASE((saquinavir), and VIRACEPT((nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE(, DAPSONE(, PENTAMIDINE(, ATOVAQUONE(, ISONIAZID(, RIFAMPIN(, PYRAZINAMIDE(, ETHAMBUTOL(, RIFABUTIN(, CLARITHROMYCIN(, AZITHROMYCIN(, GANCICLOVIR(, FOSCARNET(, CIDOFOVIR(, FLUCONAZOLE(, ITRACONAZOLE(, KETOCONAZOLE(, ACYCLOVIR(, FAMCICOLVIR(, PYRIMETHAMINE(, LEUCOVORIN(, NEUPOGEN((filgrastim/G-CSF), and LEUKINE((sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE(, DAPSONE(, PENTAMIDINE(, and/or ATOVAQUONE(to prophylactically treat or prevent an opportunistic Pneumocystis carinii pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID(, RIFAMPIN(, PYRAZINAMIDE(, and/or ETHAMBUTOL(to prophylactically treat or prevent an opportunistic Mycobacterium avium complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN(, CLARITHROMYCIN(, and/or AZITHROMYCIN(to prophylactically treat or prevent an opportunistic Mycobacterium tuberculosis infection. In another specific embodiment, Therapeutics of the invention are used in any combination with GANCICLOVIR(, FOSCARNET(, and/or CIDOFOVIR(to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE(, ITRACONAZOLE(, and/or KETOCONAZOLE(to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR(and/or FAMCICOLVIR(to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE(and/or LEUCOVORIN(to prophylactically treat or prevent an opportunistic Toxoplasma gondii infection. In another specific

embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN(and/or NEUPOGEN(to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE((OKT3), SANDIMMUNE(/NEORAL(/SANGDYA((cyclosporin), PROGRAF((tacrolimus), CELLCEPT((mycophenolate), Azathioprine, glucorticosteroids, and RAPAMUNE((sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR(, IVEEGAM(,

SANDOGLOBULIN(, GAMMAGARD S/D(, and GAMIMUNE(. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In another embodiment, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, formulations of the present invention may further comprise antagonists of P-glycoprotein (also referred to as the multiresistance protein, or PGP), including antagonists of its encoding polynucleotides (e.g., antisense oligonucleotides, ribozymes, zinc-finger proteins, etc.). P-glycoprotein is well known for decreasing the efficacy of various drug administrations due to its ability to export intracellular levels of absorbed drug to the cell exterior. While this activity has been particularly pronounced in cancer cells in response to the administration of chemotherapy regimens, a variety of other cell types and the administration of other drug classes have been noted (e.g., T-cells and anti-HIV drugs). In fact, certain mutations in the PGP gene significantly reduces PGP function, making it less able to force drugs out of cells. People who have two versions of the mutated gene--one inherited from each parent--have more than four times less PGP than those with two normal versions of the gene. People may also have one normal gene and one mutated one. Certain ethnic populations have increased incidence of such PGP mutations. Among individuals from Ghana, Kenya, the Sudan, as well as African Americans, frequency of the normal gene ranged from 73% to 84%. In contrast, the frequency was 34% to 59% among British whites, Portuguese, Southwest Asian, Chinese, Filipino and Saudi populations. As a result, certain ethnic populations may require increased administration of PGP antagonist in the formulation of the present invention to arrive at the an efficacious dose of the therapeutic (e.g., those from African descent). Conversely, certain ethnic populations, particularly those having increased frequency of the mutated PGP (e.g., of Caucasian descent, or non-African descent) may require less pharmaceutical compositions in the formulation due to an effective increase in efficacy of such compositions as a result of the increased effective absorption (e.g., less PGP activity) of said composition.

Moreover, in another specific embodiment, formulations of the present invention may further comprise antagonists of OATP2 (also referred to as the multiresistance protein, or MRP2), including antagonists of its encoding polynucleotides (e.g., antisense oligonucleotides, ribozymes, zinc-finger proteins, etc.). The invention also further comprises any additional antagonists known to inhibit proteins thought to be attributable to a multidrug resistant phenotype in proliferating cells.

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Gorwth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832;

and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE((SARGRAMOSTIM() and NEUPOGEN((FILGRASTIM().

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In additional embodiments, the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Example 25 - Method Of Treating Decreased Levels Of The Polypeptide.

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a secreted protein in an individual can be treated by administering the polypeptide of the present invention, preferably in the secreted form. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the polypeptide to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the polypeptide for six consecutive days. Preferably, the polypeptide is in the secreted form. The exact details of the dosing scheme, based on

administration and formulation, are provided herein.

Example 26 - Method Of Treating Increased Levels Of The Polypeptide.

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided herein.

Example 27 - Method Of Treatment Using Gene Therapy-Ex Vivo.

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long

terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 10 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or

after having been grown to confluence on cytodex 3 microcarrier beads.

Example 28 - Gene Therapy Using Endogenous Genes Corresponding To Polynucleotides Of The Invention.

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA, 86:8932-8935 (1989); and Zijlstra et al., Nature, 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked

polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na2 HPO4, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately 3X106 cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 μg/ml. 0.5 ml of the cell suspension (containing approximately 1.5.X106 cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 μF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 29 - Method Of Treatment Using Gene Therapy - In Vivo.

Another aspect of the present invention is using in vivo gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997); Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation

94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred

for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle in vivo is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

Example 30 - Transgenic Animals.

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pleuripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated

gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA

expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and reverse transcriptase-PCR(RT-PCR).. Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 31 - Knock-Out Animals.

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (E.g., see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or

regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention in vivo. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (e.g., see Thomas & Capecchi 1987 and Thompson 1989, supra). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site in vivo using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient in vivo. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered in vitro using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the

body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying diseases, disorders, and/or conditions associated with aberrant expression, and in screening for compounds effective in ameliorating such diseases, disorders, and/or conditions.

Example 32 - Production Of An Antibody.

a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing human phosphatase are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of human phosphatase protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for protein human phosphatase are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with human phosphatase polypeptide or, more preferably, with a secreted human phosphatase

polypeptide-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 μg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the human phosphatase polypeptide.

Alternatively, additional antibodies capable of binding to human phosphatase polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody that binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the human phosphatase protein-specific antibody can be blocked by human phosphatase. Such antibodies comprise anti-idiotypic antibodies to the human phosphatase protein-specific antibody and are used to immunize an animal to induce formation of further human phosphatase protein-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO

8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed

Against human phosphatase From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against human phosphatase to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 109 E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 μg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 108 TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 μg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 μg ampicillin/ml and 25 μg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 μm filter (Minisart NML; Sartorius) to give a final concentration of approximately 1013 transducing units/ml

(ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 μg/ml or 10 μg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 1013 TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 μg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

Example 33 - Assays Detecting Stimulation Or Inhibition Of B Cell Proliferation And Differentiation.

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may

impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Purified polypeptides of the invention, or truncated forms thereof, is assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the polypeptides of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed Staphylococcus aureus Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 105 B-cells suspended in culture medium (RPMI 1640

containing 10% FBS, 5 X 10-5M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10-5 dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of a polypeptide of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with polypeptides of the invention identify the results of the activity of the polypeptides on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with polypeptide is used to indicate whether the polypeptide specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and polypeptide-treated mice.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 34 - T Cell Proliferation Assay.

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of 3H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 (l/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-

matched control mAb (B33.1) overnight at 4 degrees C (1 (g/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5 x 104/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of polypeptides of the invention (total volume 200 ul). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100 (l of supernatant is removed and stored –20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 ul of medium containing 0.5 uCi of 3H-thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of 3H-thymidine used as a measure of proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of polypeptides of the invention.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 35 - Effect Of Polypeptides Of The Invention On The Expression Of MHC Class II, Costimulatory And Adhesion Molecules And Cell Differentiation Of Monocytes And Monocyte-Derived Human Dendritic Cells.

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF-(, causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC(RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Thl helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (106/ml) are treated with increasing concentrations of polypeptides of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit(e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc

receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of polypeptides of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Polypeptides, agonists, or antagonists of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2 x 106/ml in PBS containing PI at a final concentration of 5 (g/ml, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA

fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of 5x105 cells/ml with increasing concentrations of the a polypeptide of the invention and under the same conditions, but in the absence of the polypeptide. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of a polypeptide of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit(e.g., R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at 2-1x105 cell/well. Increasing concentrations of polypeptides of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37(C for 2 hours and the reaction is stopped by adding 20 µl 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H2O2 produced by the macrophages, a standard curve of a H2O2 solution of known molarity is performed for each experiment.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 36 - Biological Effects of human phosphatase Polypeptides of the Invention.

Astrocyte and Neuronal Assays.

Recombinant polypeptides of the invention, expressed in Escherichia coli and purified as described above, can be tested for activity in promoting the survival,

neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate a polypeptide of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons in vitro have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." Proc. Natl. Acad. Sci. USA 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of a polypeptide of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays.

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE2 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a

medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or polypeptides of the invention with or without IL-1(for 24 hours. The supernatants are collected and assayed for PGE2 by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without polypeptides of the invention IL-1(for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or polypeptides of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with polypeptides of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP+) and released. Subsequently, MPP+ is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP+ is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotidamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, polypeptides of the invention can be evaluated

to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival in vitro and it can also be tested in vivo for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of a polypeptide of the invention is first examined in vitro in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm2 on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days in vitro and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving in vitro. Therefore, if a polypeptide of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the polypeptide may be involved in Parkinson's Disease.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 37 - The Effect Of The human phosphatase Polypeptides Of The Invention On The Growth Of Vascular Endothelial Cells.

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at 2-5x104 cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnique, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. A polypeptide having the amino acid sequence of SEQ

ID NO:Y, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the polypeptide of the invention may proliferate vascular endothelial cells.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 38 - Stimulatory Effect Of Polypeptides Of The Invention On The Proliferation Of Vascular Endothelial Cells.

For evaluation of mitogenic activity of growth factors, the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)2H-tetrazolium) assay with the electron coupling reagent PMS (phenazine methosulfate) was performed (CellTiter 96 AQ, Promega). Cells are seeded in a 96-well plate (5,000 cells/well) in 0.1 mL serum-supplemented medium and are allowed to attach overnight. After serum-starvation for 12 hours in 0.5% FBS, conditions (bFGF, VEGF165 or a polypeptide of the invention in 0.5% FBS) with or without Heparin (8 U/ml) are added to wells for 48 hours. 20 mg of MTS/PMS mixture (1:0.05) are added per well and allowed to incubate for 1 hour at 37°C before measuring the absorbance at 490 nm in an ELISA plate reader. Background absorbance from control wells (some media, no cells) is subtracted, and seven wells are performed in parallel for each condition. See, Leak et al. In Vitro Cell. Dev. Biol. 30A:512-518 (1994).

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 39 - Inhibition Of PDGF-Induced Vascular Smooth Muscle Cell Proliferation Stimulatory Effect.

HAoSMC proliferation can be measured, for example, by BrdUrd incorporation. Briefly, subconfluent, quiescent cells grown on the 4-chamber slides are transfected with CRP or FITC-labeled AT2-3LP. Then, the cells are pulsed with

10% calf serum and 6 mg/ml BrdUrd. After 24 h, immunocytochemistry is performed by using BrdUrd Staining Kit (Zymed Laboratories). In brief, the cells are incubated with the biotinylated mouse anti-BrdUrd antibody at 4 degrees C for 2 h after being exposed to denaturing solution and then incubated with the streptavidin-peroxidase and diaminobenzidine. After counterstaining with hematoxylin, the cells are mounted for microscopic examination, and the BrdUrd-positive cells are counted. The BrdUrd index is calculated as a percent of the BrdUrd-positive cells to the total cell number. In addition, the simultaneous detection of the BrdUrd staining (nucleus) and the FITC uptake (cytoplasm) is performed for individual cells by the concomitant use of bright field illumination and dark field-UV fluorescent illumination. See, Hayashida et al., J. Biol. Chem... 6:271(36):21985-21992 (1996).

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 40 - Stimulation Of Endothelial Migration.

This example will be used to explore the possibility that a polypeptide of the invention may stimulate lymphatic endothelial cell migration.

Endothelial cell migration assays are performed using a 48 well microchemotaxis chamber (Neuroprobe Inc., Cabin John, MD; Falk, W., et al., J. Immunological Methods 1980;33:239-247). Polyvinylpyrrolidone-free polycarbonate filters with a pore size of 8 um (Nucleopore Corp. Cambridge, MA) are coated with 0.1% gelatin for at least 6 hours at room temperature and dried under sterile air. Test substances are diluted to appropriate concentrations in M199 supplemented with 0.25% bovine serum albumin (BSA), and 25 ul of the final dilution is placed in the lower chamber of the modified Boyden apparatus. Subconfluent, early passage (2-6) HUVEC or BMEC cultures are washed and trypsinized for the minimum time required to achieve cell detachment. After placing the filter between lower and upper chamber, 2.5 x 105 cells suspended in 50 ul M199 containing 1% FBS are seeded in the upper compartment. The apparatus is then incubated for 5 hours at 37°C in a humidified chamber with 5% CO2 to allow cell migration. After the incubation period, the filter is removed and the upper side of the filter with the non-migrated

cells is scraped with a rubber policeman. The filters are fixed with methanol and stained with a Giemsa solution (Diff-Quick, Baxter, McGraw Park, IL). Migration is quantified by counting cells of three random high-power fields (40x) in each well, and all groups are performed in quadruplicate.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 41 - Stimulation Of Nitric Oxide Production By Endothelial Cells.

Nitric oxide released by the vascular endothelium is believed to be a mediator of vascular endothelium relaxation. Thus, activity of a polypeptide of the invention can be assayed by determining nitric oxide production by endothelial cells in response to the polypeptide.

Nitric oxide is measured in 96-well plates of confluent microvascular endothelial cells after 24 hours starvation and a subsequent 4 hr exposure to various levels of a positive control (such as VEGF-1) and the polypeptide of the invention. Nitric oxide in the medium is determined by use of the Griess reagent to measure total nitrite after reduction of nitric oxide-derived nitrate by nitrate reductase. The effect of the polypeptide of the invention on nitric oxide release is examined on HUVEC.

Briefly, NO release from cultured HUVEC monolayer is measured with a NO-specific polarographic electrode connected to a NO meter (Iso-NO, World Precision Instruments Inc.) (1049). Calibration of the NO elements is performed according to the following equation:

2 KNO2 + 2 KI + 2 H2SO4 6 2 NO + I2 + 2 H2O + 2 K2SO4

The standard calibration curve is obtained by adding graded concentrations of KNO2 (0, 5, 10, 25, 50, 100, 250, and 500 nmol/L) into the calibration solution containing KI and H2SO4. The specificity of the Iso-NO electrode to NO is previously determined by measurement of NO from authentic NO gas (1050). The culture medium is removed and HUVECs are washed twice with Dulbecco's phosphate buffered saline. The cells are then bathed in 5 ml of filtered Krebs-Henseleit solution in 6-well plates, and the cell plates are kept on a slide warmer (Lab Line Instruments Inc.) To maintain the temperature at 37°C. The NO sensor probe is

inserted vertically into the wells, keeping the tip of the electrode 2 mm under the surface of the solution, before addition of the different conditions. S-nitroso acetyl penicillamin (SNAP) is used as a positive control. The amount of released NO is expressed as picomoles per 1x106 endothelial cells. All values reported are means of four to six measurements in each group (number of cell culture wells). See, Leak et al. Biochem. and Biophys. Res. Comm. 217:96-105 (1995).

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 42 - Effect Of human phosphatase Polypepides Of The Invention On Cord Formation In Angiogenesis.

Another step in angiogenesis is cord formation, marked by differentiation of endothelial cells. This bioassay measures the ability of microvascular endothelial cells to form capillary-like structures (hollow structures) when cultured in vitro.

CADMEC (microvascular endothelial cells) are purchased from Cell Applications, Inc. as proliferating (passage 2) cells and are cultured in Cell Applications' CADMEC Growth Medium and used at passage 5. For the in vitro angiogenesis assay, the wells of a 48-well cell culture plate are coated with Cell Applications' Attachment Factor Medium (200 ml/well) for 30 min. at 37°C. CADMEC are seeded onto the coated wells at 7,500 cells/well and cultured overnight in Growth Medium. The Growth Medium is then replaced with 300 mg Cell Applications' Chord Formation Medium containing control buffer or a polypeptide of the invention (0.1 to 100 ng/ml) and the cells are cultured for an additional 48 hr. The numbers and lengths of the capillary-like chords are quantitated through use of the Boeckeler VIA-170 video image analyzer. All assays are done in triplicate.

Commercial (R&D) VEGF (50 ng/ml) is used as a positive control. b-esteradiol (1 ng/ml) is used as a negative control. The appropriate buffer (without protein) is also utilized as a control.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 43 - Angiogenic Effect On Chick Chorioallantoic Membrane.

Chick chorioallantoic membrane (CAM) is a well-established system to examine angiogenesis. Blood vessel formation on CAM is easily visible and quantifiable. The ability of polypeptides of the invention to stimulate angiogenesis in CAM can be examined.

Fertilized eggs of the White Leghorn chick (Gallus gallus) and the Japanese qual (Coturnix coturnix) are incubated at 37.8°C and 80% humidity. Differentiated CAM of 16-day-old chick and 13-day-old qual embryos is studied with the following methods.

On Day 4 of development, a window is made into the egg shell of chick eggs. The embryos are checked for normal development and the eggs sealed with cellotape. They are further incubated until Day 13. Thermanox coverslips (Nunc, Naperville, IL) are cut into disks of about 5 mm in diameter. Sterile and salt-free growth factors are dissolved in distilled water and about 3.3 mg/ 5 ml are pipetted on the disks. After air-drying, the inverted disks are applied on CAM. After 3 days, the specimens are fixed in 3% glutaraldehyde and 2% formaldehyde and rinsed in 0.12 M sodium cacodylate buffer. They are photographed with a stereo microscope [Wild M8] and embedded for semi- and ultrathin sectioning as described above. Controls are performed with carrier disks alone.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 44 - Angiogenesis Assay Using A Matrigel Implant In Mouse.

In vivo angiogenesis assay of a polypeptide of the invention measures the ability of an existing capillary network to form new vessels in an implanted capsule of murine extracellular matrix material (Matrigel). The protein is mixed with the liquid Matrigel at 4 degree C and the mixture is then injected subcutaneously in mice where it solidifies. After 7 days, the solid "plug" of Matrigel is removed and examined for the presence of new blood vessels. Matrigel is purchased from Becton Dickinson Labware/Collaborative Biomedical Products.

When thawed at 4 degree C the Matrigel material is a liquid. The Matrigel is mixed with a polypeptide of the invention at 150 ng/ml at 4 degrees C and drawn into cold 3 ml syringes. Female C57Bl/6 mice approximately 8 weeks old are injected with the mixture of Matrigel and experimental protein at 2 sites at the midventral aspect of the abdomen (0.5 ml/site). After 7 days, the mice are sacrificed by cervical dislocation, the Matrigel plugs are removed and cleaned (i.e., all clinging membranes and fibrous tissue is removed). Replicate whole plugs are fixed in neutral buffered 10% formaldehyde, embedded in paraffin and used to produce sections for histological examination after staining with Masson's Trichrome. Cross sections from 3 different regions of each plug are processed. Selected sections are stained for the presence of vWF. The positive control for this assay is bovine basic FGF (150 ng/ml). Matrigel alone is used to determine basal levels of angiogenesis.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 45 - Rescue Of Ischemia In Rabbit Lower Limb Model.

To study the in vivo effects of polynucleotides and polypeptides of the invention on ischemia, a rabbit hindlimb ischemia model is created by surgical removal of one femoral arteries as described previously (Takeshita et al., Am J. Pathol 147:1649-1660 (1995)). The excision of the femoral artery results in retrograde propagation of thrombus and occlusion of the external iliac artery. Consequently, blood flow to the ischemic limb is dependent upon collateral vessels originating from the internal iliac artery (Takeshitaet al. Am J. Pathol 147:1649-1660 (1995)). An interval of 10 days is allowed for post-operative recovery of rabbits and development of endogenous collateral vessels. At 10 day post-operatively (day 0), after performing a baseline angiogram, the internal iliac artery of the ischemic limb is transfected with 500 mg naked expression plasmid containing a polynucleotide of the invention by arterial gene transfer technology using a hydrogel-coated balloon catheter as described (Riessen et al. Hum Gene Ther. 4:749-758 (1993); Leclerc et al. J. Clin. Invest. 90: 936-944 (1992)). When a polypeptide of the invention is used in the treatment, a single bolus of 500 mg polypeptide of the invention or control is

delivered into the internal iliac artery of the ischemic limb over a period of 1 min. through an infusion catheter. On day 30, various parameters are measured in these rabbits: (a) BP ratio - The blood pressure ratio of systolic pressure of the ischemic limb to that of normal limb; (b) Blood Flow and Flow Reserve - Resting FL: the blood flow during undilated condition and Max FL: the blood flow during fully dilated condition (also an indirect measure of the blood vessel amount) and Flow Reserve is reflected by the ratio of max FL: resting FL; (c) Angiographic Score - This is measured by the angiogram of collateral vessels. A score is determined by the percentage of circles in an overlaying grid that with crossing opacified arteries divided by the total number m the rabbit thigh; (d) Capillary density - The number of collateral capillaries determined in light microscopic sections taken from hindlimbs.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 46 - Effect Of Polypeptides Of The Invention On Vasodilation.

Since dilation of vascular endothelium is important in reducing blood pressure, the ability of polypeptides of the invention to affect the blood pressure in spontaneously hypertensive rats (SHR) is examined. Increasing doses (0, 10, 30, 100, 300, and 900 mg/kg) of the polypeptides of the invention are administered to 13-14 week old spontaneously hypertensive rats (SHR). Data are expressed as the mean +/- SEM. Statistical analysis are performed with a paired t-test and statistical significance is defined as $p<0.05$ vs. the response to buffer alone.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 47 - Rat Ischemic Skin Flap Model.

The evaluation parameters include skin blood flow, skin temperature, and factor VIII immunohistochemistry or endothelial alkaline phosphatase reaction. Expression of polypeptides of the invention, during the skin ischemia, is studied using in situ hybridization.

The study in this model is divided into three parts as follows:

a) Ischemic skin

b) Ischemic skin wounds

c) Normal wounds

The experimental protocol includes:

a) Raising a 3x4 cm, single pedicle full-thickness random skin flap (myocutaneous flap over the lower back of the animal).

b) An excisional wounding (4-6 mm in diameter) in the ischemic skin (skin-flap).

c) Topical treatment with a polypeptide of the invention of the excisional wounds (day 0, 1, 2, 3, 4 post-wounding) at the following various dosage ranges: 1mg to 100 mg.

d) Harvesting the wound tissues at day 3, 5, 7, 10, 14 and 21 post-wounding for histological, immunohistochemical, and in situ studies.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 48 - Peripheral Arterial Disease Model.

Angiogenic therapy using a polypeptide of the invention is a novel therapeutic strategy to obtain restoration of blood flow around the ischemia in case of peripheral arterial diseases. The experimental protocol includes:

a) One side of the femoral artery is ligated to create ischemic muscle of the hindlimb, the other side of hindlimb serves as a control.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-3 weeks.

c) The ischemic muscle tissue is collected after ligation of the femoral artery at 1, 2, and 3 weeks for the analysis of expression of a polypeptide of the invention and histology. Biopsy is also performed on the other side of normal muscle of the contralateral hindlimb.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 49 - Ischemic Myocardial Disease Model.

A polypeptide of the invention is evaluated as a potent mitogen capable of stimulating the development of collateral vessels, and restructuring new vessels after coronary artery occlusion. Alteration of expression of the polypeptide is investigated in situ. The experimental protocol includes:

a) The heart is exposed through a left-side thoracotomy in the rat. Immediately, the left coronary artery is occluded with a thin suture (6-0) and the thorax is closed.

b) a polypeptide of the invention, in a dosage range of 20 mg - 500 mg, is delivered intravenously and/or intramuscularly 3 times (perhaps more) per week for 2-4 weeks.

c) Thirty days after the surgery, the heart is removed and cross-sectioned for morphometric and in situ analyzes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 50 - Rat Corneal Wound Healing Model.

This animal model shows the effect of a polypeptide of the invention on neovascularization. The experimental protocol includes:

a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.

b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.

c) Making a pocket (its base is 1-1.5 mm form the edge of the eye).

d) Positioning a pellet, containing 50ng- 5ug of a polypeptide of the invention, within the pocket.

e) Treatment with a polypeptide of the invention can also be applied

topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 51 - Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models.

A. Diabetic db+/db+ Mouse Model.

To demonstrate that a polypeptide of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. et al., J. Surg. Res. 52:389 (1992); Greenhalgh, D.G. et al., Am. J. Pathol. 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman et al. Proc. Natl. Acad. Sci. USA 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel et al., J. Immunol. 120:1375 (1978); Debray-Sachs, M. et al., Clin. Exp. Immunol. 51(1):1-7 (1983); Leiter et al., Am. J. of Pathol. 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. et al., Exp. Neurol. 83(2):221-232 (1984); Robertson et al., Diabetes 29(1):60-67 (1980); Giacomelli et al., Lab Invest. 40(4):460-473 (1979); Coleman, D.L., Diabetes 31 (Suppl):1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel et al., J. Immunol. 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, et al., Am. J. of Pathol. 136:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Bristol-Myers Squibb Company's Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., J. Exp. Med. 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

A polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm2, the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with a polypeptide of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. et al., Am. J. Pathol. 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer can serve as a positive tissue control and human brain tissue can be used as a negative tissue control. Each specimen includes a section with omission of the primary antibody and substitution with non-immune mouse IgG.

Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

B. Steroid Impaired Rat Model

The inhibition of wound healing by steroids has been well documented in various in vitro and in vivo systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahlet al., J. Immunol. 115: 476-481 (1975); Werb et al., J. Exp. Med. 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert et al., An. Intern. Med. 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck et al., Growth Factors. 5: 295-304 (1991); Haynes et al., J. Clin. Invest. 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes et al., J. Clin. Invest. 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck et al., Growth Factors. 5: 295-304 (1991); Haynes et al., J. Clin. Invest. 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce et al., Proc. Natl. Acad. Sci. USA 86: 2229-2233 (1989)).

To demonstrate that a polypeptide of the invention can accelerate the healing process, the effects of multiple topical applications of the polypeptide on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and

received food and water ad libitum. All manipulations are performed using aseptic techniques. This study would be conducted according to the rules and guidelines of Bristol-Myers Squibb Corporations Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The polypeptide of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by

establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm2, the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with a polypeptide of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 52 - Lymphedema Animal Model.

The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of a polypeptide of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal

paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main lymphatic vessels in this area are then electrically coagulated suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosis and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized

with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and Ca2+ comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 53 - Suppression Of TNF Alpha-Induced Adhesion Molecule Expression By A Polypeptide Of The Invention.

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local

vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of a polypeptide of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO2. HUVECs are seeded in 96-well plates at concentrations of 1 x 104 cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 µl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4oC for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 µl of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-

VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37oC for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution) to each well and incubated at 37oC for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 (100) > 10-0.5 > 10-1 > 10-1.5. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNNP reagent must then be added to each of the standard wells. The plate must be incubated at 37oC for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Example 54 – Method of Creating N- and C-terminal Deletion Mutants Corresponding to the Human Phosphatase Polypeptides of the Present Invention.

As described elsewhere herein, the present invention encompasses the creation of N- and C-terminal deletion mutants, in addition to any combination of N- and C-terminal deletions thereof, corresponding to the human phosphatase polypeptides of the present invention. A number of methods are available to one skilled in the art for creating such mutants. Such methods may include a combination of PCR amplification and gene cloning methodology. Although one of skill in the art of molecular biology, through the use of the teachings provided or referenced herein, and/or otherwise known in the art as standard methods, could readily create each deletion mutant of the present invention, exemplary methods are described below using specific BMY_HPP1, BMY_HPP2, BMY_HPP5 and human RET31 deletions as examples.

Briefly, using the isolated cDNA clone encoding the full-length human BMY_HPP1, BMY_HPP2, BMY_HPP5 or RET31 phosphatase polypeptide sequence (as described elsewhere herein, for example), appropriate primers of about 15-25 nucleotides derived from the desired 5' and 3' positions of SEQ ID NO:41, SEQ ID NO:108, SEQ ID NO:149, or SEQ ID NO:151 may be designed to PCR amplify, and subsequently clone, the intended N- and/or C-terminal deletion mutant. Such primers could comprise, for example, an inititation and stop codon for the 5' and 3' primer, respectively. Such primers may also comprise restriction sites to facilitate cloning of the deletion mutant post amplification. Moreover, the primers may comprise additional sequences, such as, for example, flag-tag sequences, kozac sequences, or other sequences discussed and/or referenced herein.

For example, in the case of the N9 to L606 BMY_HPP1 N-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'-GCAGCA GCGGCCGC AATTTCGGATGGAAGGATTATGGTG –3' (SEQ ID NO:167) *NotI*
3' Primer	5'- GCAGCA GTCGAC GAGGCCAGGCTTAGGGCCATC -3' (SEQ ID NO:168) *SalI*

For example, in the case of the M1 to E500 BMY_HPP1 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'- GCAGCA GCGGCCGC ATGGAGGCTGGCATTTACTTCTAC –3' (SEQ ID NO:169) *NotI*
3' Primer	5'- GCAGCA GTCGAC CACCCAAGACCACATCAAGCTGC -3' (SEQ ID NO:170) *SalI*

For example, in the case of the L31 to K150 BMY_HPP2 N-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'-GCAGCA GCGGCCGC CTGTTGGACCTGGGCGTGCGGCACC –3' (SEQ ID NO:171) *NotI*
3' Primer	5'- GCAGCA GTCGAC TTTCGTTCGCTGGTAGAACTGGAAG -3' (SEQ ID NO:172) *SalI*

For example, in the case of the M1 to V111 BMY_HPP2 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'- GCAGCA GCGGCCGC ATGGGCGTGCAGCCCCCCAACTTC –3' (SEQ ID NO:173) *NotI*
3' Primer	5'- GCAGCA GTCGAC CACCAGGTAACAGGCCAGCATGGTG -3' (SEQ ID NO:174) *SalI*

For example, in the case of the I256 to S665 BMY_HPP5 N-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'-GCAGCA GCGGCCGC ATCGCCTACATCATGAAGAGGATGG –3' (SEQ ID NO:104) ***NotI***
3' Primer	5'- GCAGCA GTCGAC GGAGACCTCAATGATTTCCATGCTG -3' (SEQ ID NO:105) ***SalI***

For example, in the case of the M1 to Q367 BMY_HPP5 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'- GCAGCA GCGGCCGC ATGGCCCATGAGATGATTGGAACTC –3' (SEQ ID NO:106) ***NotI***
3' Primer	5'- GCAGCA GTCGAC CTGCACGCTGGGCACGCTGGGCACG -3' (SEQ ID NO:107) ***SalI***

For example, in the case of the I157 to S665 RET31 N-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'-GCAGCA GCGGCCGC ATTGGGCCAACCCGAATTCTTCCC –3' (SEQ ID NO:136) ***NotI***
3' Primer	5'- GCAGCA GTCGAC GGAGACCTCAATGATTTCCATGCTG –3' (SEQ ID NO:137) ***SalI***

For example, in the case of the M1 to K297 RET31 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'- GCAGCA GCGGCCGC ATGGCCCATGAGATGATTGGAACTC –3' (SEQ ID NO:138) ***NotI***
3' Primer	5'- GCAGCA GTCGAC CTTCTTCTCATAGTCCAGGAGTTGG -3' (SEQ ID NO:139) ***SalI***

For example, in the case of the I157 to S660 mRET31 N-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'-GCAGCA GCGGCCGC ATTGGGCCAACTCGAATTCTTCCC –3' (SEQ ID NO:140) ***NotI***
3' Primer	5'- GCAGCA GTCGAC AGAGACCTCGATGATCTCCATGCTG –3' (SEQ ID NO:141) ***SalI***

For example, in the case of the M1 to T297 mRET31 C-terminal deletion mutant, the following primers could be used to amplify a cDNA fragment corresponding to this deletion mutant:

5' Primer	5'- GCAGCA GCGGCCGC ATGGCCCATGAGATGATTGGAACTC –3' (SEQ ID NO:142) *NotI*
3' Primer	5'- GCAGCA GTCGAC CGTCTTCTCATAGTCCATGAGTTGG -3' (SEQ ID NO:143) *SalI*

Representative PCR amplification conditions are provided below, although the skilled artisan would appreciate that other conditions may be required for efficient amplification. A 100 ul PCR reaction mixture may be prepared using 10ng of the template DNA (cDNA clone of Human phosphatase polypeptides), 200 uM 4dNTPs, 1uM primers, 0.25U Taq DNA polymerase (PE), and standard Taq DNA polymerase buffer. Typical PCR cycling condition are as follows:

20-25 cycles:	45 sec, 93 degrees
	2 min, 50 degrees
	2 min, 72 degrees
1 cycle:	10 min, 72 degrees

After the final extension step of PCR, 5U Klenow Fragment may be added and incubated for 15 min at 30 degrees.

Upon digestion of the fragment with the NotI and SalI restriction enzymes, the fragment could be cloned into an appropriate expression and/or cloning vector which has been similarly digested (e.g., pSport1, among others). . The skilled artisan would appreciate that other plasmids could be equally substituted, and may be desirable in certain circumstances. The digested fragment and vector are then ligated using a DNA ligase, and then used to transform competent E.coli cells using methods provided herein and/or otherwise known in the art.

The 5' primer sequence for amplifying any additional N-terminal deletion mutants may be determined by reference to the following formula:

(S+(X * 3)) to ((S+(X * 3))+25), wherein 'S' is equal to the nucleotide position of the initiating start codon of the human BMY_HPP1, BMY_HPP2,

BMY_HPP5 or RET31 phosphatase gene (SEQ ID NO:41, SEQ ID NO:149, SEQ ID NO:151, or SEQ ID NO:108, respectively), and 'X' is equal to the most N-terminal amino acid of the intended N-terminal deletion mutant. The first term will provide the start 5' nucleotide position of the 5' primer, while the second term will provide the end 3' nucleotide position of the 5' primer corresponding to sense strand SEQ ID NO:41, SEQ ID NO:149, SEQ ID NO:151, or SEQ ID NO:108, respectively. Once the corresponding nucleotide positions of the primer are determined, the final nucleotide sequence may be created by the addition of applicable restriction site sequences to the 5' end of the sequence, for example. As referenced herein, the addition of other sequences to the 5' primer may be desired in certain circumstances (e.g., kozac sequences, etc.).

The 3' primer sequence for amplifying any additional N-terminal deletion mutants may be determined by reference to the following formula:

(S+(X * 3)) to ((S+(X * 3))-25), wherein 'S' is equal to the nucleotide position of the initiating start codon of the human BMY_HPP1, BMY_HPP2, BMY_HPP5 or RET31 phosphatase genes (SEQ SEQ ID NO:41, SEQ ID NO:149, SEQ ID NO:151, or SEQ ID NO:108, respectively), and 'X' is equal to the most C-terminal amino acid of the intended N-terminal deletion mutant. The first term will provide the start 5' nucleotide position of the 3' primer, while the second term will provide the end 3' nucleotide position of the 3' primer corresponding to the anti-sense strand of SEQ SEQ ID NO:41, SEQ ID NO:149, SEQ ID NO:151, or SEQ ID NO:108, respectively. Once the corresponding nucleotide positions of the primer are determined, the final nucleotide sequence may be created by the addition of applicable restriction site sequences to the 5' end of the sequence, for example. As referenced herein, the addition of other sequences to the 3' primer may be desired in certain circumstances (e.g., stop codon sequences, etc.). The skilled artisan would appreciate that modifications of the above nucleotide positions may be necessary for optimizing PCR amplification.

The same general formulas provided above may be used in identifying the 5' and 3' primer sequences for amplifying any C-terminal deletion mutant of the present invention. Moreover, the same general formulas provided above may be used in identifying the 5' and 3' primer sequences for amplifying any combination of N-

terminal and C-terminal deletion mutant of the present invention. The skilled artisan would appreciate that modifications of the above nucleotide positions may be necessary for optimizing PCR amplification.

As mentioned above, the same methodology described for BMY_HPP1, BMY_HPP2, BMY_HPP5 or RET31 N- and C-terminal deletion mutants could be applied to creating N- and C-terminal deletion mutants corresponding to HPP_BMY1, HPP_BMY2, HPP_BMY3, HPP_BMY4, HPP_BMY5, RET31, and/or mRET31 as would be appreciated by the skilled artisan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides of the invention (e.g., gene therapy), agonists, and/or antagonists of polynucleotides or polypeptides of the invention.

Example 55 – Method of Mutating The Human Phosphatases Of The Present Invention Using Site Directed/Site-Specific Mutagenesis.

In vitro site-directed mutagenesis is an invaluable technique for studying protein structure-function relationships and gene expression, for example, as well as for vector modification. Approaches utilizing single stranded DNA (ssDNA) as the template have been reported (e.g., T.A. Kunkel et al., 1985, *Proc. Natl. Acad. Sci. USA*), 82:488-492; M.A. Vandeyar et al., 1988, *Gene*, 65(1):129-133; M. Sugimoto et al., 1989, *Anal. Biochem.*, 179(2):309-311; and J.W. Taylor et al., 1985, *Nuc. Acids. Res.*, 13(24):8765-8785).

The use of PCR in site-directed mutagenesis accomplishes strand separation by using a denaturing step to separate the complementary strands and to allow efficient polymerization of the PCR primers. PCR site-directed mutagenesis methods thus permit site specific mutations to be incorporated in virtually any double stranded plasmid, thus eliminating the need for re-subcloning into M13-based bacteriophage vectors or single-stranded rescue. (M.P. Weiner et al., 1995, *Molecular Biology: Current Innovations and Future Trends*, Eds. A.M. Griffin and H.G. Griffin, Horizon Scientific Press, Norfolk, UK; and C. Papworth et al., 1996, *Strategies*, 9(3):3-4).

A protocol for performing site-directed mutagenesis, particularly employing the QuikChange™ site-directed mutagenesis kit (Stratagene, La Jolla, CA; U.S. Patent Nos. 5,789,166 and 5,923,419) is provided for making point mutations, to switch or

substitute amino acids, and to delete or insert single or multiple amino acids in the RATL1d6 amino acid sequence of this invention.

Primer Design

For primer design using this protocol, the mutagenic oligonucleotide primers are designed individually according to the desired mutation. The following considerations should be made for designing mutagenic primers: 1) Both of the mutagenic primers must contain the desired mutation and anneal to the same sequence on opposite strands of the plasmid; 2) Primers should be between 25 and 45 bases in length, and the melting temperature (T_m) of the primers should be greater than, or equal to, 78°C. The following formula is commonly used for estimating the T_m of primers: $T = 81.5 + 0.41$ (%GC) – $675/N$ - %mismatch. For calculating T_m, N is the primer length in bases; and values for %GC and % mismatch are whole numbers. For calculating T_m for primers intended to introduce insertions or deletions, a modified version of the above formula is employed: $T = 81.5 + 0.41$ (%GC) – $675/N$, where N does not include the bases which are being inserted or deleted; 3) The desired mutation (deletion or insertion) should be in the middle of the primer with approximately 10-15 bases of correct sequence on both sides; 4) The primers optimally should have a minimum GC content of 40%, and should terminate in one or more C or G bases; 5) Primers need not be 5'-phosphorylated, but must be purified either by fast polynucleotide liquid chromatography (FPLC) or by polyacrylamide gel electrophoresis (PAGE). Failure to purify the primers results in a significant decrease in mutation efficiency; and 6) It is important that primer concentration is in excess. It is suggested to vary the amount of template while keeping the concentration of the primers constantly in excess (QuikChange™ Site-Directed Mutagenesis Kit, Stratagene, La Jolla, CA).

Protocol for Setting Up the Reactions

Using the above-described primer design, two complimentary oligonucleotides containing the desired mutation, flanked by unmodified nucleic acid sequence, are synthesized. The resulting oligonucleotide primers are purified.

A control reaction is prepared using 5 µl 10x reaction buffer (100mM KCl; 100mM $(NH_4)_2SO_4$; 200mM Tris-HCl, pH 8.8; 20mM $MgSO_4$; 1% Triton® X-100; 1 mg/ml nuclease-free bovine serum albumin, BSA); 2 µl (10ng) of pWhitescript™, 4.5-kb control plasmid (5 ng/µl); 1.25 µl (125 ng) of oligonucleotide control primer #1 (34-mer, 100 ng/µl); 1.25 µl (125 ng) of oligonucleotide control primer #2 (34-mer, 100 ng/µl); 1 µl of dNTP mix; double distilled H_2O; to a final volume of 50 µl. Thereafter, 1 µl of DNA polymerase (*PfuTurbo*® DNA Polymerase, Stratagene), (2.5U/µl) is added. *PfuTurbo*® DNA Polymerase is stated to have 6-fold higher fidelity in DNA synthesis than does *Taq* polymerase. To maximize temperature cycling performance, use of thin-walled test tubes is suggested to ensure optimum contact with the heating blocks of the temperature cycler.

The sample reaction is prepared by combining 5 µl of 10x reaction buffer; x µl (5-50 ng) of dsDNA template; x µl (125 ng) of oligonucleotide primer #1; x µl (5-50 ng) of dsDNA template; x µl (125 ng) of oligonucleotide primer #2; 1 µl of dNTP mix; and ddH_2O to a final volume of 50 µl. Thereafter, 1 µl of DNA polymerase (*PfuTurbo* DNA Polymerase, Stratagene), (2.5U/µl) is added.

It is suggested that if the thermal cycler does not have a hot-top assembly, each reaction should be overlaid with approximately 30 µl of mineral oil.

Cycling the Reactions

Each reaction is cycled using the following cycling parameters:

Segment	Cycles	Temperature	Time
1	1	95°C	30 seconds
2	12-18	95°C	30 seconds
		55°C	1 minute
		68°C	2 minutes/kb of plasmid length

For the control reaction, a 12-minute extension time is used and the reaction is run for 12 cycles. Segment 2 of the above cycling parameters is adjusted in accordance with

the type of mutation desired. For example, for point mutations, 12 cycles are used; for single amino acid changes, 16 cycles are used; and for multiple amino acid deletions or insertions, 18 cycles are used. Following the temperature cycling, the reaction is placed on ice for 2 minutes to cool the reaction to ≤37°C.

Digesting the Products and Transforming Competent Cells

One µl of the *Dpn*I restriction enzyme (10U/µl) is added directly (below mineral oil overlay) to each amplification reaction using a small, pointed pipette tip. The reaction mixture is gently and thoroughly mixed by pipetting the solution up and down several times. The reaction mixture is then centrifuged for 1 minute in a microcentrifuge. Immediately thereafter, each reaction is incubated at 37°C for 1 hour to digest the parental (i.e., the non-mutated) supercoiled dsDNA.

Competent cells (i.e., XL1-Blue supercompetent cells, Stratagene) are thawed gently on ice. For each control and sample reaction to be transformed, 50 µl of the supercompetent cells are aliquotted to a prechilled test tube (Falcon 2059 polypropylene). Next, 1 µl of the *Dpn*I-digested DNA is transferred from the control and the sample reactions to separate aliquots of the supercompetent cells. The transformation reactions are gently swirled to mix and incubated for 30 minutes on ice. Thereafter, the transformation reactions are heat-pulsed for 45 seconds at 42°C for 2 minutes.

0.5 ml of NZY+ broth, preheated to 42°C is added to the transformation reactions which are then incubated at 37°C for 1 hour with shaking at 225-250 rpm. An aliquot of each transformation reaction is plated on agar plates containing the appropriate antibiotic for the vector. For the mutagenesis and transformation controls, cells are spread on LB-ampicillin agar plates containing 80 µg/ml of X-gal and 20mM MIPTG. Transformation plates are incubated for >16 hours at 37°C.

Example 56 - Complementary Polynucleotides Of The BMY_HPP2 Phosphatase Of The Present Invention.

Antisense molecules or nucleic acid sequences complementary to the BMY_HPP2 protein-encoding sequence, or any part thereof, is used to decrease or to

inhibit the expression of naturally occurring BMY_HPP2. Although the use of antisense or complementary oligonucleotides comprising about 15 to 35 base-pairs is described, essentially the same procedure is used with smaller or larger nucleic acid sequence fragments. An oligonucleotide based on the coding sequence of BMY_HPP2 protein, as shown in Figure 21, or as depicted in SEQ ID NO:151, for example, is used to inhibit expression of naturally occurring BMY_HPP2. The complementary oligonucleotide is typically designed from the most unique 5' sequence and is used either to inhibit transcription by preventing promoter binding to the coding sequence, or to inhibit translation by preventing the ribosome from binding to the BMY_HPP2 protein-encoding transcript. However, other regions may also be targeted.

Using an appropriate portion of the signal and/or 5' sequence of SEQ ID NO:151, an effective antisense oligonucleotide includes any of about 15-35 nucleotides spanning the region which translates into the signal or 5' coding sequence, among other regions, of the polypeptide as shown in Figure 21 (SEQ ID NO:152). Appropriate oligonucleotides are designed using OLIGO 4.06 software and the BMY_HPP2 protein coding sequence (SEQ ID NO:151). Preferred oligonucleotides are dideoxy based and are provided below. The oligonucleotides were synthesized using chemistry essentially as described in U.S. Patent No. 5,849,902; which is hereby incorporated herein by reference in its entirety.

ID#	Sequence
13600	GGAUAUCACUACUGCAUUGCCUGGA (SEQ ID NO:179)
13601	UACAGCAGAUCUGUGCAGGCCAGGU (SEQ ID NO:180)
13602	UGAUCACACAGUAGCGGAAGAUGCU (SEQ ID NO:181)
13603	AGGAGUAGCAGAAUGGUUAGCCUUC (SEQ ID NO:182)
13604	UGAAAGCAGGCGAGAUUCGAUCCGA (SEQ ID NO:183)

The BMY_HPP2 polypeptide has been shown to be involved in the regulation of the mammalian cell cycle. Subjecting cells with an effective amount of a pool of all five of the above antisense oligoncleotides resulted in a significant increase in Cyclin D expression/activity providing convincing evidence that BMY_HPP2 at least regulates the activity and/or expression of Cyclin D either directly, or indirectly. Moreover, the results suggest the physiological role of BMY_HPP2 is the negative

regulation of Cyclin D activity and/or expression, either directly or indirectly. The Cyclin D assay used is described below and was based upon the analysis of Cyclin D activity as a downstream marker for proliferative signal transduction events.

Transfection of post-quiescent A549 cells With AntiSense Oligonucleotides.

Materials needed:

- A549 cells maintained in DMEM with high glucose (Gibco-BRL) supplemented with 10% Fetal Bovine Serum, 2mM L-Glutamine, and 1X penicillin/streptomycin.
- Opti-MEM (Gibco-BRL)
- Lipofectamine 2000 (Invitrogen)
- Antisense oligomers (Sequitur)
- Polystyrene tubes.
- Tissue culture treated plates.

Quiescent cells were prepared as follows:

Day 0: 300, 000 A549 cells were seeded in a T75 tissue culture flask in 10 ml of A549 media, and incubated in at 37°C, 5% CO_2 in a humidified incubator for 48 hours.

Day 2: The T75 flasks were rocked to remove any loosely adherent cells, and the A549 growth media removed and replenished with 10 ml of fresh A549 media. The cells were cultured for six days without changing the media to create a quiescent cell population.

Day 8: Quiescent cells were plated in multi-well format and transfected with antisense oligonucleotides.

A549 cells were transfected according to the following:

1. Trypsinize T75 flask containing quiescent population of A549 cells.
2. Count the cells and seed 24-well plates with 60K quiescent A549 cells per well.

3. Allow the cells to adhere to the tissue culture plate (approximately 4 hours).
4. Transfect the cells with antisense and control oligonucleotides according to the following:

a. A 10X stock of lipofectamine 2000 (10 ug/ml is 10X) was prepared, and diluted lipid was allowed to stand at RT for 15 minutes.
Stock solution of lipofectamine 2000 was 1 mg/ml.
10 X solution for transfection was 10 ug/ml.
To prepare 10X solution, dilute 10 ul of lipofectamine 2000 stock per 1 ml of Opti-MEM (serum free media).

b. A 10X stock of each oligomer was prepared to be used in the transfection.
Stock solutions of oligomers were at 100 uM in 20 mM HEPES, pH 7.5.
10X concentration of oligomer was 0.25 uM.
To prepare the 10X solutions, dilute 2.5 ul of oligomer per 1 ml of Opti-MEM.

c. Equal volumes of the 10X lipofectamine 2000 stock and the 10X oligomer solutions were mixed well, and incubated for 15 minutes at RT to allow complexation of the oligomer and lipid. The resulting mixture was 5X.

d. After the 15 minute complexation, 4 volumes of full growth media was added to the oligomer/lipid complexes (solution was 1X).

e. The media was aspirated from the cells, and 0.5 ml of the 1X oligomer/lipid complexes added to each well.

f. The cells were incubated for 16-24 hours at 37°C in a humidified CO_2 incubator.

g. Cell pellets were harvested for RNA isolation and TaqMan analysis of downstream marker genes.

TaqMan Reactions

Quantitative RT-PCR analysis was performed on total RNA preps that had been treated with DNaseI or poly A selected RNA. The Dnase treatment may be performed using methods known in the art, though preferably using a Qiagen Rneasy kit to purify the RNA samples, wherein DNAse I treatment is performed on the column.

Briefly, a master mix of reagents was prepared according to the following table:

Dnase I Treatment

Reagent	Per r'xn (in uL)
10x Buffer	2.5
Dnase I (1 unit/ul @1 unit per ug sample)	2
DEPC H_2O	0.5
RNA sample @ 0.1 ug/ul (2-3 ug total)	20
Total	25

Next, 5 ul of master mix was aliquoted per well of a 96-well PCR reaction plate (PE part # N801-0560). RNA samples were adjusted to 0.1 ug/ul with DEPC treated H_2O (if necessary), and 20 ul was added to the aliquoted master mix for a final reaction volume of 25 ul.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and briefly spun in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient

The plates were incubated at 37°C for 30 mins. Then, an equal volume of 0.1mM EDTA in 10mM Tris was added to each well, and heat inactivated at 70°C for 5 min. The plates were stored at -80°C upon completion.

RT reaction

A master mix of reagents was prepared according to the following table:

RT reaction

Reagent	RT Per Rx'n (in ul)	No RT Per Rx'n (in ul)
10x RT buffer	5	2.5
$MgCl_2$	11	5.5
DNTP mixture	10	5
Random Hexamers	2.5	1.25
Rnase inhibitors	1.25	0.625
RT enzyme	1.25	-
Total RNA 500ng (100ng no RT)	19.0 max	10.125 max
DEPC H_2O	-	-
Total	50uL	25uL

Samples were adjusted to a concentration so that 500ng of RNA was added to each RT rx'n (100ng for the no RT). A maximum of 19 ul can be added to the RT rx'n mixture (10.125 ul for the no RT.) Any remaining volume up to the maximum values was filled with DEPC treated H_2O, so that the total reaction volume was 50 ul (RT) or 25 ul (no RT).

On a 96-well PCR reaction plate (PE part # N801-0560), 37.5 ul of master mix was aliquoted (22.5 ul of no RT master mix), and the RNA sample added for a total reaction volume of 50ul (25 ul, no RT). Control samples were loaded into two or even three different wells in order to have enough template for generation of a standard curve.

The wells were capped using strip well caps (PE part # N801-0935), placed in a plate, and spin briefly in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient.

For the RT-PCR reaction, the following thermal profile was used:

- 25°C for 10 min
- 48°C for 30 min
- 95°C for 5 min
- 4°C hold (for 1 hour)
- Store plate @-20°C or lower upon completion.

TaqMan reaction (Template comes from RT plate.)

A master mix was prepared according to the following table:

TaqMan reaction (per well)

Reagent	Per Rx'n (in ul)
TaqMan Master Mix	4.17
100 uM Probe (SEQ ID NO:186)	.025
100 uM Forward primer (SEQ ID NO:184)	.05
100 uM Reverse primer (SEQ ID NO:185)	.05
Template	-
DEPC H_2O	18.21
Total	22.5

The primers used for the RT-PCR reaction is as follows:

Cyclin D primer and probes:

Forward Primer: ACTACCGCCTCACACGCTTC (SEQ ID NO:184)

Reverse Primer: CTTGACTCCAGCAGGGCTTC (SEQ ID NO:185)

TaqMan Probe: ATCAAGTGTGACCCAGACTGCCTCCG (SEQ ID NO:186)

Using a Gilson P-10 repeat pipetter, 22.5 ul of master mix was aliquouted per well of a 96-well optical plate. Then, using P-10 pipetter, 2.5 ul of sample was added to individual wells. Generally, RT samples are run in triplicate with each primer/probe set used, and no RT samples are run once and only with one primer/probe set, often gapdh (or other internal control).

A standard curve is then constructed and loaded onto the plate. The curve has five points plus one no template control (NTC, =DEPC treated H_2O). The curve was made with a high point of 50 ng of sample (twice the amount of RNA in unknowns), and successive samples of 25, 10, 5, and 1 ng. The curve was made from a control sample(s) (see above).

The wells were capped using optical strip well caps (PE part # N801-0935), placed in a plate, and spun in a centrifuge to collect all volume in the bottom of the tubes. Generally, a short spin up to 500rpm in a Sorvall RT is sufficient.

Plates were loaded onto a PE 5700 sequence detector making sure the plate is aligned properly with the notch in the upper right hand corner. The lid was tightened down and run using the 5700 and 5700 quantitation programes and the SYBR probe using the following thermal profile:

- 50°C for 2 min
- 95°C for 10 min
- and the following for 40 cycles:
  - 95°C for 15 sec
  - 60°C for 1 min
- Change the reaction volume to 25ul.

Once the reaction was complete, a manual threshold of around 0.1 was set to minimuze the background signal.Additional information relative to operation of the GeneAmp 5700 machine may be found in reference to the following manuals: "GeneAmp 5700 Sequence Detection System Operator Training CD"; and the "User's Manual for 5700 Sequence Detection System"; available from Perkin-Elmer and hereby incorporated by reference herein in their entirety.

Cyclin D1 is a critical regulator of the process of cell division. It has been identified as an early modulator of the G1 phase of the cell cycle, and cyclin D1 expression increases as cells enter that phase of the cell cycle. It has long been thought that an ability to pharmacologically block cancerous cells in any part of the cell cycle will have a negative impact on the tumor and be beneficial for managing the disease. Support for this rationale comes from the observation that effective drugs such as Taxol block the cell cycle in G2 phase. Importantly, the rapidly dividing cells found in the cancerous state require abundant levels of cyclin D1 to maintain an accelerated rate of proliferation and proceed to S-phase. Most noteably, overexpression of cyclinD1 is a hallmark of several types of human tumors, especially breast tumors (J Mammary Gland Biol Neoplasia 1996 Apr;1(2):153-62). As such, it

is thought that drugs that affect cyclin D1, directly or indirectly, would block cancer cells from dividing and have a beneficial effect for patients. Such drug targets could lie within the signal transduction pathway between the oncogene ras and the nucleus, where cell cycle modulators control DNA synthesis (J. Biol Chem 2000, Oct 20;275 (42):32649-57). Even more evidence exists suggesting that the Wnt pathway, mediated by the tumor suppressor betacatenin, regulates the cell cycle via transcriptional control of cyclinD1 (Oncogene 2001 Aug 23;20(37):5093-9: PNAS 2000 Apr11;97(8):4262-6). Thus targets influencing beta catenin/TCF4 function could also affect cyclin D1 transcript levels. As mutations in oncogenes such as ras, and tumor suppressors such as beta catenin are common to may cancers, it is obvious that cyclinD1 levels are indicative of the condition of the cell and its preparedness to proliferate, and affecting cyclinD1 levels and activity could be achieved by numerous mechanisms embodied in multiple pathways .

Antisense inhibition of the HPP_BMY2 phosphatase levels provokes a response in A549 cells that indicates the regulatory pathways controlling cydlinD1 levels are affected. This implicates HPP_BMY2 in pathways important for maintenance of the proliferative state and progression through the cell cycle. As stated above, there are numerous pathways that could have either indirect or direct affects on the transcriptional levels of cyclin D1. Importantly, a major part of the pathways implicated involve the regulation of protein activity through phosphorylation. In as much as HPP_BMY2 is a phosphatase enzyme, it is readily conceivable that dephosphorylation of proteins, the counter activity to the kinases in the signal transduction cascades, contributes to the signals determining cell cycle regulation and proliferation, including regulating cyclin D1 levels. Additionally, the complexity of the interactions between proteins in the pathways described also allow for affects on the pathway eliciting compensatory responses. That is, inhibition of one pathway affecting cyclinD1 activity could provoke a more potent response and signal from another pathway of the same end, resulting in upregulation of cyclin D1. Thus, the effect of inhibition of HPP_BMY2 resulting in slight increases in cyclin D1 levels could indicate that one pathway important to cancer is effected in a way to implicate HPP_BMY2 as a potential target for pharmacologic inhibition for cancer treatment,

yet a parallel pathway in the context of the experiment would replace HPP_BMY2 and propagate dysregulation of Cyclin D1.

Example 57 – Method of Creating RET31 and Truncated RET31 Fusion Protein Constructs And Methods of Expression and Purification Of The Same.

The GST fusion proteins were designed to contain the full-length RET31 protein sequence (SEQ ID NO:109), as well as a C-terminal deletion mutant of the RET31 protein sequence corresponding to amino acids M1 to T302 of SEQ ID NO:109 which was truncated after the phosphatase homology domain ending at about amino acid residue 297 of SEQ ID NO:109.

In order to generate the RET31 fusion proteins, three PCR primers were designed and received from Life Technologies (Gaithersburg, MD). The oligos were:

Oligo number	Name	Sequence
S5972B08	RET31for	5'-CATATGGGATCCATGGCCCATGAGATTG (SEQ ID NO:187)
S5972B09	RET31rev	5'-GGTACCCTCGAGTCAGGAGACCTCAATGAT (SEQ ID NO:188)
S6311A01	RET31rev2-2	5'-GGTACCCTCGAGTCAAGTCTGGTTCTTAAT (SEQ ID NO:189)

Clones containing the original gene sequence of the full-length RET 31 polynucleotide (SEQ ID NO:108) were used as a template for the subsequent PCR. The clone was linearized using a restriction enzyme prior to PCR. PCR was performed using random hexamers and the Expand High Fidelity PCR System (ROCHE). Amplification was achieved using RET31 forward primer (SEQ ID NO:187) paired with either RET31 Rev (SEQ ID NO:188) or Rev2-2 (SEQ ID NO:189), for the full-length cDNA or truncated cDNA respectively. The thermocycler settings were 94°C for 30 sec, 55°C for 30 sec and 72°C for 60 seconds for 25 cycles. The amplimers were gel purified by the QIAgen Extraction kit (QIAgen, Valencia, CA) and ligated, using T4 DNA ligase, into the pGEX 4T3 Vector (Amersham Pharmacia Biotech) and sequenced using standard methods.

Appropriate clones were chosen based upon the sequencing data, and were used for subsequent steps. Protein expression was induced with 0.1mM IPTG over a 5-hour period. The fusion protein was isolated following the methods outlined in Ausubel, et al., 1992, Short Protocols in Molecular Biology, John Wiley and Sons, Inc., pp.16-28 to 16-31., using GST beads (Pierce) and reduced Glutathione (Sigma). The predicted proteins were approximately 100kD for the full-length protein and 60

kD for the truncated protein. To confirm that GST fusion protein was present, the proteins, along with appropriate markers, were run on a 4-12% NuPage BIS TRIS Gel with Mops buffer and transferred to a PVD membrane at 4°C. The membrane was blocked with 5% nonfat dry milk in TBS, and probed with a rabbit anti-GST antibody (developed in house). A goat anti-rabbit conjugated to HRP secondary antibody (Biorad) was used and the blot was developed with ECL reagent (Amersham Pharmacia Biotech) – data not shown.

Example 57 – Method of Assaying the Phosphatase Activity Of The RET31 Polypeptide.

The phosphatase activity for the full-length RET31 and the M1 to T302 C-terminal RET31 GST fusion proteins were measured by assaying the ability of the proteins to hydrolyze para-nitrophenylphosphate, a compound known to be a substrate for phosphatases, as described in Krejsa, C. et al., J. Biol. Chem.. Vol. 272, p.11541-11549, 1997 (which is hereby incorporated in its entirety herein). The proteins are incubated with para-nitrophenylphosphate in a solution containing 10 mM imidazole, pH 7.0, 1 mM EDTA, 2 mM dithiothreitol, and 5 μg/ml BSA for 2 hours with and without sodium orthovanadate (Fisher) prepared in distilled water. The progress of the phosphatase reaction in a 96-well format was monitored by the OD405 nm on a plate reader (Molecular Devices) at 10-minute intervals in the kinetic mode.

The RET31-GST full length (FL), M1 to T302 C-terminal RET31-GST (trunc), or GST alone were purified and assayed for cleavage of para-nitrophenylphosphate (pNPP). The bars represent the average of triplicate determinations, and the standard deviations are as shown. Each protein preparation was assayed in the absence and presence of 2 mM of the phosphatase inhibitor orthovanadate. The full length and truncated versions clearly demonstrated activity compared to the GST protein as shown in Figure 36. In addition, the full length and truncated protein phosphatase activity was blocked by the phosphatase inhibitor vanadate, as shown.

Of particular significance is the unexpected five fold increase in phosphatase activity of the M1 to T302 C-terminal RET31-GST (trunc) fusion protein relative to the RET31-GST full length (FL) fusion protein.

While the described phosphatase assay elucidated the phosphatase activity of the full-length RET31 (SEQ ID NO:109) and M1 to T302 RET31 C-terminal deletion mutant (amino acids 1 to 302 of SEQ ID NO:109), subsequent sequencing of the RET31-GST full length (FL) and M1 to T302 C-terminal RET31-GST (trunc) fusion protein constructs determined that several amino acid mutations were unintentionally introduced during their construction. The sequences of the RET31 portions of both fusion proteins are provided below. Since the location of these mutations are not within the conserved phosphatase domain nor near any active site residues, it is not believed they would have any effect on the phosphatase activity of either construct. Rather, the observed phosphatase activity is believed to be representative of the wild type RET31 polypeptide sequence (SEQ ID NO:109) for the RET31-GST full length (FL), while the observed phosphatase activity of the M1 to T302 C-terminal RET31-GST (trunc) fusion protein is believed to be representative of the wild type M1 to T302 C-terminal RET31 C-terminal deletion (amino acids M1 to T302 of SEQ ID NO:109). One skilled in the art of molecular biology could easily correct the mutations of both constructs using known methods in conjunction with the information and teachings described herein. Nonetheless, the polypeptide sequences of the RET31 portion of both fusion proteins are encompassed by the present invention.

In preferred embodiments, the following RET31 polypeptide is encompassed by the present invention:

MAHEIGTQIVTERLVALLESGTEKVLLIDSRPFVEYNTSHILEAININCSKLMKR
RLQQDKVLITELIQHSAKHKVDIDCSQKVVVYDQSSQDVASLSSDCFLTVLLG
KLEKSFNSVHLLAGGFAEFSRCFPGLCEGKSTLVPTCISQPCLPVANIGPTRILP
NLYLGCQRDVLNKELMQQNGIGYVLNASNTCPKPDFIPESHFLRVPVNDSFCE
KILPWLDKSVDFIEKAKASNGCVLVHCLAGISRSATIAIAYIMKRMDMSLDEA
YRFVKEKRPTISPSFNFLGQLLDYEKKIKNQAGASGPKSKLKLLHLEKPNEPVP
AVSEGGQKSETPLSPPCADSATSEAAGQRPVHPASVPSVPSVQPSLLEDSPLVQ
ALSGLHLSADRLEDSNKLKRSFSLDIKSVSYSASMAASLHGFSSSEDALEYYK
PSTTLDGTNKLCQFSPVQELSEQTPETSPDKEEASIPKKLQTARPSDSQSKRLH
SVRTSSSGTAQRSLLSPLHRSGSVEDNYHTSFLFGLSTSQQHLTKSAGLGLKG
WHSDILAPQTSTPSLTSSWYFATESSHFYSASAIYGGSASYSAYSRSQLPTCGD

QVYSVRRRQKPSDRADSRRSWHEESPFEKQFKRRSCQMEFGESIMSENRSREE LGKVGSQSSFSGSMEIIEVS (SEQ ID NO:190). Polynucleotides encoding this polypeptide are also provided.

In preferred embodiments, the following M1 to T302 RET31 polypeptide is encompassed by the present invention:

MAHEIVGTQIVTERLVALLESGTEKVLLIDSRPFVEYNTSHILEAININCSKLMKRRLQQDKVLI TELIQHSAKHKVDIDCSQKVVVYDQSSQDVASLSSDCFLTVLLGKLEKSFNSVHLLAGGFAEF SRCFPGLCEGKSTLVPTCISQPCLPVANIGPTRILPNLYLGCQRDVLNKELMQQNGIGYVLNAS NTCPKPDFIPESHFLRVPVNDSFCEKILPWLDKSVDFIEKAKASNGCVLVHCLAGISRSATIAIA YIMKRMDMSLDEAYRFVKEKRPTISPSFNFLGQLLDYEKKIKNQT (SEQ ID NO:191). Polynucleotides encoding this polypeptide are also provided.

The present invention encompasses the application of this phosphatase activity assay to the other phosphatases of the present invention.

Example 58 – Method Of Assessing The Expression Profile Of The RET31 Phosphatase Polypeptides Of The Present Invention At The Level Of The Protein Using Immunohistochemistry

Peptide Selection and Antibody Production

The sequence for the RET31 polypeptide (SEQ ID NO:109) was analyzed by the algorithm of Hopp and Woods to determine potential peptides for synthesis and antibody production. The peptides were then BLASTed against the SWISS-PROT database to determine the uniqueness of the identified peptide and to help predict the specificity of the resulting antibodies. The following RET31 polypeptide fragments were selected according to the methods above for peptide synthesis:

KNQTGASGPKSKKLKLLHLE (SEQ ID NO:192); and

CKKLQTARPSDSQSKRLHS (SEQ ID NO:193). Rabbit polyclonal antisera was generated for both synthesized RET31 peptides. In order to allow for peptide conjugation to the carrier protein, a cysteine residue was added to the N-terminus of the SEQ ID NO:193 peptide. The third bleeds were subjected to peptide affinity purification, and the resulting antisera were then used as primary antibodies in immunohistochemistry experiments. The antisera for the SEQ ID NO:192 peptide was labeled RET31 antibody 299, while the antisera for the SEQ ID NO:193 peptide was labeled RET31 antibody 469 antibody.

Antibody Titration Protocol and Positive Control Study Results

Antibody titration experiments were conducted with RET31 antibodies 299 and 469 (both rabbit polyclonals) to establish concentrations that would result in minimal background and maximal detection of signal. Serial dilutions were performed at 1:50. 1:100, 1:250, 1:500, and 1:1000. The serial dilution study demonstrated the highest signal-to-noise ratios at dilutions 1:250 and 1:400, on paraffin-embedded, formalin-fixed tissues for both antibodies. These concentrations were used for the study. RET31 antibodies 299 and 469 were used as primary antibodies, and the principal detection system consisted of a Vector anti-rabbit secondary (BA-1000; DAKO Corp.), a Vector ABC-AP Kit (AK-5000; DAKO Corp.) with a Vector Red substrate kit (SK-5100; DAKO Corp.), which was used to produce a fuchsia-colored deposit. Tissues were also stained with a positive control antibody (CD31) to ensure that the tissue antigens were preserved and accessible for immunohistochemical analysis. Only tissues that stained positive for CD31 were chosen for the remainder of the study. The negative control consisted of performing the entire immunohistochemistry procedure on adjacent sections in the absence of primary antibody. Slides were imaged using a DVC 1310C digital camera coupled to a Nikon microscope. Images were stored as TIFF files using Adobe PhotoShop.

Immunohistochemistry Procedure

Slides containing paraffin sections (LifeSpan BioSciences, Inc.; Seattle, WA) were deparaffinized through xylene and alcohol, rehydrated, and then subjected to the steam method of target retrieval (#S1700; DAKO Corp.; Carpenteria, CA). Immunohistochemical assay techniques are commonly known in the art and are described briefly herein. Immunocytochemical (ICC) experiments were performed on a DAKO autostainer following the procedures and reagents developed by DAKO. Specifically, the slides were blocked with avidin, rinsed, blocked with biotin, rinsed, protein blocked with DAKO universal protein block, machine blown dry, primary antibody, incubated, and the slides rinsed. Biotinylated secondary antibody was applied using the manufacturer's instructions (1 drop/10 ml, or approximately 0.75 μg/mL), incubated, rinsed slides, and applied Vectastain ABC-AP reagent for 30

minutes. Vector Red was used as substrate and prepared according to the manufacturer's instructions just prior to use.

Immunohistochemistry Results

The immunohistochemistry results were consistent with the Northern Blot and RT-PCR expression profiles described elsewhere herein for the RET31 polypetide. Specifically, moderate to strong staining was observed in normal respiratory epithelial cell bodies and cilia. Types I and II pneumocytes were also moderately positive, as were neutrophils, mast cells, and macrophages in normal lung. In asthmatic patients, respiratory epithelial cell bodies stained less intensely, but cilia continued to stain strongly. Pneumocytes also stained less intensely than normal tissue. Inflammatory cell staining did not differ from normal tissue. Bronchial smooth muscle stained faintly in normal and asthmatic lungs. Cytoplasmic, diffuse nucleoplasmic, and nucleolar staining was observed in several cell types, including vascular endothelial and respiratory epithelial cells.

Moderate to strong staining was seen in chondrocytes and rimming osteoblasts in degenerative arthritis. In constrast, osteocytes were negative, as was the osteoid matrix. Hematopoetic tissue showed strongly positive cytoplasm and nucleus in myeloid series cells at all stages of maturation. Megakaryocytic and erythroid cells were negative.

Schwann cells and vascular endothelial cells were moderately to strongly positive in normal colon, in contrast to epithelial cells and ganglion cells, which were negative. Inflammatory cells, such as neutrophils, eosinophils, macrophages, and mast wells were strongly positive. Plasma cells showed blush to faint staining. Lymphocytes in norml colon showed strong punctate nuclear and nucleolar staining. In contrast to normal colon, the colon sections with ulcerative colitis showed less prominent nucleolar staining in lymphocytes. Neuroendocrine cells in the epithelium were faintly positive.

Normal lung showed strong cilial staining in the respiratory epithelial cells, with only blush, diffuse, nuclear staining in the cell body of these cells. Pneumocytes were faintly to moderately positive, as were alveolar macrophages and vascular endothelium. Asthmatic lungs continued to show strong cilial staining, but showed

blush positivity in normal lung, and were predominately negative in diseased lung. Pneumocyte staining varied from blush to moderately positive in asthmatic lungs. Pneumocyte staining wa unchanged from normal lung. Inflammatory cell staining was similar to normal tissue.

Moderate staining was seen in the stratum granulosum in normal skin, whereas the other layers were negative or showed blush positivity. Melanocytes were moderately to strongly positive, as were hair follicles and eccrine and sebaceous glands. Skin with psoriasis showed 'strong staining in the stratum granulosum, increased from normal skin. In contrast to normal skin, melanocytes in skin were negative. In the psoriasisform dermatitis sample, the staining pattern was similar to that observed in normal skin.

In synovium, the reactive synoviocytes in one sample of rheumatoid arthritis were faintly to moderately positive, in contrast to normal synoviocytes, which were negative or showed blush staining. In the second sample of rheumatoid arthritis, the difference in synoviocyte staining was smaller than in the first sample.

Interesting observations in this study included the very prominent staining of the nucleolus of lymphocytes and other cell types. In inflammatory bowel disease, the lymphocytes did not show nucleolar staining as prominately as in normal colon. Skin with psorisis had very prominent staining of the stratum granulosum, in comparison to normal skin or to the psoriasiform dermatitis sample.

The present invention encompasses the application of this phosphatase activity assay to the other phosphatases of the present invention.

Example 59 – Method Of Assessing The Expression Profile Of The Novel Phosphatases of Polypeptides Of The Present Invention Using Expanded mRNA Tissue and Cell Sources

Total RNA from tissues was isolated using the TriZol protocol (Invitrogen) and quantified by determining its absorbance at 260nM. An assessment of the 18s and 28s ribosomal RNA bands was made by denaturing gel electrophoresis to determine RNA integrity.

The specific sequence to be measured was aligned with related genes found in GenBank to identity regions of significant sequence divergence to maximize primer

and probe specificity. Gene-specific primers and probes were designed using the ABI primer express software to amplify small amplicons (150 base pairs or less) to maximize the likelihood that the primers function at 100% efficiency. All primer/probe sequences were searched against Public Genbank databases to ensure target specificity. Primers and probes were obtained from ABI.

For BMY_HPP1, the primer probe sequences were as follows

Forward Primer 5'-TCAGAGAATGGGCCAACAAGA-3' (SEQ ID NO:194)
Reverse Primer 5'-CGAAAACGCTCGAGGAATGA-3' (SEQ ID NO:195)
TaqMan Probe5' –CAGGCCTAGGTTCCTCCTCTCGGAAA-3' (SEQ ID NO:196)

For BMY_HPP2, the primer probe sequences were as follows

Forward Primer 5'-TCAGAGAATGGGCCAACAAGA-3' (SEQ ID NO:197)
Reverse Primer 5'-CGAAAACGCTCGAGGAATGA-3' (SEQ ID NO:198)
TaqMan Probe5' –CAGGCCTAGGTTCCTCCTCTCGGAAA-3' (SEQ ID NO:199)

For BMY_HPP4, the primer probe sequences were as follows

Forward Primer 5'-TCAGAGAATGGGCCAACAAGA-3' (SEQ ID NO:200)
Reverse Primer 5'-CGAAAACGCTCGAGGAATGA-3' (SEQ ID NO:201)
TaqMan Probe5' –CAGGCCTAGGTTCCTCCTCTCGGAAA-3' (SEQ ID NO:202)

For BMY_HPP5 (RET31), the primer probe sequences were as follows

Forward Primer 5'-TCAGAGAATGGGCCAACAAGA-3' (SEQ ID NO:203)
Reverse Primer 5'-CGAAAACGCTCGAGGAATGA-3' (SEQ ID NO:204)
TaqMan Probe5' –CAGGCCTAGGTTCCTCCTCTCGGAAA-3' (SEQ ID NO:205)

The same BMY_HPP5 primer probe sequences hybridize to the RET31 mRNA sequences as well. Therefore, the expression profiling for BMY_HPP5 is also representative of the RET31 expression profile as well.

DNA contamination

To access the level of contaminating genomic DNA in the RNA, the RNA was divided into 2 aliquots and one half was treated with Rnase-free Dnase (Invitrogen). Samples from both the Dnase-treated and non-treated were then subjected to reverse transcription reactions with (RT+) and without (RT-) the presence of reverse transcriptase. TaqMan assays were carried out with gene-specific primers (see above) and the contribution of genomic DNA to the signal detected was evaluated by comparing the threshold cycles obtained with the RT+/RT- non-Dnase treated RNA to that on the RT+/RT- Dnase treated RNA. The amount of signal contributed by genomic DNA in the Dnased RT- RNA must be less that 10% of that obtained with Dnased RT+ RNA. If not the RNA was not used in actual experiments.

Reverse Transcription reaction and Sequence Detection

100ng of Dnase-treated total RNA was annealed to 2.5 μM of the respective gene-specific reverse primer in the presence of 5.5 mM Magnesium Chloride by heating the sample to 72°C for 2 min and then cooling to 55° C for 30 min. 1.25 U/μl of MuLv reverse transcriptase and 500μM of each dNTP was added to the reaction and the tube was incubated at 37° C for 30 min. The sample was then heated to 90°C for 5 min to denature enzyme.

Quantitative sequence detection was carried out on an ABI PRISM 7700 by adding to the reverse transcribed reaction 2.5μM forward and reverse primers, 500μM of each dNTP, buffer and 5U AmpliTaq Gold™. The PCR reaction was then held at 94°C for 12 min, followed by 40 cycles of 94° C for 15 sec and 60° C for 30 sec.

Data handling

The threshold cycle (Ct) of the lowest expressing tissue (the highest Ct value) was used as the baseline of expression and all other tissues were expressed as the

relative abundance to that tissue by calculating the difference in Ct value between the baseline and the other tissues and using it as the exponent in $2^{(\Delta Ct)}$

mRNA levels were assayed in samples from three individual donors for each tissue for each human phosphatase polypeptide. Values presented represent the average abundance of each human phosphatase polypeptide for each tissue divided by the average abundance of said polypeptide in the tissue with the lowest level of expression. For example, the lowest expression level detected for each polypeptide is as follows: BMY_HPP1 = blood mononuclear cells; BMY_HPP2 = umbilical cord; BMY_HPP4 = blood mononuclear cells; and BMY_HPP5 (RET31) = umbilical cord.

The expanded expression profile of BMY_HPP1, BMY_HPP2, BMY_HPP4, and BMY_HPP5 (RET31), are provided in Figures 26, 30, 34, and 35 and are described elsewhere herein.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties.

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO:1 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: PTA-2966, which is hybridizable to SEQ ID NO:41;

(b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:42 or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No: PTA-2966, which is hybridizable to SEQ ID NO:41;

(c) a polynucleotide encoding a polypeptide domain of SEQ ID NO:42 or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No: PTA-2966, which is hybridizable to SEQ ID NO:41;

(d) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:42 or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No: PTA-2966, which is hybridizable to SEQ ID NO:41;

(e) a polynucleotide encoding a polypeptide of SEQ ID NO:42 or the cDNA sequence included in ATCC Deposit No: PTA-2966, which is hybridizable to SEQ ID NO:41, having biological activity;

(f) a polynucleotide which is a variant of SEQ ID NO:41;

(g) a polynucleotide which is an allelic variant of SEQ ID NO:41;

(h) an isolated polynucleotide comprising nucleotides 473 to 2464 of SEQ ID NO:41, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 665 of SEQ ID NO:42 minus the start codon;

(i) an isolated polynucleotide comprising nucleotides 470 to 2464 of SEQ ID NO:41, wherein said nucleotides encode a polypeptide corresponding to amino acids 1 to 665 of SEQ ID NO:109 including the start codon;

(j) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:41;

(k) a polynucleotide fragment of SEQ ID NO:108 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: PTA-3434, which is hybridizable to SEQ ID NO:108;

(l) (b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:109 or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No: PTA-3434, which is hybridizable to SEQ ID NO:108;

(m) a polynucleotide encoding a polypeptide domain of SEQ ID NO:109 or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No: PTA-3434, which is hybridizable to SEQ ID NO:108;

(n) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:109 or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No: PTA-3434, which is hybridizable to SEQ ID NO:108;

(o) a polynucleotide encoding a polypeptide of SEQ ID NO:109 or the cDNA sequence included in ATCC Deposit No: PTA-3434, which is hybridizable to SEQ ID NO:108, having biological activity;

(p) a polynucleotide which is a variant of SEQ ID NO:108;

(q) a polynucleotide which is an allelic variant of SEQ ID NO:108;

(r) an isolated polynucleotide comprising nucleotides 541 to 2532 of SEQ ID NO:108, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 665 of SEQ ID NO:109 minus the start codon;

(s) an isolated polynucleotide comprising nucleotides 538 to 2532 of SEQ ID NO:108, wherein said nucleotides encode a polypeptide corresponding to amino acids 1 to 665 of SEQ ID NO:109 including the start codon;

(t) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:108;

(u) an isolated polynucleotide comprising nucleotides 541 to 1443 of SEQ ID NO:108, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 302 of SEQ ID NO:109 minus the start codon;

(v) an isolated polynucleotide comprising nucleotides 538 to 1443 of SEQ ID NO:108, wherein said nucleotides encode a polypeptide corresponding to amino acids 1 to 302 of SEQ ID NO:109 including the start codon;

(w) a polynucleotide fragment of SEQ ID NO:149 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: XXXXX, which is hybridizable to SEQ ID NO:149;

(x) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:150 or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No: PTA-XXXXX, which is hybridizable to SEQ ID NO:149;

(y) a polynucleotide encoding a polypeptide domain of SEQ ID NO:150 or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No: PTA-XXXXX, which is hybridizable to SEQ ID NO:149;

(z) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:150 or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No: PTA-XXXXX, which is hybridizable to SEQ ID NO:149;

(aa) a polynucleotide encoding a polypeptide of SEQ ID NO:150 or the cDNA sequence included in ATCC Deposit No: XXXXX, which is hybridizable to SEQ ID NO:149, having biological activity;

(bb) a polynucleotide which is a variant of SEQ ID NO:149;

(cc) a polynucleotide which is an allelic variant of SEQ ID NO:149;

(dd) an isolated polynucleotide comprising nucleotides 631 to 2448 of SEQ ID NO:149, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 607 of SEQ ID NO:150 minus the start codon;

(ee) an isolated polynucleotide comprising nucleotides 628 to 2448 of SEQ ID NO:149, wherein said nucleotides encode a polypeptide corresponding to amino acids 1 to 607 of SEQ ID NO:150 including the start codon;

(ff) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:149;

(gg) a polynucleotide fragment of SEQ ID NO:151 or a polynucleotide fragment of the cDNA sequence included in ATCC Deposit No: XXXXX, which is hybridizable to SEQ ID NO:151;

(hh) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:152 or a polypeptide fragment encoded by the cDNA sequence included in ATCC Deposit No: XXXXX, which is hybridizable to SEQ ID NO:151;

(ii) a polynucleotide encoding a polypeptide domain of SEQ ID NO:152 or a polypeptide domain encoded by the cDNA sequence included in ATCC Deposit No: XXXXX, which is hybridizable to SEQ ID NO:151;

(jj) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:152 or a polypeptide epitope encoded by the cDNA sequence included in ATCC Deposit No: XXXXX, which is hybridizable to SEQ ID NO:151;

(kk) a polynucleotide encoding a polypeptide of SEQ ID NO:152 or the cDNA sequence included in ATCC Deposit No: XXXXX, which is hybridizable to SEQ ID NO:151, having biological activity;

(ll) a polynucleotide which is a variant of SEQ ID NO:151;

(mm) a polynucleotide which is an allelic variant of SEQ ID NO:151;

(nn) an isolated polynucleotide comprising nucleotides 92 to 538 of SEQ ID NO:151, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 150 of SEQ ID NO:152 minus the start codon;

(oo) an isolated polynucleotide comprising nucleotides 89 to 538 of SEQ ID NO:151, wherein said nucleotides encode a polypeptide corresponding to amino acids 1 to 150 of SEQ ID NO:152 including the start codon;

(pp) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:151; and

(qq) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(pp), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a human phosphatase protein.

3. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

4. The recombinant host cell of claim 3 comprising vector sequences.

5. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:42 or the encoded sequence included in ATCC Deposit No: PTA-2966;

(b) a polypeptide fragment of SEQ ID NO:42 or the encoded sequence included in ATCC Deposit No: PTA-2966, having biological activity;

(c) a polypeptide domain of SEQ ID NO:42 or the encoded sequence included in ATCC Deposit No: PTA-2966;

(d) a polypeptide epitope of SEQ ID NO:42 or the encoded sequence included in ATCC Deposit No: PTA-2966;

(e) a full length protein of SEQ ID NO:42 or the encoded sequence included in ATCC Deposit No: PTA-2966;

(f) a variant of SEQ ID NO:42;

(g) an allelic variant of SEQ ID NO:42;

(h) a species homologue of SEQ ID NO:42;

(i) a polypeptide comprising amino acids 2 to 665 of SEQ ID NO:42, wherein said amino acids 2 to 665 comprise a polypeptide of SEQ ID NO:42 minus the start methionine;

(j) a polypeptide comprising amino acids 1 to 665 of SEQ ID NO:42; and

(k) a polypeptide encoded by the cDNA contained in ATCC Deposit No. PTA-2966;

(l) a polypeptide fragment of SEQ ID NO:109 or the encoded sequence included in ATCC Deposit No: PTA-3434;

(m)a polypeptide fragment of SEQ ID NO:109 or the encoded sequence included in ATCC Deposit No: PTA-3434, having biological activity;

(n) a polypeptide domain of SEQ ID NO:109 or the encoded sequence included in ATCC Deposit No: PTA-3434;

(o) a polypeptide epitope of SEQ ID NO:109 or the encoded sequence included in ATCC Deposit No: PTA-3434;

(p) a full length protein of SEQ ID NO:109 or the encoded sequence included in ATCC Deposit No: PTA-3434;

(q) a variant of SEQ ID NO:109;

(r) an allelic variant of SEQ ID NO:109;

(s) a species homologue of SEQ ID NO:109;

(t) a polypeptide comprising amino acids 2 to 665 of SEQ ID NO:109, wherein said amino acids 2 to 665 comprise a polypeptide of SEQ ID NO:109 minus the start methionine;

(u) a polypeptide comprising amino acids 1 to 665 of SEQ ID NO:109;

(v) a polypeptide encoded by the cDNA contained in ATCC Deposit No. PTA-3434;

(w) a polypeptide fragment of SEQ ID NO:150 or the encoded sequence included in ATCC Deposit No: XXXXX;

(x) a polypeptide fragment of SEQ ID NO:150 or the encoded sequence included in ATCC Deposit No: XXXXX, having biological activity;

(y) a polypeptide domain of SEQ ID NO:150 or the encoded sequence included in ATCC Deposit No: XXXXX;

(z) a polypeptide epitope of SEQ ID NO:150 or the encoded sequence included in ATCC Deposit No: XXXXX;

(aa) a full length protein of SEQ ID NO:150 or the encoded sequence included in ATCC Deposit No: XXXXX;

(bb) a variant of SEQ ID NO:150;

(cc) an allelic variant of SEQ ID NO:150;

(dd) a species homologue of SEQ ID NO:150;

(ee) a polypeptide comprising amino acids 2 to 607 of SEQ ID NO:150, wherein said amino acids 2 to 607 comprise a polypeptide of SEQ ID NO:150 minus the start methionine;

(ff) a polypeptide comprising amino acids 1 to 607 of SEQ ID NO:150;

(gg) a polypeptide encoded by the cDNA contained in ATCC Deposit No. XXXX;

(hh) a polypeptide fragment of SEQ ID NO:152 or the encoded sequence included in ATCC Deposit No: XXXXX;

(ii) a polypeptide fragment of SEQ ID NO:152 or the encoded sequence included in ATCC Deposit No: XXXXX, having biological activity;

(jj) a polypeptide domain of SEQ ID NO:152 or the encoded sequence included in ATCC Deposit No: XXXXX;

(kk) a polypeptide epitope of SEQ ID NO:152 or the encoded sequence included in ATCC Deposit No: XXXXX;

(ll) a full length protein of SEQ ID NO:152 or the encoded sequence included in ATCC Deposit No: XXXXX;

(mm) a variant of SEQ ID NO:152;

(nn) an allelic variant of SEQ ID NO:152;

(oo) a species homologue of SEQ ID NO:152;

(pp) a polypeptide comprising amino acids 2 to 150 of SEQ ID NO:152, wherein said amino acids 2 to 150 comprise a polypeptide of SEQ ID NO:152 minus the start methionine;

(qq) a polypeptide comprising amino acids 1 to 150 of SEQ ID NO:152; and

(rr) a polypeptide encoded by the cDNA contained in ATCC Deposit No. XXXX.

6. The isolated polypeptide of claim 5, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

7. An isolated antibody that binds specifically to the isolated polypeptide of claim 5.

8. A recombinant host cell that expresses the isolated polypeptide of claim 15

9. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 8 under conditions such that said polypeptide is expressed; and

(b) recovering said polypeptide.

10. The polypeptide produced by claim 9.

11. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 5 or the polynucleotide of claim 1.

12. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

13. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 5 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

14. A process for making polynucleotide sequences encoding a gene product having altered phosphatase activity comprising,

a) shuffling a nucleotide sequence of claim 1,

b) expressing the resulting shuffled nucleotide sequences and,

c) selecting for altered phosphatase activity as compared to the phosphatase activity of the gene product of said unmodified nucleotide sequence.

15. A shuffled polynucleotide sequence produced from the process of claim 14.

16. An isolated nucleic acid molecule consisting of a polynucleotide having a nucleotide sequence selected from the group consisting of:

(a) a polynucleotide encoding a polypeptide of SEQ ID NO:42;

(b) an isolated polynucleotide comprising nucleotides 473 to 2464 of SEQ ID NO:41, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 665 of SEQ ID NO:42 minus the start codon;

(c) an isolated polynucleotide comprising nucleotides 473 to 2464 of SEQ ID NO:41, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 665 of SEQ ID NO:42 including the start codon;

(d) a polynucleotide encoding the BMY_HPP5 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. PTA-2966;

(e) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:41;

(f) a polynucleotide encoding a polypeptide of SEQ ID NO:109;

(g) an isolated polynucleotide comprising nucleotides 473 to 2464 of SEQ ID NO:41, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 665 of SEQ ID NO:42 minus the start codon;

(h) an isolated polynucleotide comprising nucleotides 473 to 2464 of SEQ ID NO:41, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 665 of SEQ ID NO:42 including the start codon;

(i) a polynucleotide encoding the RET31 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. PTA-3434;

(j) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:109;

(k) an isolated polynucleotide comprising nucleotides 541 to 1443 of SEQ ID NO:108, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 302 of SEQ ID NO:109 minus the start codon;

(l) an isolated polynucleotide comprising nucleotides 538 to 1443 of SEQ ID NO:108, wherein said nucleotides encode a polypeptide corresponding to amino acids 1 to 302 of SEQ ID NO:109 including the start codon;

(m)a polynucleotide encoding a polypeptide of SEQ ID NO:150;

(n) an isolated polynucleotide comprising nucleotides 631 to 2448 of SEQ ID NO:149, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 607 of SEQ ID NO:150 minus the start codon;

(o) an isolated polynucleotide comprising nucleotides 628 to 2448 of SEQ ID NO:149, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 607 of SEQ ID NO:150 including the start codon;

(p) a polynucleotide encoding the BMY_HPP5 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. XXXXX;

(q) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:149;

(r) a polynucleotide encoding a polypeptide of SEQ ID NO:152;

(s) an isolated polynucleotide comprising nucleotides 92 to 538 of SEQ ID NO:151, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 150 of SEQ ID NO:152 minus the start codon;

(t) an isolated polynucleotide comprising nucleotides 89 to 538 of SEQ ID NO:151, wherein said nucleotides encode a polypeptide corresponding to amino acids 2 to 150 of SEQ ID NO:152 including the start codon;

(u) a polynucleotide encoding the BMY_HPP5 polypeptide encoded by the cDNA clone contained in ATCC Deposit No. XXXXX; and

(v) a polynucleotide which represents the complimentary sequence (antisense) of SEQ ID NO:151.

17. The isolated nucleic acid molecule of claim 16, wherein the polynucleotide comprises a nucleotide sequence encoding a human phosphatase protein.

18. A recombinant vector comprising the isolated nucleic acid molecule of claim 16.

19. A recombinant host cell comprising the recombinant vector of claim 18.

20. An isolated polypeptide consisting of an amino acid sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:42 having phosphatase activity;

(b) a polypeptide domain of SEQ ID NO:42 having phosphatase activity;

(c) a full length protein of SEQ ID NO:42;

(d) a polypeptide corresponding to amino acids 2 to 665 of SEQ ID NO:42, wherein said amino acids 2 to 665 comprise a polypeptide of SEQ ID NO:42 minus the start methionine;

(e) a polypeptide corresponding to amino acids 1 to 665 of SEQ ID NO:42;

(f) a polypeptide encoded by the cDNA contained in ATCC Deposit No. PTA-2966;

(g) a polypeptide fragment of SEQ ID NO:109 having phosphatase activity;

(h) a polypeptide domain of SEQ ID NO:109 having phosphatase activity;

(i) a full length protein of SEQ ID NO:109;

(j) a polypeptide corresponding to amino acids 2 to 665 of SEQ ID NO:109, wherein said amino acids 2 to 665 comprise a polypeptide of SEQ ID NO:109 minus the start methionine;

(k) a polypeptide corresponding to amino acids 1 to 665 of SEQ ID NO:109;

(l) a polypeptide encoded by the cDNA contained in ATCC Deposit No. PTA-3434;

(m) a polypeptide corresponding to amino acids 2 to 302 of SEQ ID NO:109, wherein said amino acids 2 to 302 comprise a polypeptide of SEQ ID NO:109 minus the start methionine;

(n) a polypeptide corresponding to amino acids 1 to 302 of SEQ ID NO:109;

(o) a polypeptide fragment of SEQ ID NO:150 having phosphatase activity;

(p) a polypeptide domain of SEQ ID NO:150 having phosphatase activity;

(q) a full length protein of SEQ ID NO:150;

(r) a polypeptide corresponding to amino acids 2 to 607 of SEQ ID NO:150, wherein said amino acids 2 to 607 comprise a polypeptide of SEQ ID NO:150 minus the start methionine;

(s) a polypeptide corresponding to amino acids 1 to 607 of SEQ ID NO:150;

(t) a polypeptide encoded by the cDNA contained in ATCC Deposit No. XXXXX;

(u) a polypeptide fragment of SEQ ID NO:152 having phosphatase activity;

(v) a polypeptide domain of SEQ ID NO:152 having phosphatase activity;

(w) a full length protein of SEQ ID NO:152;

(x) a polypeptide corresponding to amino acids 2 to 150 of SEQ ID NO:152, wherein said amino acids 2 to 150 comprise a polypeptide of SEQ ID NO:152 minus the start methionine;

(y) a polypeptide corresponding to amino acids 1 to 150 of SEQ ID NO:152; and

(z) a polypeptide encoded by the cDNA contained in ATCC Deposit No. XXXX.

21. A method of phosphorylating a protein comprising the step of incubating said protein with the isolated polypeptide of claim 5.

22. The method for preventing, treating, or ameliorating a medical condition of claim 21, wherein the medical condition is a proliferative disorder.

23. A computer for producing a three-dimensional representation of a molecule or molecular complex, wherein said molecule or molecular complex comprises the structural coordinates of a member of the group consisting of

(a) BMY_HPP1 model provided in Figure 28 in accordance with Table VIII

(b) BMY_HPP2 model provided in Figure 32 in accordance with Table IX; and

(c) BMY_HPP5 model provided in Figure 38 in accordance with Table X,

wherein said computer comprises:

(a) A machine-readable data storage medium, comprising a data storage material encoded with machine readable data, wherein the data is defined by the set of structure coordinates of the model;

(b) a working memory for storing instructions for processing said machine-readable data;

(c) a central-processing unit coupled to said working memory and to said machine-readable data storage medium for processing said machine readable data into said three-dimensional representation; and

(d) a display coupled to said central-processing unit for displaying said three-dimensional representation.

24. A method for identifying a mutant with altered biological properties, function, or activity of a member of the group consisting of:

(a) BMY_HPP1;

(b) BMY_HPP2; and

(c) BMY_HPP5,

Wherein said method comprises the steps of:

(a) using a model of said polypeptide according to the structural coordinates of said model to identify amino acids to mutate; and

(b) mutating said amino acids to create a mutant protein with altered biological function or properties.

25. A method for designing or selecting compounds as potential modulators of a member of the group consisting of:

(a) BMY_HPP1;

(b) BMY_HPP2; and

(c) BMY_HPP5,

Wherein said method comprises the steps of:

(a) identifying a structural or chemical feature of said member using the structural coordinates of said member; and

(b) rationally designing compounds that bind to said feature.