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(54) Title: MAMMALIAN ALPHA-HELICAL PROTEIN - 12		
(57) Abstract The present invention relates to polynucleotide and polypeptide molecules for mammalian alpha helix-12 (Zalpha12). The polypeptides, and polynucleotides encoding them, are hormonal and may be used to regulate the functioning of the immune system. The present invention also includes antibodies to the Zalpha12 polypeptides. Antagonists may be used to treat inflammation and inflammation-related diseases.		

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MAMMALIAN ALPHA-HELICAL PROTEIN - 12

5

BACKGROUND OF THE INVENTION

Inflammation normally is a localized, protective response to trauma or microbial invasion that destroys, dilutes, or walls-off the injurious agent and the injured tissue. It is characterized in the acute form by the classic signs of pain, heat, redness, swelling, and loss of function. Microscopically it involves a complex series of events, including dilation of arterioles, capillaries, and venules, with increased permeability and blood flow, exudation of fluids, including plasma proteins, and leukocyte migration into the area of inflammation.

Diseases characterized by inflammation are significant causes of morbidity and mortality in humans. Commonly, inflammation occurs as a defensive response to invasion of the host by foreign, particularly microbial, material. Responses to mechanical trauma, toxins, and neoplasia also may results in inflammatory reactions. The accumulation and subsequent activation of leukocytes are central events in the pathogenesis of most forms of inflammation. Deficiencies of inflammation compromise the host. Excessive inflammation caused by abnormal recognition of host tissue as foreign or prolongation of the inflammatory process may lead to inflammatory diseases as diverse as diabetes, arteriosclerosis, cataracts, reperfusion injury, and cancer, to post-infectious syndromes such as in infectious meningitis, rheumatic fever, and to rheumatic diseases such as systemic lupus erythematosus and rheumatoid arthritis. The centrality of the inflammatory response in these varied disease processes makes its regulation a major element in the prevention control or cure of human disease. Thus, there

is a need to discover cytokines which contribute to inflammation and inflammatory related diseases so that antagonists such as antibodies can be administered to down-regulate the cytokine so as to ameliorate the
5 inflammation.

SUMMARY OF THE INVENTION

10 The present invention addresses this need by providing novel polypeptides and related compositions and methods and their antagonists. Within one aspect, the present invention provides an isolated polynucleotide encoding a mammalian cytokine termed Zalpha12. The data
15 show that the cytokine is involved in the inflammation response. Thus, antagonists of Zalpha12 can be used to lessen inflammation especially inflammation associated with coronary heart disease, arteriosclerosis, Crohn's disease and inflammatory bowel disease.

20 Three variants have been discovered as shown in SEQ ID NOs:1, 2, 3, 4, 5 and 6. Each Zalpha12 polypeptide has a signal sequence which extends from amino acid residue 1, a methionine to and including amino acid
25 residue 34, a serine of SEQ ID NOs:2, 4 and 6. Thus, the Zalpha12 polypeptide represented by SEQ ID NO:2 has a mature sequence extending from amino acid residue 35, an alanine, to and including amino acid residue 202, an asparagine, also represented by SEQ ID NO:8. The Zalpha12
30 polypeptide represented by SEQ ID NO:4 has a mature sequence extending from amino acid residue 35, an alanine to and including amino acid residue 288, an asparagine also represented by SEQ ID NO:9. The Zalpha12 polypeptide represented by SEQ ID NO:6 has a mature sequence extending
35 from amino acid residue 35, an alanine to and including amino acid residue 158, an asparagine, also represented by SEQ ID NO:10.

Within a second aspect of the invention there is provided an expression vector comprising (a) a transcription promoter; (b) a DNA segment encoding Zalpha12 polypeptide, and (c) a transcription terminator, 5 wherein the promoter, DNA segment, and terminator are operably linked.

Within a third aspect of the invention there is provided a cultured eukaryotic or prokaryotic cell into which has been introduced an expression vector as 10 disclosed above, wherein said cell expresses a protein polypeptide encoded by the DNA segment.

Within a further aspect of the invention there is provided a chimeric polypeptide consisting essentially 15 of a first portion and a second portion joined by a peptide bond. The first portion of the chimeric polypeptide consists essentially of (a) a Zalpha12 polypeptide (b) allelic variants of Zalpha12; and (c) protein polypeptides that are at least 80% identical to 20 (a) or (b). The second portion of the chimeric polypeptide consists essentially of another polypeptide such as an affinity tag. Within one embodiment the affinity tag is an immunoglobulin F_C polypeptide. The invention also provides expression vectors encoding the 25 chimeric polypeptides and host cells transfected to produce the chimeric polypeptides.

Within an additional aspect of the invention there is provided an antibody that specifically binds to a Zalpha12 polypeptide as disclosed above, and also an anti- 30 idiotypic antibody which neutralizes the antibody to a Zalpha12 polypeptide.

An additional embodiment of the present invention relates to a peptide or polypeptide which has the amino acid sequence of an epitope-bearing portion of a 35 Zalpha12 polypeptide having an amino acid sequence described above. Peptides or polypeptides having the amino acid sequence of an epitope-bearing portion of a Zalpha12

polypeptide of the present invention include portions of such polypeptides with at least nine, preferably at least 15 and more preferably at least 30 to 50 amino acids, although epitope-bearing polypeptides of any length up to 5 and including the entire amino acid sequence of a polypeptide of the present invention described above are also included in the present invention. Examples of such epitope binding regions are SEQ ID NOs: 18, 19, 20, 25, 26, 27, 28, 29, 30, 35, 36, 37, 38, 39, 40, 41, 42, 43 and 10 44. Also claimed are any of these polypeptides that are fused to another polypeptide or carrier molecule.

These and other aspects of the invention will become evident upon reference to the following detailed description of the invention.

15

DETAILED DESCRIPTION OF THE INVENTION

The teachings of all the references cited herein are incorporated in their entirety herein by reference.

20 Prior to setting forth the invention in detail, it may be helpful to the understanding thereof to define the following terms:

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second 25 polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. 30 Affinity tags include a poly-histidine tract, protein A, Nilsson et al., *EMBO J.* 4:1075 (1985); Nilsson et al., *Methods Enzymol.* 198:3 (1991), glutathione S transferase, Smith and Johnson, *Gene* 67:31 (1988), Glu-Glu affinity tag, Grussenmeyer et al., *Proc. Natl. Acad. Sci. USA* 35 82:7952-4 (1985), substance P, FlagTM peptide, Hopp et al., *Biotechnology* 6:1204-1210 (1988), streptavidin binding

peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., *Protein Expression and Purification 2*: 95-107 (1991). DNAs encoding affinity tags are available from commercial suppliers (e.g.,
5 Pharmacia Biotech, Piscataway, NJ).

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in
10 phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic
15 variant of a gene.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of
20 a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus
25 of the complete polypeptide.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are
30 prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the
35 complement/anti-complement pair is desirable, the

complement/anti-complement pair preferably has a binding affinity of $<10^9 \text{ M}^{-1}$.

The term "complements of a polynucleotide molecule" is a polynucleotide molecule having a
5 complementary base sequence and reverse orientation as compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a polynucleotide that
10 has a contiguous stretch of identical or complementary sequence to another polynucleotide. Contiguous sequences are said to "overlap" a given stretch of polynucleotide sequence either in their entirety or along a partial stretch of the polynucleotide. For example,
15 representative contigs to the polynucleotide sequence 5'-ATGGCTTAGCTT-3' are 5'-TAGCTTgagtct-3' and 3'-gtcgactACCGA-5'.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or
20 more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

The term "expression vector" is used to denote a
25 DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments include promoter and terminator
30 sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a
35 polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free

of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, *Nature* 316:774-78 (1985)).

An "isolated" polypeptide or protein is a polypeptide or protein that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. When used in this context, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The term "operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g., transcription initiates in the promoter and proceeds through the coding segment to the terminator.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, a-globin, b-globin, and myoglobin are paralogs of each other.

5 A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a
10 combination of natural and synthetic molecules. Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or double-
15 stranded. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term "base pairs". It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide may differ
20 slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired. Such unpaired ends will in general not exceed 20 nt in length.

25 A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides".

30 The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5'
35 non-coding regions of genes.

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-

peptidic components, such as carbohydrate groups.

Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins
5 are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule (i.e., a
10 ligand) and mediates the effect of the ligand on the cell. Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of
15 ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell. This interaction in turn leads to an alteration in the metabolism of the cell. Metabolic events that are linked
20 to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of
25 phospholipids. In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor,
30 erythropoietin receptor and IL-6 receptor).

The term "secretory signal sequence" denotes a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway
35 of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Molecular weights and lengths of polymers determined by imprecise analytical methods (e.g., gel electrophoresis) will be understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to $\pm 10\%$.

Introduction

The Zalpha12 protein is an alpha helical protein. SEQ ID NO:2 has four alpha helices, A, B, C and D. Helix A of SEQ ID NO:2 extends from amino acid residue 56, a leucine, to and including amino acid residue 70, an isoleucine also defined by SEQ ID NO:21. Helix B of SEQ ID NO:2 extends from amino acid residue 96, a leucine to and including amino acid residue 110, a tyrosine, also defined by SEQ ID NO:22. Helix C of SEQ ID NO:2 extends from amino acid residue 130, a leucine, to and including amino acid residue 144, a leucine, also defined by SEQ ID NO:23. Helix D of SEQ ID NO: 2 extends from amino acid residue 162, a methionine, to and including amino acid residue 176, a serine, also defined by SEQ ID NO:24. SEQ ID NO:25 contains helices A + B; SEQ ID NO:26 contains helices A + B + C; SEQ ID NO:27 contains helices A + B + C + D; SEQ ID NO:28 contains helices B + C + D; SEQ ID NO:29 contains helices B + C; and SEQ ID NO:30 contains helices C + D of SEQ ID NO:2.

The polypeptide of SEQ ID NO:4 also contains four helices A, B, C and D. Helix A of SEQ ID NO:4 extends from amino acid residue 45, a histidine, to and including amino acid residue 59, a leucine, also defined by SEQ ID NO:31.

5 Helix B of SEQ ID NO:4 extends from amino acid residue 116, a valine, to and including amino acid residue 130, a lysine, also defined by SEQ ID NO:32. Helix C of SEQ ID NO:4 extends from amino acid residue 142, a leucine, to and including amino acid residue 156, an isoleucine, also

10 defined by SEQ ID NO:33. Helix D of SEQ ID NO:4 extends from amino acid residue 182, a leucine, to and including amino acid residue 196, a tyrosine, also defined by SEQ ID NO:34. SEQ ID NO:35 contains helices A + B of SEQ ID NO:4. SEQ ID NO:36 contains helices A + B + C of SEQ ID NO:4.

15 SEQ ID NO:37 contains helices A + B + C + D of SEQ ID NO:4. SEQ ID NO: 38 contains helices B + C + D of SEQ ID NO:4. SEQ ID NO:39 contains helices B + C of SEQ ID NO:4. SEQ ID NO:40 contains helices C + D of SEQ ID NO:4.

Expression of the *Zalpha12* gene is seen in a number

20 of different tissues including the spleen, thymus, testis, small intestines, colon, peripheral blood lymphocytes (PBL), stomach, trachea, T-cells including CD4+ and CD8+ cells, and bone marrow. This pattern of expression suggests that *zalpha12* may play a general role in

25 development and exert important regulatory control of testicular differentiation, of the hypothalamic-pituitary-gonadal axis, and of gonadal steroidogenesis and spermatogenesis.

Development of testicular hormone production can be

30 divided into early and late steps, with the latter dependent on the activation of functionally-determined Leydig cell precursors by luteinizing hormone (LH). However, the factors that control the early steps in this process remain unknown, Huhtaniemi , *Reprod. Fertil. Dev.*

35 7: 1025-1035 (1995) suggesting that *zalpha12* might be

responsible for activation of a non-steroidogenic, non-LH responsive precursor cell.

Once Leydig cell differentiation has occurred, production of steroid hormones in the testis is dependent on the secretion of the gonadotrophins, LH and follicle-stimulating hormone (FSH), by the pituitary. LH stimulates production of testosterone by the Leydig cells, whereas spermatogenesis depends on both FSH and high intratesticular testosterone concentrations. LH and FSH secretion is in turn under control of gonadotrophin releasing hormone (GnRH) produced in the hypothalamus, Kaufman, *The neuro endocrine regulation of male reproduction*. in: *Male Infertility. Clinical Investigation, Cause Evaluation and Treatment.*, FH Comhaire, ed., pp 29-54 (Chapman and Hall, London, 1996). Since testicular products have been shown to control LH and FSH production, this suggests that zalpha12 might regulate hormone production by the hypothalamus.

It is well known that steroidogenesis and spermatogenesis take place within two different cellular compartments of the testes, with Leydig and Sertoli cells responsible for the former and latter, respectively, Saez, *Endocrin. Rev.* 15: 574-626 (1994). The activity of each of these cell types appears to be regulated by the secretory products of the other. Sertoli cell derived tumor necrosis factor- α , fibroblast growth factor, interleukin-1, transforming growth factor-B, epidermal growth factor/transforming growth factor- α , activin, inhibin, insulin-like growth factor-1, platelet derived growth factor, endothelin, and arginine-vasopressin have all been shown to regulate Leydig cell function, Saez, *Endocrin. Rev.* 15: 574-626 (1994). Thus, zalpha12 might control or modulate the activities of one or more of these genes.

In men, aging is associated with a progressive decline in testicular function. These changes are

manifest clinically by decreased virility, vigor, and libido that point towards a relative testicular deficiency, Vermeulen, *Ann. Med.* 25:531-534 (1993); Pugeat et al., *Horm. Res.* 43: 104-110 (1995). Hormone

5 replacement therapy in elderly men is not currently recommended which suggests that a new therapy for the male climacterium would be very valuable.

POLYNUCLEOTIDES:

10 The present invention also provides polynucleotide molecules, including DNA and RNA molecules, that encode the Zalpha12 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable
15 sequence variation is possible among these polynucleotide molecules.

Polynucleotides, generally a cDNA sequence, of the present invention encode the described polypeptides
20 herein. A cDNA sequence which encodes a polypeptide of the present invention is comprised of a series of codons, each amino acid residue of the polypeptide being encoded by a codon and each codon being comprised of three nucleotides. The amino acid residues are encoded by their
25 respective codons as follows.

Alanine (Ala) is encoded by GCA, GCC, GCG or GCT;

Cysteine (Cys) is encoded by TGC or TGT;

30 Aspartic acid (Asp) is encoded by GAC or GAT;

Glutamic acid (Glu) is encoded by GAA or GAG;

Phenylalanine (Phe) is encoded by TTC or TTT;

Glycine (Gly) is encoded by GGA, GGC, GGG or

GGT;

35 Histidine (His) is encoded by CAC or CAT;

Isoleucine (Ile) is encoded by ATA, ATC or ATT;

Lysine (Lys) is encoded by AAA, or AAG;
Leucine (Leu) is encoded by TTA, TTG, CTA, CTC,
CTG or CTT;
Methionine (Met) is encoded by ATG;
5 Asparagine (Asn) is encoded by AAC or AAT;
Proline (Pro) is encoded by CCA, CCC, CCG or
CCT;
Glutamine (Gln) is encoded by CAA or CAG;
Arginine (Arg) is encoded by AGA, AGG, CGA, CGC,
10 CGG or CGT;
Serine (Ser) is encoded by AGC, AGT, TCA, TCC,
TCG or TCT;
Threonine (Thr) is encoded by ACA, ACC, ACG or
ACT;
15 Valine (Val) is encoded by GTA, GTC, GTG or GTT;
Tryptophan (Trp) is encoded by TGG; and
Tyrosine (Tyr) is encoded by TAC or TAT.

It is to be recognized that according to the
20 present invention, when a polynucleotide is claimed as
described herein, it is understood that what is claimed
are both the sense strand, the anti-sense strand, and the
DNA as double-stranded having both the sense and anti-
sense strand annealed together by their respective
25 hydrogen bonds. Also claimed is the messenger RNA (mRNA)
which encodes the polypeptides of the present invention,
and which mRNA is encoded by the cDNA described herein.
Messenger RNA (mRNA) will encode a polypeptide using the
same codons as those defined herein, with the exception
30 that each thymine nucleotide (T) is replaced by a uracil
nucleotide (U).

One of ordinary skill in the art will also
appreciate that different species can exhibit
"preferential codon usage." In general, see, Grantham, et
35 *al.*, *Nuc. Acids Res.* 8:1893-1912 (1980); Haas, *et al.*
Curr. Biol. 6:315-324 (1996); Wain-Hobson, *et al.*, *Gene*

13:355-364 (1981); Grosjean and Fiers, *Gene* 18:199-209
(1982); Holm, *Nuc. Acids Res.* 14:3075-3087 (1986);
Ikemura, *J. Mol. Biol.* 158:573-597 (1982). As used
herein, the term "preferential codon usage" or
5 "preferential codons" is a term of art referring to
protein translation codons that are most frequently used
in cells of a certain species, thus favoring one or a few
representatives of the possible codons encoding each amino
acid (See Table 2). For example, the amino acid Threonine
10 (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in
mammalian cells ACC is the most commonly used codon; in
other species, for example, insect cells, yeast, viruses
or bacteria, different Thr codons may be preferential.
Preferential codons for a particular species can be
15 introduced into the polynucleotides of the present
invention by a variety of methods known in the art.
Introduction of preferential codon sequences into
recombinant DNA can, for example, enhance production of
the protein by making protein translation more efficient
20 within a particular cell type or species. Sequences
containing preferential codons can be tested and optimized
for expression in various species, and tested for
functionality as disclosed herein.

Within preferred embodiments of the invention
25 the isolated polynucleotides will hybridize to similar
sized regions of SEQ ID NOs:1, 3 or 5 or a sequence
complementary thereto, under stringent conditions. In
general, stringent conditions are selected to be about 5°C
lower than the thermal melting point (T_m) for the specific
30 sequence at a defined ionic strength and pH. The T_m is
the temperature (under defined ionic strength and pH) at
which 50% of the target sequence hybridizes to a perfectly
matched probe. Typical stringent conditions are those in
which the salt concentration is up to about 0.03 M at pH 7
35 and the temperature is at least about 60°C.

As previously noted, the isolated polynucleotides of the present invention include DNA and RNA. Methods for preparing DNA and RNA are well known in the art. In general, RNA is isolated from a tissue or
5 cell that produces large amounts of Zalpha12 RNA. Such tissues and cells are identified by Northern blotting, Thomas, *Proc. Natl. Acad. Sci. USA* 77:5201 (1980) and are discussed below. Total RNA can be prepared using guanidine HCl extraction followed by isolation by centrifugation in
10 a CsCl gradient, Chirgwin *et al.*, *Biochemistry* 18:52-94 (1979). Poly (A)⁺ RNA is prepared from total RNA using the method of Aviv and Leder, *Proc. Natl. Acad. Sci. USA* 69:1408-1412 (1972). Complementary DNA (cDNA) is prepared from poly(A)⁺ RNA using known methods. In the
15 alternative, genomic DNA can be isolated. Polynucleotides encoding Zalpha12 polypeptides are then identified and isolated by, for example, hybridization or PCR.

A full-length clone encoding Zalpha12 can be obtained by conventional cloning procedures.
20 Complementary DNA (cDNA) clones are preferred, although for some applications (e.g., expression in transgenic animals) it may be preferable to use a genomic clone, or to modify a cDNA clone to include at least one genomic intron. Methods for preparing cDNA and genomic clones are
25 well known and within the level of ordinary skill in the art, and include the use of the sequence disclosed herein, or parts thereof, for probing or priming a library. Expression libraries can be probed with antibodies to Zalpha12, receptor fragments, or other specific binding
30 partners.

The polynucleotides of the present invention can also be synthesized using DNA synthesizers. Currently the method of choice is the phosphoramidite method. If chemically synthesized double stranded DNA is required for
35 an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made

separately. The production of short genes (60 to 80 bp) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 bp),
5 however, special strategies must be invoked, because the coupling efficiency of each cycle during chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to
10 100 nucleotides in length.

See Glick and Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA*, (ASM Press, Washington, D.C. 1994); Itakura et al., *Annu. Rev. Biochem.* 53: 323-356 (1984) and Climie et al.,
15 *Proc. Natl. Acad. Sci. USA* 87:633-637 (1990).

The present invention further provides counterpart polypeptides and polynucleotides from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish,
20 insect and other vertebrate and invertebrate species. Of particular interest are Zalpha12 polypeptides from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides. Orthologs of human Zalpha12 can be cloned
25 using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses Zalpha12 as disclosed herein. Suitable sources of mRNA
30 can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A Zalpha12-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or
35 partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also

be cloned using the polymerase chain reaction, or PCR (Mullis, U.S. Patent No. 4,683,202), using primers designed from the representative human Zalpha12 sequence disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to Zalpha12 polypeptide. Similar techniques can also be applied to the isolation of genomic clones.

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NOs:1, 3 or 5 represent specific alleles of human Zalpha12 and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO:1, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NOs:2, 4 or 6. cDNAs generated from alternatively spliced mRNAs, which retain the properties of the Zalpha12 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

The present invention also provides isolated Zalpha12 polypeptides that are substantially identical to the polypeptides of SEQ ID NOs:2, 4, 6, 8, 9 or 10 and their orthologs. The term "substantially identical" is used herein to denote polypeptides having 50%, 60%, 70%, 80% and most preferably at least 90%, 95% or 99% sequence identity to the sequences shown in SEQ ID NOs:2, 4, 6, 8, 9 or 10 or their orthologs. Percent sequence identity is

determined by conventional methods. See, for example, Altschul *et al.*, *Bull. Math. Bio.* 48: 603-616 (1986) and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915-10919 (1992). Briefly, two amino acid sequences
5 are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM 62" scoring matrix of Henikoff and Henikoff (*ibid.*) as shown in Table 1 (amino acids are indicated by the standard one-letter codes). The percent identity is
10 then calculated as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

15

Those skilled in the art appreciate that there are many established algorithms to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence and the amino acid sequence of a putative variant. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA* 85:2444 (1988), and by Pearson, *Meth. Enzymol.* 183:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (e.g., SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions or deletions. The ten regions with the highest density of identities are then re-scored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol.* 48:444 (1970); Sellers, *SIAM J. Appl. Math.* 26:787 (1974), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by

modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol.* 183:63 (1990).

FASTA can also be used to determine the sequence
5 identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the k_{tp} value can range between one to six, preferably from four to six.

10 The present invention includes nucleic acid molecules that encode a polypeptide having one or more conservative amino acid changes, compared with the amino acid sequence of SEQ ID NOs:2, 4, 6, 8, 9 or 10. The BLOSUM62 table is an amino acid substitution matrix derived from about 2,000
15 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins [Henikoff and Henikoff, *Proc. Nat'l Acad. Sci. USA* 89:10915 (1992)]. Accordingly, the BLOSUM62 substitution frequencies can be used to define
20 conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. As used herein, the language "conservative amino acid substitution" refers to a substitution represented by a BLOSUM62 value of greater than -1. For
25 example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0,1,2, or 3. Preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (e.g., 1,2 or 3), while more preferred
30 conservative substitutions are characterized by a BLOSUM62 value of at least 2 (e.g., 2 or 3).

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

Variant Zalpha12 polypeptides or substantially homologous Zalpha12 polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 2) and other substitutions that do not significantly affect the folding or activity of the polypeptide; small deletions, typically of one to about 30 amino acids; and small amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag. The present invention thus includes polypeptides of from 20 to 30 amino acid residues that comprise a sequence that is at least 90%, preferably at least 95%, and more preferably 99% or more identical to the corresponding region of SEQ ID NOs:2, 4, 6, 8, 9 or 10. Polypeptides comprising affinity tags can further comprise a proteolytic cleavage site between the Zalpha12 polypeptide and the affinity tag. Preferred such sites include thrombin cleavage sites and factor Xa cleavage sites.

Table 2

Conservative amino acid substitutions

25

Basic: arginine

lysine

histidine

Acidic: glutamic acid

aspartic acid

30

Polar: glutamine

asparagine

Table 2 cont.

	Hydrophobic:	leucine
		isoleucine
		valine
5	Aromatic:	phenylalanine
		tryptophan
		tyrosine
	Small:	glycine
		alanine
10		serine
		threonine
		methionine

The present invention further provides a variety
15 of other polypeptide fusions [and related multimeric
proteins comprising one or more polypeptide fusions]. For
example, a Zalpha12 polypeptide can be prepared as a
fusion to a dimerizing protein as disclosed in U.S.
Patents Nos. 5,155,027 and 5,567,584. Preferred
20 dimerizing proteins in this regard include immunoglobulin
constant region domains. Immunoglobulin-Zalpha12
polypeptide fusions can be expressed in genetically
engineered cells [to produce a variety of multimeric
Zalpha12 analogs]. Auxiliary domains can be fused to
25 Zalpha12 polypeptides to target them to specific cells,
tissues, or macromolecules (e.g., collagen). For example,
a Zalpha12 polypeptide or protein could be targeted to a
predetermined cell type by fusing a Zalpha12 polypeptide
to a ligand that specifically binds to a receptor on the
30 surface of the target cell. In this way, polypeptides and
proteins can be targeted for therapeutic or diagnostic
purposes. A Zalpha12 polypeptide can be fused to two or
more moieties, such as an affinity tag for purification
and a targeting domain. Polypeptide fusions can also
35 comprise one or more cleavage sites, particularly between

domains. See, Tuan *et al.*, *Connective Tissue Research* 34:1-9 (1996).

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. 5 Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, 10 nitroglutamine, homoglutamine, pipercolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several 15 methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs.

20 Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other 25 reagents. Proteins are purified by chromatography. See, for example, Robertson *et al.*, *J. Am. Chem. Soc.* 113:2722 (1991); Ellman *et al.*, *Methods Enzymol.* 202:301 (1991); Chung *et al.*, *Science* 259:806-809 (1993); and Chung *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10145-1019 (1993). In 30 a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs, Turcatti *et al.*, *J. Biol. Chem.* 271:19991-19998 (1996). Within a third method, *E. coli* cells are cultured in the absence of a natural amino

acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide et al., *Biochem.* 33:7470-7476 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification.

10 Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions, Wynn and Richards, *Protein Sci.* 2:395-403 (1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for Zalpha12 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis, Cunningham and Wells, *Science* 244: 1081-1085 (1989); Bass et al., *Proc. Natl. Acad. Sci. USA* 88:4498-502 (1991). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity as disclosed below to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., *J. Biol. Chem.* 271:4699-708, 1996. Sites of ligand-receptor interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., *Science* 255:306-312 (1992); Smith et al., *J.*

Mol. Biol. 224:899-904 (1992); Wlodaver et al., *FEBS Lett.* 309:59-64 (1992).

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer, *Science* 241:53-57 (1988) or Bowie and Sauer, *Proc. Natl. Acad. Sci. USA* 86:2152-2156 (1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display, e.g., Lowman et al., *Biochem.* 30:10832-10837 (1991); Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204 and region-directed mutagenesis, Derbyshire et al., *Gene* 46:145 (1986); Ner et al., *DNA* 7:127 (1988).

Variants of the disclosed Zalpha12 DNA and polypeptide sequences can be generated through DNA shuffling as disclosed by Stemmer, *Nature* 370:389-391, (1994), Stemmer, *Proc. Natl. Acad. Sci. USA* 91:10747-10751 (1994) and WIPO Publication WO 97/20078. Briefly, variant DNAs are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNAs, such as allelic variants or DNAs from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods

to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active polypeptides can be recovered from the host cells and rapidly sequenced using modern equipment. These methods
5 allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed herein, one of
10 ordinary skill in the art can identify and/or prepare a variety of polypeptide fragments or variants of SEQ ID NOs:2, 4 or 6 or that retain the properties of the wild-type Zalpha12 protein.

For any Zalpha12 polypeptide, including variants
15 and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 above.

20 PROTEIN PRODUCTION

The Zalpha12 polypeptides of the present invention, including full-length polypeptides, biologically active fragments, and fusion polypeptides, can be produced in genetically engineered host cells
25 according to conventional techniques. Suitable host cells are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured
30 cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd ed., (Cold Spring Harbor Laboratory
35 Press, Cold Spring Harbor, NY, 1989), and Ausubel et al.,

eds., *Current Protocols in Molecular Biology* (John Wiley and Sons, Inc., NY, 1987).

In general, a DNA sequence encoding a Zalpha12 polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a Zalpha12 polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of Zalpha12, or may be derived from another secreted protein (e.g., t-PA) or synthesized *de novo*. The secretory signal sequence is operably linked to the Zalpha12 DNA sequence, i.e., the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830).

Alternatively, the secretory signal sequence contained in the polypeptides of the present invention is

used to direct other polypeptides into the secretory pathway. The present invention provides for such fusion polypeptides. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein, such as a receptor. Such fusions may be used *in vivo* or *in vitro* to direct peptides through the secretory pathway.

Cultured mammalian cells are suitable hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection, Wigler *et al.*, *Cell* 14:725 (1978); Corsaro and Pearson, *Somatic Cell Genetics* 7:603 (1981); Graham and Van der Eb, *Virology* 52:456 (1973), electroporation, Neumann *et al.*, *EMBO J.* 1:841-845 (1982), DEAE-dextran mediated transfection (Ausubel *et al.*, *ibid.*, and liposome-mediated transfection, Hawley-Nelson *et al.*, *Focus* 15:73 (1993); Ciccarone *et al.*, *Focus* 15:80 (1993), and viral vectors, Miller and Rosman, *BioTechniques* 7:980 (1989); Wang and Finer, *Nature Med.* 2:714 (1996). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson *et al.*, U.S. Patent No. 4,713,339; Hagen *et al.*, U.S. Patent No. 4,784,950; Palmiter *et al.*, U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham *et al.*, *J. Gen. Virol.* 36:59 (1977) and Chinese hamster ovary (*e.g.* CHO-K1; ATCC No. CCL 61) cell

lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

10 Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternative markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Other higher eukaryotic cells can also be used as hosts, including plant cells, insect cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., *J. Biosci. (Bangalore)* 11:47 (1987).
5 Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222 and WIPO publication WO 94/06463. Insect cells can be infected with recombinant baculovirus,
10 commonly derived from *Autographa californica* nuclear polyhedrosis virus (AcNPV). DNA encoding the Zalpha12 polypeptide is inserted into the baculoviral genome in place of the AcNPV polyhedrin gene coding sequence by one of two methods. The first is the traditional method of
15 homologous DNA recombination between wild-type AcNPV and a transfer vector containing the Zalpha12 flanked by AcNPV sequences. Suitable insect cells, e.g. SF9 cells, are infected with wild-type AcNPV and transfected with a transfer vector comprising a Zalpha12 polynucleotide
20 operably linked to an AcNPV polyhedrin gene promoter, terminator, and flanking sequences. See, King, L.A. and Possee, R.D., *The Baculovirus Expression System: A Laboratory Guide*, (Chapman & Hall, London); O'Reilly, D.R. et al., *Baculovirus Expression Vectors: A Laboratory*
25 *Manual* (Oxford University Press, New York, New York, 1994); and, Richardson, C. D., Ed., *Baculovirus Expression Protocols. Methods in Molecular Biology*, (Humana Press, Totowa, NJ 1995). Natural recombination within an insect cell will result in a recombinant baculovirus which
30 contains Zalpha12 driven by the polyhedrin promoter. Recombinant viral stocks are made by methods commonly used in the art.

The second method of making recombinant baculovirus utilizes a transposon-based system described
35 by Luckow, V.A, et al., *J Virol* 67:4566 (1993). This

system is sold in the Bac-to-Bac kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the Zalpha12 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." The pFastBac1™ transfer vector utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case Zalpha12. However, pFastBac1™ can be modified to a considerable degree. The polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as Pcor, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins. See, Hill-Perkins, M.S. and Possee, R.D., *J Gen Virol* 71:971 (1990); Bonning, B.C. et al., *J Gen Virol* 75:1551 (1994); and, Chazenbalk, G.D., and Rapoport, B., *J Biol Chem* 270:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed which replace the native Zalpha12 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen, Carlsbad, CA), or baculovirus gp67 (PharMingen, San Diego, CA) can be used in constructs to replace the native Zalpha12 secretory signal sequence. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed Zalpha12 polypeptide, for example, a Glu-Glu epitope tag, Grussenmeyer, T. et al., *Proc Natl Acad Sci.* 82:7952 (1985). Using a technique known in the art, a transfer vector containing Zalpha12 is transformed into *E. coli*, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant

baculovirus. The bacmid DNA containing the recombinant baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, e.g. Sf9 cells. Recombinant virus that expresses Zalpha12 is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall army worm, *Spodoptera frugiperda*. See, in general, Glick and Pasternak, *Molecular Biotechnology: Principles and Applications of Recombinant DNA* (ASM Press, Washington, D.C., 1994). Another suitable cell line is the High FiveO™ cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent #5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Excell0405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. The cells are grown up from an inoculation density of approximately 2-5 x 10⁵ cells to a density of 1-2 x 10⁶ cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. The recombinant virus-infected cells typically produce the recombinant Zalpha12 polypeptide at 12-72 hours post-infection and secrete it with varying efficiency into the medium. The culture is usually harvested 48 hours post-infection. Centrifugation is used to separate the cells from the medium (supernatant). The supernatant containing the Zalpha12 polypeptide is filtered through micropore filters, usually 0.45 μm pore size. Procedures used are generally described in available laboratory manuals (King, L. A. and Possee, R.D., *ibid.*; O'Reilly, D.R. *et al.*, *ibid.*; Richardson, C. D., *ibid.*). Subsequent purification

of the Zalpha12 polypeptide from the supernatant can be achieved using methods described herein.

Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., *J. Gen. Microbiol.* 132:3459 (1986) and Cregg, U.S. Patent No. 4,882,279.

Aspergillus cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

The use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed in WIPO Publications WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, it is preferred that the promoter and terminator in the plasmid be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (*DHAS*), formate dehydrogenase (*FMD*), and catalase (*CAT*) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A preferred selectable marker for use in *Pichia methanolica* is a *P. methanolica* *ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (*AIRC*; EC 4.1.1.21), which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (*PEP4* and *PRB1*) are preferred. Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of

interest into *P. methanolica* cells. It is preferred to transform *P. methanolica* cells by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 5 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention. 10 Techniques for transforming these hosts and expressing foreign DNA sequences cloned therein are well known in the art, see, e.g., Sambrook et al., *ibid.*). When expressing a Zalpha12 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as 15 insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded 20 and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic 25 space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

30 Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, 35 are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins

and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in
5 an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell. *P. methanolica* cells are cultured in a medium comprising adequate sources of carbon, nitrogen and trace nutrients at a temperature of
10 about 25°C to 35°C. Liquid cultures are provided with sufficient aeration by conventional means, such as shaking of small flasks or sparging of fermentors. A preferred culture medium for *P. methanolica* is YEPD (2% D-glucose, 2% Bacto™ Peptone (Difco Laboratories, Detroit, MI), 1%
15 Bacto™ yeast extract (Difco Laboratories), 0.004% adenine and 0.006% L-leucine).

Another embodiment of the present invention provides for a peptide or polypeptide comprising an epitope-bearing portion of a Zalpha12 polypeptide of the
20 invention. The epitope of the this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. A region of a protein to which an antibody can bind is defined as an "antigenic epitope". See for instance, Geysen, H.M. et al., *Proc. Natl. Acad. Sci. USA*
25 *81:3998-4002* (1984).

As to the selection of peptides or polypeptides bearing an antigenic epitope (*i.e.*, that contain a region of a protein molecule to which an antibody can bind), it is well known in the art that relatively short synthetic
30 peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See Sutcliffe, J.G. et al. *Science* *219:660-666* (1983). Peptides capable of eliciting protein-reactive sera are frequently represented
35 in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined

neither to immunodominant regions of intact proteins
(*i.e.*, immunogenic epitopes) nor to the amino or carboxyl
terminals. Peptides that are extremely hydrophobic and
those of six or fewer residues generally are ineffective
5 at inducing antibodies that bind to the mimicked protein;
longer soluble peptides, especially those containing
proline residues, usually are effective.

Antigenic epitope-bearing peptides and
polypeptides of the invention are therefore useful to
10 raise antibodies, including monoclonal antibodies, that
bind specifically to a polypeptide of the invention.
Antigenic epitope-bearing peptides and polypeptides of the
present invention contain a sequence of at least nine,
preferably between 15 to about 30 amino acids contained
15 within the amino acid sequence of a polypeptide of the
invention. However, peptides or polypeptides comprising a
larger portion of an amino acid sequence of the invention,
containing from 30 to 50 amino acids, or any length up to
and including the entire amino acid sequence of a
20 polypeptide of the invention, also are useful for inducing
antibodies that react with the protein. Preferably, the
amino acid sequence of the epitope-bearing peptide is
selected to provide substantial solubility in aqueous
solvents (*i.e.*, the sequence includes relatively
25 hydrophilic residues and hydrophobic residues are
preferably avoided); and sequences containing proline
residues are particularly preferred. All of the
polypeptides shown in the sequence listing contain
antigenic epitopes to be used according to the present
30 invention. The present invention also provides polypeptide
fragments or peptides comprising an epitope-bearing
portion of a Zalpha12 polypeptide described herein. Such
fragments or peptides may comprise an "immunogenic
epitope," which is a part of a protein that elicits an
35 antibody response when the entire protein is used as an
immunogen. Immunogenic epitope-bearing peptides can be
identified using standard methods [see, for example,

Geysen *et al.*, *supra*. See also U.S. Patent No. 4,708,781 (1987) further describes how to identify a peptide bearing an immunogenic epitope of a desired protein.

5 Protein Isolation

It is preferred to purify the polypeptides of the present invention to $\geq 80\%$ purity, more preferably to $\geq 90\%$ purity, even more preferably $\geq 95\%$ purity, and particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

Expressed recombinant Zalpha12 polypeptides (or chimeric Zalpha12 polypeptides) can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which

they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988).

The polypeptides of the present invention can be isolated by exploitation of their properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate, Sulkowski, *Trends in Biochem.* 3:1 (1985). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography. *Methods in Enzymol.*, Vol. 182, "Guide to Protein Purification", M. Deutscher, (ed.), page 529-539 (Acad. Press, San Diego, 1990). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (e.g.,

maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Moreover, using methods described in the art, polypeptide fusions, or hybrid Zalpha12 proteins, are
5 constructed using regions or domains of the inventive Zalpha12, Sambrook et al., *ibid.*, Altschul et al., *ibid.*, Picard, *Cur. Opin. Biology*, 5:511 (1994). These methods allow the determination of the biological importance of larger domains or regions in a polypeptide of interest.
10 Such hybrids may alter reaction kinetics, binding, constrict or expand the substrate specificity, or alter tissue and cellular localization of a polypeptide, and can be applied to polypeptides of unknown structure.

Fusion proteins can be prepared by methods known
15 to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding both components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the
20 methods described herein. For example, part or all of a domain(s) conferring a biological function may be swapped between Zalpha12 of the present invention with the functionally equivalent domain(s) from another family member. Such domains include, but are not limited to, the
25 secretory signal sequence, conserved, and significant domains or regions in this family. Such fusion proteins would be expected to have a biological functional profile that is the same or similar to polypeptides of the present invention or other known family proteins, depending on the
30 fusion constructed. Moreover, such fusion proteins may exhibit other properties as disclosed herein.

Zalpha12 polypeptides or fragments thereof may also be prepared through chemical synthesis. Zalpha12 polypeptides may be monomers or multimers; glycosylated or
35 non-glycosylated; pegylated or non-pegylated; and may or may not include an initial methionine amino acid residue.

Chemical Synthesis of Polypeptides

Polypeptides, especially polypeptides of the present invention can also be synthesized by exclusive
5 solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. The polypeptides are preferably prepared by solid phase peptide synthesis, for example as described by Merrifield, *J. Am. Chem. Soc.* 85:2149 (1963).

10

ASSAYS

The activity of molecules of the present invention can be measured using a variety of assays. Of particular interest are changes in steroidogenesis,
15 spermatogenesis, in the testis, LH and FSH production and GnRH in the hypothalamus. Such assays are well known in the art.

Proteins of the present invention are useful for increasing sperm production. Zalpha12 can be measured *in vitro* using cultured cells or *in vivo* by administering
20 molecules of the claimed invention to the appropriate animal model. For instance, Zalpha12 transfected (or co-transfected) expression host cells may be embedded in an alginate environment and injected (implanted) into
25 recipient animals. Alginate-poly-L-lysine microencapsulation, permselective membrane encapsulation and diffusion chambers have been described as a means to entrap transfected mammalian cells or primary mammalian cells. These types of non-immunogenic "encapsulations" or
30 microenvironments permit the transfer of nutrients into the microenvironment, and also permit the diffusion of proteins and other macromolecules secreted or released by the captured cells across the environmental barrier to the recipient animal. Most importantly, the capsules or
35 microenvironments mask and shield the foreign, embedded cells from the recipient animal's immune response. Such

microenvironments can extend the life of the injected cells from a few hours or days (naked cells) to several weeks (embedded cells).

Alginate threads provide a simple and quick means for generating embedded cells. The materials needed to generate the alginate threads are readily available and relatively inexpensive. Once made, the alginate threads are relatively strong and durable, both *in vitro* and, based on data obtained using the threads, *in vivo*. The alginate threads are easily manipulable and the methodology is scalable for preparation of numerous threads. In an exemplary procedure, 3% alginate is prepared in sterile H₂O, and sterile filtered. Just prior to preparation of alginate threads, the alginate solution is again filtered. An approximately 50% cell suspension (containing about 5×10^5 to about 5×10^7 cells/ml) is mixed with the 3% alginate solution. One ml of the alginate/cell suspension is extruded into a 100 mM sterile filtered CaCl₂ solution over a time period of ~15 min, forming a "thread". The extruded thread is then transferred into a solution of 50 mM CaCl₂, and then into a solution of 25 mM CaCl₂. The thread is then rinsed with deionized water before coating the thread by incubating in a 0.01% solution of poly-L-lysine. Finally, the thread is rinsed with Lactated Ringer's Solution and drawn from solution into a syringe barrel (without needle attached). A large bore needle is then attached to the syringe, and the thread is intraperitoneally injected into a recipient in a minimal volume of the Lactated Ringer's Solution.

An alternative *in vivo* approach for assaying proteins of the present invention involves viral delivery systems. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review,

see T.C. Becker *et al.*, *Meth. Cell Biol.* 43:161 (1994);
and J.T. Douglas and D.T. Curiel, *Science & Medicine* 4:44
(1997). The adenovirus system offers several advantages:
adenovirus can (i) accommodate relatively large DNA
5 inserts; (ii) be grown to high-titer; (iii) infect a broad
range of mammalian cell types; and (iv) be used with a
large number of available vectors containing different
promoters. Also, because adenoviruses are stable in the
bloodstream, they can be administered by intravenous
10 injection.

By deleting portions of the adenovirus genome,
larger inserts (up to 7 kb) of heterologous DNA can be
accommodated. These inserts can be incorporated into the
viral DNA by direct ligation or by homologous
15 recombination with a co-transfected plasmid. In an
exemplary system, the essential E1 gene has been deleted
from the viral vector, and the virus will not replicate
unless the E1 gene is provided by the host cell (the human
293 cell line is exemplary). When intravenously
20 administered to intact animals, adenovirus primarily
targets the liver. If the adenoviral delivery system has
an E1 gene deletion, the virus cannot replicate in the
host cells. However, the host's tissue (e.g., liver) will
express and process (and, if a secretory signal sequence
25 is present, secrete) the heterologous protein. Secreted
proteins will enter the circulation in the highly
vascularized liver, and effects on the infected animal can
be determined.

The adenovirus system can also be used for
30 protein production *in vitro*. By culturing adenovirus-
infected non-293 cells under conditions where the cells
are not rapidly dividing, the cells can produce proteins
for extended periods of time. For instance, BHK cells are
grown to confluence in cell factories, then exposed to the
35 adenoviral vector encoding the secreted protein of
interest. The cells are then grown under serum-free

conditions, which allows infected cells to survive for several weeks without significant cell division. Alternatively, adenovirus vector infected 293S cells can be grown in suspension culture at relatively high cell density to produce significant amounts of protein (see 5 Garnier et al., *Cytotechnol.* 15:145 (1994)). With either protocol, an expressed, secreted heterologous protein can be repeatedly isolated from the cell culture supernatant. Within the infected 293S cell production protocol, non- 10 secreted proteins may also be effectively obtained.

Agonists and Antagonists

In view of the tissue distribution observed for Zalpha12, agonists (including the natural ligand/ 15 substrate/ cofactor/ etc.) and antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as Zalpha12 agonists are useful for stimulating the immune system or spermatogenesis. For example, Zalpha12 and agonist compounds are useful as 20 components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture.

Antagonists

25 Antagonists are also useful as research reagents for characterizing sites of ligand-receptor interaction. Antagonists of Zalpha12 can also be used to down-regulate inflammation as discussed in more further detail below. Inhibitors of Zalpha12 activity (Zalpha12 antagonists) 30 include anti-Zalpha12 antibodies and soluble Zalpha12 receptors, as well as other peptidic and non-peptidic agents (including ribozymes).

Zalpha12 can also be used to identify inhibitors (antagonists) of its activity. Test compounds are added 35 to the assays disclosed herein to identify compounds that inhibit the activity of Zalpha12. In addition to those assays disclosed herein, samples can be tested for

inhibition of Zalpha12 activity within a variety of assays designed to measure receptor binding or the stimulation/inhibition of Zalpha12-dependent cellular responses. For example, Zalpha12-responsive cell lines
5 can be transfected with a reporter gene construct that is responsive to a Zalpha12-stimulated cellular pathway. Reporter gene constructs of this type are known in the art, and will generally comprise a Zalpha12-DNA response element operably linked to a gene encoding a protein which
10 can be assayed, such as luciferase. DNA response elements can include, but are not limited to, cyclic AMP response elements (CRE), hormone response elements (HRE) insulin response element (IRE), Nasrin *et al.*, *Proc. Natl. Acad. Sci. USA* 87:5273 (1990) and serum response elements (SRE)
15 (Shaw *et al.* *Cell* 56: 563 (1989). Cyclic AMP response elements are reviewed in Roestler *et al.*, *J. Biol. Chem.* 263 (19):9063 (1988) and Habener, *Molec. Endocrinol.* 4 (8):1087 (1990). Hormone response elements are reviewed in Beato, *Cell* 56:335 (1989). Candidate compounds,
20 solutions, mixtures or extracts are tested for the ability to inhibit the activity of Zalpha12 on the target cells as evidenced by a decrease in Zalpha12 stimulation of reporter gene expression. Assays of this type will detect compounds that directly block Zalpha12 binding to cell-
25 surface receptors, as well as compounds that block processes in the cellular pathway subsequent to receptor-ligand binding. In the alternative, compounds or other samples can be tested for direct blocking of Zalpha12 binding to receptor using Zalpha12 tagged with a
30 detectable label (e.g., ¹²⁵I, biotin, horseradish peroxidase, FITC, and the like). Within assays of this type, the ability of a test sample to inhibit the binding of labeled Zalpha12 to the receptor is indicative of inhibitory activity, which can be confirmed through
35 secondary assays. Receptors used within binding assays

may be cellular receptors or isolated, immobilized receptors.

A Zalpha12 polypeptide can be expressed as a fusion with an immunoglobulin heavy chain constant region, typically an F_C fragment, which contains two constant region domains and lacks the variable region. Methods for preparing such fusions are disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Such fusions are typically secreted as multimeric molecules wherein the F_C portions are disulfide bonded to each other and two non-Ig polypeptides are arrayed in closed proximity to each other. Fusions of this type can be used to affinity purify the ligand. For use in assays, the chimeras are bound to a support via the F_C region and used in an ELISA format.

A Zalpha12 ligand-binding polypeptide can also be used for purification of ligand. The polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

An assay system that uses a ligand-binding receptor (or an antibody, one member of a complement/anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore, Pharmacia Biosensor, Piscataway, NJ) may be advantageously

employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, *J. Immunol. Methods* 5 145:229 (1991) and Cunningham and Wells, *J. Mol. Biol.* 234:554 (1993). A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed 10 through the cell. If a ligand, epitope, or opposite member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a 15 change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

Ligand-binding receptor polypeptides can also be 20 used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity, Scatchard, *Ann. NY Acad. Sci.* 51: 660 (1949) and calorimetric assays, Cunningham *et al.*, *Science* 253:545 (1991); Cunningham *et al.*, *Science* 245:821 (1991).

25 Zalpha12 polypeptides can also be used to prepare antibodies that specifically bind to Zalpha12 epitopes, peptides or polypeptides. The Zalpha12 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal and elicit an immune 30 response. Suitable antigens would be the Zalpha12 polypeptides encoded by SEQ ID NOs: 2, 4, 6, 8- 10, 18- 20, 25-30 and 35-44. Antibodies generated from this immune response can be isolated and purified as described herein. Methods for preparing and isolating polyclonal and 35 monoclonal antibodies are well known in the art. See, for example, *Current Protocols in Immunology*, Cooligan, *et al.*

(eds.), National Institutes of Health, (John Wiley and Sons, Inc., 1995); Sambrook et al., *Molecular Cloning: A Laboratory Manual, Second Edition* (Cold Spring Harbor, NY, 1989); and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma*
5 *Antibodies: Techniques and Applications* (CRC Press, Inc., Boca Raton, FL, 1982).

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as
10 horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats with a Zalpha12 polypeptide or a fragment thereof. The immunogenicity of a Zalpha12 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete
15 adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of Zalpha12 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If
20 the polypeptide portion is "haptene-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes
25 polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as F(ab')₂, Fab proteolytic fragments, Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain
30 antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking"
35 them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody).

In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics.

Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced.

Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to Zalpha12 protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled Zalpha12 protein or peptide). Genes encoding polypeptides having potential Zalpha12 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner et al., US Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the Zalpha12 sequences disclosed herein to identify proteins which bind to Zalpha12. These "binding proteins" which interact

with Zalpha12 polypeptides can be used for tagging cells; for isolating homolog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like.

5 These binding proteins can also be used in analytical methods such as for screening expression libraries and neutralizing activity. The binding proteins can also be used for diagnostic assays for determining circulating levels of polypeptides; for detecting or quantitating

10 soluble polypeptides as marker of underlying pathology or disease. These binding proteins can also act as Zalpha12 "antagonists" to block Zalpha12 binding and signal transduction *in vitro* and *in vivo*.

Antibodies are determined to be specifically

15 binding if: (1) they exhibit a threshold level of binding activity, and (2) they do not significantly cross-react with related polypeptide molecules. First, antibodies herein specifically bind if they bind to a Zalpha12 polypeptide, peptide or epitope with a binding affinity

20 (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis.

25 Second, antibodies are determined to specifically bind if they do not significantly cross-react with related polypeptides. Antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect Zalpha12 but not known related

30 polypeptides using a standard Western blot analysis (Ausubel *et al.*, *ibid.*). Examples of known related polypeptides are orthologs, proteins from the same species that are members of a protein family (e.g. IL-16), Zalpha12 polypeptides, and non-human Zalpha12. Moreover,

35 antibodies may be "screened against" known related

polypeptides to isolate a population that specifically binds to the inventive polypeptides. For example, antibodies raised to Zalpha12 are adsorbed to related polypeptides adhered to insoluble matrix; antibodies
5 specific to Zalpha12 will flow through the matrix under the proper buffer conditions. Such screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to closely related polypeptides, *Antibodies: A Laboratory Manual*, Harlow and Lane (eds.) (Cold Spring
10 Harbor Laboratory Press, 1988); *Current Protocols in Immunology*, Cooligan, et al. (eds.), National Institutes of Health (John Wiley and Sons, Inc., 1995). Screening and isolation of specific antibodies is well known in the art. See, *Fundamental Immunology*, Paul (eds.) (Raven
15 Press, 1993); Getzoff et al., *Adv. in Immunol.* 43: 1-98 (1988); *Monoclonal Antibodies: Principles and Practice*, Goding, J.W. (eds.), (Academic Press Ltd., 1996); Benjamin et al., *Ann. Rev. Immunol.* 2: 67-101 (1984).

A variety of assays known to those skilled in
20 the art can be utilized to detect antibodies which specifically bind to Zalpha12 proteins or peptides. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.) (Cold Spring
Harbor Laboratory Press, 1988). Representative examples
25 of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich
30 binding to wild-type versus mutant Zalpha12 protein or polypeptide.

Antibodies to Zalpha12 may be used for tagging cells that express Zalpha12; for isolating Zalpha12 by affinity purification; for diagnostic assays for
35 determining circulating levels of Zalpha12 polypeptides;

for detecting or quantitating soluble Zalpha12 as marker of underlying pathology or disease; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block Zalpha12 *in vitro* and *in vivo*. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. Moreover, antibodies to Zalpha12 or fragments thereof may be used *in vitro* to detect denatured Zalpha12 or fragments thereof in assays, for example, Western Blots or other assays known in the art.

20 BIOACTIVE CONJUGATES:

Antibodies or polypeptides herein can also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. For instance, polypeptides or antibodies of the present invention can be used to identify or treat tissues or organs that express a corresponding anti-complementary molecule (receptor or antigen, respectively, for instance). More specifically, Zalpha12 polypeptides or anti-Zalpha12 antibodies, or bioactive fragments or portions thereof, can be coupled to detectable or cytotoxic molecules and delivered to a mammal having cells, tissues or organs that express the anti-complementary molecule.

35 Suitable detectable molecules may be directly or indirectly attached to the polypeptide or antibody, and

include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like. Suitable cytotoxic molecules may be directly or indirectly attached to the polypeptide or antibody, and include bacterial or plant toxins (for instance, diphtheria toxin, *Pseudomonas* exotoxin, ricin, abrin and the like), as well as therapeutic radionuclides, such as iodine-131, rhenium-188 or yttrium-90 (either directly attached to the polypeptide or antibody, or indirectly attached through means of a chelating moiety, for instance). Polypeptides or antibodies may also be conjugated to cytotoxic drugs, such as adriamycin. For indirect attachment of a detectable or cytotoxic molecule, the detectable or cytotoxic molecule can be conjugated with a member of a complementary/anticomplementary pair, where the other member is bound to the polypeptide or antibody portion. For these purposes, biotin/streptavidin is an exemplary complementary/anticomplementary pair.

In another embodiment, polypeptide-toxin fusion proteins or antibody-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation (for instance, to treat cancer cells or tissues). Alternatively, if the polypeptide has multiple functional domains (i.e., an activation domain or a ligand binding domain, plus a targeting domain), a fusion protein including only the targeting domain may be suitable for directing a detectable molecule, a cytotoxic molecule or a complementary molecule to a cell or tissue type of interest. In instances where the domain only fusion protein includes a complementary molecule, the anti-complementary molecule can be conjugated to a detectable or cytotoxic molecule. Such domain-complementary molecule fusion proteins thus represent a generic targeting vehicle for cell/tissue-specific delivery of generic anti-complementary-detectable/ cytotoxic molecule conjugates.

In another embodiment, Zalpha12-cytokine fusion proteins or antibody-cytokine fusion proteins can be used for enhancing *in vivo* killing of target tissues (for example, blood and bone marrow cancers), if the Zalpha12 polypeptide or anti-Zalpha12 antibody targets the hyperproliferative blood or bone marrow cell. See, generally, Hornick *et al.*, *Blood* 89:4437 (1997). The described fusion proteins enable targeting of a cytokine to a desired site of action, thereby providing an elevated local concentration of cytokine. Suitable Zalpha12 polypeptides or anti-Zalpha12 antibodies target an undesirable cell or tissue (*i.e.*, a tumor or a leukemia), and the fused cytokine mediated improved target cell lysis by effector cells. Suitable cytokines for this purpose include interleukin 2 and granulocyte-macrophage colony-stimulating factor (GM-CSF), for instance.

In yet another embodiment, if the Zalpha12 polypeptide or anti-Zalpha12 antibody targets vascular cells or tissues, such polypeptide or antibody may be conjugated with a radionuclide, and particularly with a beta-emitting radionuclide, to reduce restenosis. Such therapeutic approach poses less danger to clinicians who administer the radioactive therapy. For instance, iridium-192 impregnated ribbons placed into stented vessels of patients until the required radiation dose was delivered showed decreased tissue growth in the vessel and greater luminal diameter than the control group, which received placebo ribbons. Further, revascularisation and stent thrombosis were significantly lower in the treatment group. Similar results are predicted with targeting of a bioactive conjugate containing a radionuclide, as described herein.

The bioactive polypeptide or antibody conjugates described herein can be delivered intravenously, intraarterially or intraductally, or may be introduced locally at the intended site of action.

USES OF POLYNUCLEOTIDE/POLYPEPTIDE:

Molecules of the present invention can be used to identify and isolate receptors involved in spermatogenesis, steroidogenesis, testicular differentiation and regulatory control of the hypothalamic-pituitary-gonadal axis or receptors of the immune system. For example, proteins and peptides of the present invention can be immobilized on a column and membrane preparations run over the column, *Immobilized Affinity Ligand Techniques*, Hermanson et al., eds., pp.195-202 (Academic Press, San Diego, CA, 1992,). Proteins and peptides can also be radiolabeled, *Methods in Enzymol.*, vol. 182, "Guide to Protein Purification", M. Deutscher, ed., pp 721-737 (Acad. Press, San Diego, 1990) or photoaffinity labeled, Brunner et al., *Ann. Rev. Biochem.* 62:483-514 (1993) and Fedan et al., *Biochem. Pharmacol.* 33:1167 (1984) and specific cell-surface proteins can be identified.

The molecules of the present invention will be useful for testing disorders of the reproductive system and immunological systems.

GENE THERAPY:

Polynucleotides encoding Zalpha12 polypeptides are useful within gene therapy applications where it is desired to increase or inhibit Zalpha12 activity. If a mammal has a mutated or absent Zalpha12 gene, the Zalpha12 gene can be introduced into the cells of the mammal. In one embodiment, a gene encoding a Zalpha12 polypeptide is introduced *in vivo* in a viral vector. Such vectors include an attenuated or defective DNA virus, such as, but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective

viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes simplex virus 1 (HSV1) vector, Kaplitt et al., *Molec. Cell. Neurosci.* 2:320 (1991); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., *J. Clin. Invest.* 90:626 (1992); and a defective adeno-associated virus vector, Samulski et al., *J. Virol.* 61:3096 (1987); Samulski et al., *J. Virol.* 63:3822 (1989).

In another embodiment, a Zalpha12 gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al. *Cell* 33:153, 1983; Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., *J. Virol.* 62:1120 (1988); Temin et al., U.S. Patent No. 5,124,263; International Patent Publication No. WO 95/07358, published March 16, 1995 by Dougherty et al.; and Kuo et al., *Blood* 82:845 (1993). Alternatively, the vector can be introduced by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker, Felgner et al., *Proc. Natl. Acad. Sci. USA* 84:7413 (1987); Mackey et al., *Proc. Natl. Acad. Sci. USA* 85:8027 (1988). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. More particularly, directing transfection to particular cells represents one area of benefit. For

instance, directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

It is possible to remove the target cells from the body; to introduce the vector as a naked DNA plasmid; and then to re-implant the transformed cells into the body. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter. See, e.g., Wu et al., *J. Biol. Chem.* 267:963 (1992); Wu et al., *J. Biol. Chem.* 263:14621-4, 1988.

Antisense methodology can be used to inhibit Zalpha12 gene transcription, such as to inhibit cell proliferation in vivo. Polynucleotides that are complementary to a segment of a Zalpha12-encoding polynucleotide (e.g., a polynucleotide as set forth in SEQ ID NO:1) are designed to bind to Zalpha12-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of Zalpha12 polypeptide-encoding genes in cell culture or in a subject.

The present invention also provides reagents which will find use in diagnostic applications. For example, the Zalpha12 gene, a probe comprising Zalpha12 DNA or RNA or a subsequence thereof can be used to determine if the Zalpha12 gene is present on chromosome 22q13.1 or if a mutation has occurred. Detectable chromosomal aberrations at the Zalpha12 gene locus

include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by
5 employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook *et al.*, *ibid.*; Ausubel *et al.*,
10 *ibid.*; Marian, *Chest* 108:255 (1995).

Transgenic mice, engineered to express the *Zalpha12* gene, and mice that exhibit a complete absence of *Zalpha12* gene function, referred to as "knockout mice", Snouwaert *et al.*, *Science* 257:1083 (1992), may also be
15 generated, Lowell *et al.*, *Nature* 366:740-42 (1993). These mice may be employed to study the *Zalpha12* gene and the protein encoded thereby in an *in vivo* system.

CHROMOSOMAL LOCALIZATION:

20 *Zalpha12* has been mapped to chromosome 22q13.1. Radiation hybrid mapping is a somatic cell genetic technique developed for constructing high-resolution, contiguous maps of mammalian chromosomes (Cox *et al.*, *Science* 250:245 (1990). Partial or full knowledge of a
25 gene's sequence allows one to design PCR primers suitable for use with chromosomal radiation hybrid mapping panels. Radiation hybrid mapping panels are commercially available which cover the entire human genome, such as the Stanford G3 RH Panel and the GeneBridge 4 RH Panel (Research
30 Genetics, Inc., Huntsville, AL). These panels enable rapid, PCR-based chromosomal localizations and ordering of genes, sequence-tagged sites (STSs), and other nonpolymorphic and polymorphic markers within a region of interest. This includes establishing directly
35 proportional physical distances between newly discovered

genes of interest and previously mapped markers. The precise knowledge of a gene's position can be useful for a number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining
5 additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3)
10 cross-referencing model organisms, such as mouse, which may aid in determining what function a particular gene might have.

Sequence tagged sites (STSS) can also be used independently for chromosomal localization. An STS is a DNA sequence that is unique in the human genome and can be
15 used as a reference point for a particular chromosome or region of a chromosome. An STS is defined by a pair of oligonucleotide primers that are used in a polymerase chain reaction to specifically detect this site in the presence of all other genomic sequences. Since STSS are
20 based solely on DNA sequence they can be completely described within an electronic database, for example, Database of Sequence Tagged Sites (dbSTS), GenBank, (National Center for Biological Information, National Institutes of Health, Bethesda, MD
25 <http://www.ncbi.nlm.nih.gov>), and can be searched with a gene sequence of interest for the mapping data contained within these short genomic landmark STS sequences.

For pharmaceutical use, the proteins of the present invention are formulated for parenteral,
30 particularly intravenous or subcutaneous, delivery according to conventional methods. Intravenous administration will be by bolus injection or infusion over a typical period of one to several hours. In general, pharmaceutical formulations will include a Zalpha12
35 protein in combination with a pharmaceutically acceptable vehicle, such as saline, buffered saline, 5% dextrose in water or the like. Formulations may further include one

or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc. Methods of formulation are well known in the art and are disclosed, for example, in Remington: *The Science and*
5 *Practice of Pharmacy*, Gennaro, ed., (Mack Publishing Co., Easton, PA, 19th ed., 1995). Therapeutic doses will generally be in the range of 0.1 to 100 µg/kg of patient weight per day, preferably 0.5-20 mg/kg per day, with the exact dose determined by the clinician according to
10 accepted standards, taking into account the nature and severity of the condition to be treated, patient traits, etc. Determination of dose is within the level of ordinary skill in the art. The proteins may be administered for acute treatment, over one week or less,
15 often over a period of one to three days or may be used in chronic treatment, over several months or years. Administration of the protein can be subcutaneous, intraperitoneal or rectal depending on the disease to be treated.

20

Tissue Expression and Use

Zalpha12 represents a novel polypeptide with a putative signal peptide leader sequence and alpha helical structure. Several putative isoforms have been
25 identified. Therefore this gene may encode a secreted polypeptide with secondary structure indicating it is a member of the four helix bundle cytokine family. Alternatively, this polypeptide may have other activities associated with other biological functions including:
30 enzymatic activity, association with the cell membrane, or function as a carrier protein.

Northern blot analysis detects transcripts for zalpha12 in spleen, thymus, testis, small intestine, colon, PBL, stomach, lymph node, trachea, and bone marrow.
35 Many of these organs have important immunological function or contain cells which play a role in the immune system.

Zalpha12 is also expressed in CD4+ enriched peripheral T cells and to a lesser degree in CD8+ T cells. There is very weak expression in CD19+ B cells suggesting that Zalpha12 has expression in T lymphoid lineage cells and little if any in B lymphoid lineage cells. There is elevated expression of zalpha12 in RNA derived from a 7 day human mixed lymphocyte reaction. This suggests that zalpha12 expression is regulated and increases after T cell activation.

10

Use of Zalpha12

Zalpha12 can be administered to an immunocompromised mammal, preferably a human, such as cancer patients who have undergone chemotherapy, AIDS patients and the elderly. This will stimulate their immune systems. Zalpha12 can also be used as a vaccine adjuvant to be administered before, with or after the administration of a vaccine. Zalpha12 may also be administered to stimulate the immune system to attack tumors.

20

Use of Antagonists of Zalpha12

An antagonist to Zalpha12, such as an antibody, soluble receptor or small molecule antagonist can be administered to a mammal, preferably a human, to alleviate an inflammatory response. Antagonists, such as antibodies, to Zalpha12 can be used to treat patients having inflammatory related diseases such arteriosclerotic heart disease [see Paulsson, G. et al., *Arterioscler Thromb. Vasc. Biol.*, 20:10-17 (2000)], inflammatory bowel disease, Crohn's disease, rheumatoid arthritis and pancreatitis.

30

The invention is further illustrated by the following non-limiting examples.

Example 1

Cloning of Zalpha12

35

Zalpha12 was discovered by using the Expressed Sequence Tag of SEQ ID NO:7 as a probe in a spleen cDNA library. The library was constructed using 1 microgram

(μ g) of polyA RNA isolated from the spleen tissue of a 2-year-old Hispanic male, who died from cerebral anoxia. cDNA synthesis was initiated using a NotI-oligo(dT) primer. Double-stranded cDNA was blunted, ligated to EcoRI
5 adaptors, digested with NotI, size-selected, and cloned into the NotI and EcoRI sites of a vector. The sequenced clone resulted in the full-length DNA and polypeptide sequences of SEQ ID NOs: 1 and 2.

The EST of SEQ ID NO: 17 resulted in the
10 discovery of a second clone. The clone was sequence and found to have an unspliced intron. Oligonucleotide primers SEQ ID NOs: 11 and 12 were designed to amplify the full-length sequence without the intron sequence. The PCR was performed using lymph node MARATHON READY[®] cDNA (Clontech,
15 Palo Alto, CA) as template. An 823 bp was cut out and gel purified using the QIAQUICK[®] gel extraction kit (Qiagen, Santa Clarita, CA). The excised PCR fragment was sequenced and did not contain the intron sequence. The next step was to see if the clone of SEQ ID NO:17 had another initiation
20 codon upstream. A RACE[®] reaction using lymph node cDNA was performed to obtain 5' sequence. Oligonucleotide primers SEQ ID NOs:13 and 14 were designed for 5' RACE[®] reactions and nested RACE[®]. The RACE[®] reactions revealed an additional initiation codon upstream. The actual full-
25 length was obtained through PCR using primers SEQ ID NOs:15 and 16 which flanked the coding sequence. Six bands were amplified and each was gel purified using the QIAQUICK[®] gel extraction kit and subcloned into plasmid PCR2.1TOPO vector using the TOPO TA[®] cloning kit
30 (Invitrogen, Carlsbad, CA). The clones were sequenced and resulted in the full-length sequences of SEQ ID NOs: 3, 4, 5 and 6.

Thus, three variants were discovered, namely SEQ ID NO:1 and 2, SEQ ID NO:3 and 4, and SEQ ID NOs: 5 and 6.

Example 2Northern Blot Analysis of *Zalpha12*

A northern blot analysis was carried out by
5 standard techniques using the polynucleotides of SEQ ID
NOs: 1 and 3. The results indicate that *Zalpha12* is
expressed in lymph nodes, activated mixed lymphocytes,
spleen, thymus, testis, small intestines, human aortic
endothelial cells, smooth muscle, kidney, mast cells,
10 eosinophils, tonsils, pancreas, colon, peripheral blood
lymphocytes (PBL), stomach, trachea, T-cells including CD4⁺
and CD8⁺ cells, and bone marrow.

Zalpha12 is also expressed by the following cell
lines: HL60 (ATCC 45500), an acute promyelocytic leukemia
15 cell line; Jurkat cells (ATCC TIB-152) a T lymphocyte from
acute T cell leukemia; MOLT-4 cells (ATCC CRL-1582) a T
lymphoblast from acute lymphoblastic leukemia; and HuT 78
cells (ATCC TIB-161) a cutaneous T lymphocyte from a
lymphoma.

20 From these data, it can be concluded that *Zalpha12* is
a cytokine involved in the inflammation cascade.
Antagonists of *Zalpha12* can be used to alleviate
inflammation related to a number of diseases such as
arteriosclerotic heart disease, cardiovascular disease,
25 rheumatoid arthritis, inflammatory bowel disease, and
Crohn's disease.

Example 3*Zalpha12* Anti-peptide Antibodies

30

Polyclonal anti-peptide antibodies were prepared
by immunizing two female New Zealand white rabbits with
the peptide huzalpha12X1-1
AQQHKGSLQKDPLLSQACVGCLEALLDYLDAR (SEQ ID NO:41) or the
35 peptide from SEQ ID NO:4 PLPATKDTVLAPLRMSQVRSLVIGLQNLVLC
(SEQ ID NO:42) or the peptide from SEQ ID NO:4
CEGLPPSTSSGQPPLQDMLCCLGGVAVSLSHIRN (SEQ ID NO:43) or the

peptide from SEQ ID NO:4 FMRYRSSSVLSHEEC (SEQ ID NO:44) .
The peptides were synthesized using an Applied Biosystems
Model 431A peptide synthesizer (Applied Biosystems, Inc.,
Foster City, CA) according to manufacturer's instructions.
5 The peptides were then conjugated to the carrier protein
maleimide-activated keyhole limpet hemocyanin (KLH)
through cysteine residues according to manufacturer's
instructions. (Pierce, Rockford, IL). The rabbits were
each given an initial intraperitoneal (IP) injection of
10 200 µg of the conjugated peptide in Complete Freund's
Adjuvant (Pierce, Rockford, IL) followed by booster IP
injections of 100 µg conjugated peptide in Incomplete
Freund's Adjuvant every three weeks. Seven to ten days
after the administration of the third booster injection,
15 the animals were bled and the serum was collected. The
rabbits were then boosted and bled every three weeks.

The zalpha12 peptide-specific Rabbit seras were
characterized by an ELISA titer check using 1 µg/ml of the
peptide used to make the antibody as an antibody target.
20 The 2 rabbit seras to the SEQ ID NO:41 peptide have titer
to their specific peptide at a dilution of 1:5E5
(1:100000). The 2 rabbit seras to the SEQ ID NO:42
peptide had titer to their specific peptide at a dilution
of 1:5E4 (1:10,000). The 2 rabbit seras to the SEQ ID
25 NO:43 peptide had titer to their specific peptide at a
dilution of 1:5E4. The 2 rabbit seras to the SEQ ID NO:44
peptide had titer to their specific peptide at a dilution
of 1:5E5.

30

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

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9, 10, 18, 19, 20, 25, 26, 27, 28, 29, 30, 35, 36, 37, 38, 39, 40, 41, 42, 43 and 44 or a polypeptide which is at least 80% identical to said polypeptide.
2. An isolated polypeptide comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9, 10 or a polypeptide which is at least 70% identical to said polypeptide.
3. An isolated polynucleotide which encodes a polypeptide, wherein said polypeptide is comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9, 10, 18, 19, 20, 25, 26, 27, 28, 29, 30, 35, 36, 37, 38, 39 and 40 or a polypeptide which is at least 80% identical to said polypeptide.
4. An isolated polynucleotide which encodes a polypeptide, wherein said polypeptide is comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9 and 10 or a polypeptide which is at least 70% identical to said polypeptide.
5. An antibody which specifically binds to a polypeptide, wherein said polypeptide is comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9, 10, 18, 19, 20, 25, 26, 27, 28, 29, 30, 35, 36, 37, 38, 39 and 40.
6. An antibody of claim 5 which specifically binds to a polypeptide, wherein said polypeptide is comprised of an

amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9, and 10.

7. An anti-idiotypic antibody which specifically binds to an antibody which specifically binds to a polypeptide, wherein said polypeptide is comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9, 10, 18, 19, 20, 25, 26, 27, 28, 29, 30, 35, 36, 37, 38, 39 and 40.

8. An anti-idiotypic antibody of claim 7 wherein the polypeptide is comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9 and 10.

9. The use of an antagonist to a polypeptide for treating inflammation wherein the polypeptide is comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9 and 10.

10. The use of claim 9 wherein the antagonist is an antibody which binds to a polypeptide comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9 and 10.

11. The use of an antagonist to a polypeptide for manufacturing a medicament for treating inflammation wherein the polypeptide is comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9 and 10.

12. The use of claim 11 wherein the antagonist is an antibody which binds to a polypeptide comprised of an amino acid sequence selected from the group consisting of SEQ ID NOs. 2, 4, 6, 8, 9 and 10.

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Lys Ala Leu Ser Phe Pro Lys Lys Lys Ala Ala Leu Leu Ser Ala Ala	
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atc tta tgc ttc ctg cgg aca gcc ctg cga caa agc ttt tcc tct gcc	294
Ile Leu Cys Phe Leu Arg Thr Ala Leu Arg Gln Ser Phe Ser Ser Ala	
75 80 85	
ctg gta gcc ctg gtg ccc tca ggg gcc cag cca ctg cca gcc acc aag	342
Leu Val Ala Leu Val Pro Ser Gly Ala Gln Pro Leu Pro Ala Thr Lys	
90 95 100	
gac act gtc cta gct cca ctg cga atg tcg caa gtc cgg tcc ctg gtc	390
Asp Thr Val Leu Ala Pro Leu Arg Met Ser Gln Val Arg Ser Leu Val	
105 110 115 120	
att ggg ctg cag aac ctc ctg gtg cag aag gac cct cta ttg tcc cag	438
Ile Gly Leu Gln Asn Leu Leu Val Gln Lys Asp Pro Leu Leu Ser Gln	
125 130 135	
gcc tgt gtt ggc tgc ctg gag gcc ttg ctt gac tac ctg gat gcc cgg	486
Ala Cys Val Gly Cys Leu Glu Ala Leu Leu Asp Tyr Leu Asp Ala Arg	
140 145 150	
agc cca gac att gct ctc cac gtg gcc tcc cag cct tgg aat cgg ttt	534
Ser Pro Asp Ile Ala Leu His Val Ala Ser Gln Pro Trp Asn Arg Phe	
155 160 165	

ttg ctg ttt acc ctc ttg gat gct gga gag aat tcc ttc ctc aga cct 582
 Leu Leu Phe Thr Leu Leu Asp Ala Gly Glu Asn Ser Phe Leu Arg Pro
 170 175 180

gag att ttg agg ctc atg acc ctg ttt atg cgg tac cgg agt agc agt 630
 Glu Ile Leu Arg Leu Met Thr Leu Phe Met Arg Tyr Arg Ser Ser Ser
 185 190 195 200

gtc ctc tct cat gaa gag gtg ggt gat gtt ctg caa ggt gtg gct ttg 678
 Val Leu Ser His Glu Glu Val Gly Asp Val Leu Gln Gly Val Ala Leu
 205 210 215

gct gac ctg tct acc ctc tcg aac acc aca ctc cag gcc ctg cat ggc 726
 Ala Asp Leu Ser Thr Leu Ser Asn Thr Thr Leu Gln Ala Leu His Gly
 220 225 230

ttc ttc cag cag ctc cag agc atg gga cac ctg gct gac cac agc atg 774
 Phe Phe Gln Gln Leu Gln Ser Met Gly His Leu Ala Asp His Ser Met
 235 240 245

gcc cag acc ctg cag gcc tcc ttg gag ggc ctt ccc cct agc acc tcc 822
 Ala Gln Thr Leu Gln Ala Ser Leu Glu Gly Leu Pro Pro Ser Thr Ser
 250 255 260

tca ggc cag cca ccc ctg cag gac atg ctc tgc ctg gga ggg gtg gct 870
 Ser Gly Gln Pro Pro Leu Gln Asp Met Leu Cys Leu Gly Gly Val Ala
 265 270 275 280

gta tcc ctg tcc cac atc aga aac tgatc 899
 Val Ser Leu Ser His Ile Arg Asn
 285

<210> 4

<211> 288

<212> PRT

<213> Homo sapiens

<400> 4

Met Glu Leu Leu Glu Lys Met Leu Ala Leu Thr Leu Ala Lys Ala Asp
 1 5 10 15
 Ser Pro Arg Thr Ala Leu Leu Cys Ser Ala Trp Leu Leu Thr Ala Ser
 20 25 30
 Phe Ser Ala Gln Gln His Lys Gly Ser Leu Gln Val His Gln Thr Leu
 35 40 45

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

<210> 5
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 <213> Homo sapiens

<220>
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 <222> (32)...(505)

<400> 5

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 Met Glu Leu Leu Glu Lys Met

ctg gcc ctc acc ttg gca aag gca gat tct ccc agg act gca ctc ctc 100
 Leu Ala Leu Thr Leu Ala Lys Ala Asp Ser Pro Arg Thr Ala Leu Leu
 10 15 20

tgc tct gcc tgg ctg ctc act gcc tcc ttc tct gcc cag cag cac aag 148
 Cys Ser Ala Trp Leu Leu Thr Ala Ser Phe Ser Ala Gln Gln His Lys
 25 30 35

ggc agt ttg cag aag gac cct cta ttg tcc cag gcc tgt gtt ggc tgc 196
 Gly Ser Leu Gln Lys Asp Pro Leu Leu Ser Gln Ala Cys Val Gly Cys
 40 45 50 55

ctg gag gcc ttg ctt gac tac ctg gat gcc cgg agc cca gac att gct 244
 Leu Glu Ala Leu Leu Asp Tyr Leu Asp Ala Arg Ser Pro Asp Ile Ala
 60 65 70

ctc cac gtg gcc tcc cag cct tgg aat cgg ttt ttg ctg ttt acc ctc 292
 Leu His Val Ala Ser Gln Pro Trp Asn Arg Phe Leu Leu Phe Thr Leu
 75 80 85

ttg gat gct gga gag aat tcc ttc ctc aga cct gag att ttg agg ctc 340
 Leu Asp Ala Gly Glu Asn Ser Phe Leu Arg Pro Glu Ile Leu Arg Leu
 90 95 100

atg acc ctg ctc cag agc atg gga cac ctg gct gac cac agc atg gcc 388
 Met Thr Leu Leu Gln Ser Met Gly His Leu Ala Asp His Ser Met Ala
 105 110 115

cag acc ctg cag gcc tcc ttg gag ggc ctt ccc cct agc acc tcc tca 436
 Gln Thr Leu Gln Ala Ser Leu Glu Gly Leu Pro Pro Ser Thr Ser Ser
 120 125 130 135

ggc cag cca ccc ctg cag gac atg ctc tgc ctg gga ggg gtg gct gta 484
 Gly Gln Pro Pro Leu Gln Asp Met Leu Cys Leu Gly Gly Val Ala Val
 140 145 150

tcc ctg tcc cac atc aga aac tgatc 510
 Ser Leu Ser His Ile Arg Asn
 155

<210> 6
 <211> 158
 <212> PRT
 <213> Homo sapiens

<400> 6
 Met Glu Leu Leu Glu Lys Met Leu Ala Leu Thr Leu Ala Lys Ala Asp
 1 5 10 15
 Ser Pro Arg Thr Ala Leu Leu Cys Ser Ala Trp Leu Leu Thr Ala Ser
 20 25 30
 Phe Ser Ala Gln Gln His Lys Gly Ser Leu Gln Lys Asp Pro Leu Leu
 35 40 45
 Ser Gln Ala Cys Val Gly Cys Leu Glu Ala Leu Leu Asp Tyr Leu Asp
 50 55 60
 Ala Arg Ser Pro Asp Ile Ala Leu His Val Ala Ser Gln Pro Trp Asn
 65 70 75 80
 Arg Phe Leu Leu Phe Thr Leu Leu Asp Ala Gly Glu Asn Ser Phe Leu
 85 90 95
 Arg Pro Glu Ile Leu Arg Leu Met Thr Leu Leu Gln Ser Met Gly His
 100 105 110
 Leu Ala Asp His Ser Met Ala Gln Thr Leu Gln Ala Ser Leu Glu Gly
 115 120 125
 Leu Pro Pro Ser Thr Ser Ser Gly Gln Pro Pro Leu Gln Asp Met Leu
 130 135 140
 Cys Leu Gly Gly Val Ala Val Ser Leu Ser His Ile Arg Asn
 145 150 155

<210> 7
 <211> 522
 <212> DNA
 <213> Homo sapiens

<400> 7
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 agtgctgggc agtctgtgg cctctgtatc tcaactttt catcttaaaa aaacaaatag 120
 gggtgtgtgt gtggctgggt gtcataaggt ctttctggc tctaataacc tgagcttctg 180
 ttatgaagct gggaccctta gacctcagg atgacctct gtttgtttgt gaacccaat 240
 caggtgctaa gcacatagtg gacttagct gaagctcctc tgtaactcct gtgggccctg 300
 cattgccac ccccgacagc tgctgcagtg ctctgagca gcacaggcct gatggagctt 360
 ctggagaaga tgctggccct caccttgca aaggcagatt ctcccaggac tgcactcctc 420
 tgctctgct ggctgctcac tgctcctc tctgccagc agcacaaggg cagtttgag 480
 aaggaccctc tattgtccca ggctgtgtt ggctgcctgg ag 522

<210> 8
 <211> 168
 <212> PRT
 <213> Homo sapiens

<400> 8

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

- <210> 9
- <211> 254
- <212> PRT
- <213> Homo sapiens

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

Gln Pro Trp Asn Arg Phe Leu Leu Phe Thr Leu Leu Asp Ala Gly Glu
 130 135 140
 Asn Ser Phe Leu Arg Pro Glu Ile Leu Arg Leu Met Thr Leu Phe Met
 145 150 155 160
 Arg Tyr Arg Ser Ser Ser Val Leu Ser His Glu Glu Val Gly Asp Val
 165 170 175
 Leu Gln Gly Val Ala Leu Ala Asp Leu Ser Thr Leu Ser Asn Thr Thr
 180 185 190
 Leu Gln Ala Leu His Gly Phe Phe Gln Gln Leu Gln Ser Met Gly His
 195 200 205
 Leu Ala Asp His Ser Met Ala Gln Thr Leu Gln Ala Ser Leu Glu Gly
 210 215 220
 Leu Pro Pro Ser Thr Ser Ser Gly Gln Pro Pro Leu Gln Asp Met Leu
 225 230 235 240
 Cys Leu Gly Gly Val Ala Val Ser Leu Ser His Ile Arg Asn
 245 250

<210> 10
 <211> 124
 <212> PRT
 <213> Homo sapiens

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

<210> 11
 <211> 19
 <212> DNA
 <213> Homo sapiens

<400> 11 agtttgcagg ttcaccaga	19
<210> 12 <211> 20 <212> DNA <213> Homo sapiens	
<400> 12 caattatgcc ctttgtctcc	20
<210> 13 <211> 24 <212> DNA <213> Homo sapiens	
<400> 13 ccagggtac cagggcagag gaaa	24
<210> 14 <211> 24 <212> DNA <213> Homo sapiens	
<400> 14 ccgcaggaag cataagatgg cagc	24
<210> 15 <211> 23 <212> DNA <213> Homo sapiens	
<400> 15 cttctgggcc ttcaagtcct gag	23
<210> 16 <211> 20 <212> DNA <213> Homo sapiens	
<400> 16 ccttgccac ccccgacagc	20
<210> 17 <211> 197	

<212> DNA
 <213> Homo sapiens

<400> 17
 gcagtttgca ggttcaccag gcactctctg tggaaatgga ccaagtattg aaggctctca 60
 gctttccaaa gaaaaaggct gcactactct cagctgccat cttatgcttc ctgcgacag 120
 cccttcgaca aagcttttcc tctgccctgg tagccctggt gccctcaggg gccagccac 180
 tgccagccac caaggac 197

<210> 18
 <211> 71
 <212> PRT
 <213> Homo sapiens

<400> 18
 Gln Gln His Lys Gly Ser Leu Gln Val His Gln Thr Leu Ser Val Glu
 1 5 10 15
 Met Asp Gln Val Leu Lys Ala Leu Ser Phe Pro Lys Lys Lys Ala Ala
 20 25 30
 Leu Leu Ser Ala Ala Ile Leu Cys Phe Leu Arg Thr Ala Leu Arg Gln
 35 40 45
 Ser Phe Ser Ser Ala Leu Val Ala Leu Val Pro Ser Gly Ala Gln Pro
 50 55 60
 Leu Pro Ala Thr Lys Asp Thr
 65 70

<210> 19
 <211> 69
 <212> PRT
 <213> Homo sapiens

<400> 19
 Gln Lys Asp Pro Leu Leu Ser Gln Ala Cys Val Gly Cys Leu Glu Ala
 1 5 10 15
 Leu Leu Asp Tyr Leu Asp Ala Arg Ser Pro Asp Ile Ala Leu His Val
 20 25 30
 Ala Ser Gln Pro Trp Asn Arg Phe Leu Leu Phe Thr Leu Leu Asp Ala
 35 40 45
 Gly Glu Asn Ser Phe Leu Arg Pro Glu Ile Leu Arg Leu Met Thr Leu
 50 55 60
 Phe Met Arg Tyr Arg
 65

<210> 20
 <211> 76

<212> PRT
 <213> Homo sapiens

<400> 20
 Arg Ser Ser Ser Val Leu Ser His Glu Glu Val Gly Asp Val Leu Gln
 1 5 10 15
 Gly Val Ala Leu Ala Asp Leu Ser Thr Leu Ser Asn Thr Thr Leu Gln
 20 25 30
 Ala Leu His Gly Phe Phe Gln Gln Leu Gln Ser Met Gly His Leu Ala
 35 40 45
 Asp His Ser Met Ala Gln Thr Leu Gln Ala Ser Leu Glu Gly Leu Pro
 50 55 60
 Pro Ser Thr Ser Ser Gly Gln Pro Pro Leu Gln Asp
 65 70 75

<210> 21
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 21
 Leu Glu Ala Leu Leu Asp Tyr Leu Asp Ala Arg Ser Pro Asp Ile
 1 5 10 15

<210> 22
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 22
 Leu Arg Pro Glu Ile Leu Arg Leu Met Thr Leu Phe Met Arg Tyr
 1 5 10 15

<210> 23
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 23
 Leu Ala Asp Leu Ser Thr Leu Ser Asn Thr Thr Leu Gln Ala Leu
 1 5 10 15

<210> 24
 <211> 15
 <212> PRT

14

<213> Homo sapiens

<400> 24

Met Ala Gln Thr Leu Gln Ala Ser Leu Glu Gly Leu Pro Pro Ser
 1 5 10 15

<210> 25

<211> 55

<212> PRT

<213> Homo sapiens

<400> 25

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

<210> 26

<211> 89

<212> PRT

<213> Homo sapiens

<400> 26

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

<210> 27

<211> 121

<212> PRT

<213> Homo sapiens

<400> 27

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

<210> 28

<211> 81

<212> PRT

<213> Homo sapiens

<400> 28

Leu Arg Pro Glu Ile Leu Arg Leu Met Thr Leu Phe Met Arg Tyr Arg
 1 5 10 15
 Ser Ser Ser Val Leu Ser His Glu Glu Val Gly Asp Val Leu Gln Gly
 20 25 30
 Val Ala Leu Ala Asp Leu Ser Thr Leu Ser Asn Thr Thr Leu Gln Ala
 35 40 45
 Leu His Gly Phe Phe Gln Gln Leu Gln Ser Met Gly His Leu Ala Asp
 50 55 60
 His Ser Met Ala Gln Thr Leu Gln Ala Ser Leu Glu Gly Leu Pro Pro
 65 70 75 80
 Ser

<210> 29

<211> 49

<212> PRT

<213> Homo sapiens

<400> 29

Leu Arg Pro Glu Ile Leu Arg Leu Met Thr Leu Phe Met Arg Tyr Arg
 1 5 10 15

Ser Ser Ser Val Leu Ser His Glu Glu Val Gly Asp Val Leu Gln Gly
 20 25 30
 Val Ala Leu Ala Asp Leu Ser Thr Leu Ser Asn Thr Thr Leu Gln Ala
 35 40 45
 Leu

<210> 30
 <211> 47
 <212> PRT
 <213> Homo sapiens

<400> 30
 Leu Ala Asp Leu Ser Thr Leu Ser Asn Thr Thr Leu Gln Ala Leu His
 1 5 10 15
 Gly Phe Phe Gln Gln Leu Gln Ser Met Gly His Leu Ala Asp His Ser
 20 25 30
 Met Ala Gln Thr Leu Gln Ala Ser Leu Glu Gly Leu Pro Pro Ser
 35 40 45

<210> 31
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 31
 His Gln Thr Leu Ser Val Glu Met Asp Gln Val Leu Lys Ala Leu
 1 5 10 15

<210> 32
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 32
 Val Arg Ser Leu Val Ile Gly Leu Gln Asn Leu Leu Val Gln Lys
 1 5 10 15

<210> 33
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 33
 Leu Glu Ala Leu Leu Asp Tyr Leu Asp Ala Arg Ser Pro Asp Ile

17

1 5 10 15

<210> 34
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 34

Leu Arg Pro Glu Ile Leu Arg Leu Met Thr Leu Phe Met Arg Tyr
 1 5 10 15

<210> 35
 <211> 86
 <212> PRT
 <213> Homo sapiens

<400> 35

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

<210> 36
 <211> 112
 <212> PRT
 <213> Homo sapiens

<400> 36

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

18

65				70					75				80		
Asn	Leu	Leu	Val	Gln	Lys	Asp	Pro	Leu	Leu	Ser	Gln	Ala	Cys	Val	Gly
				85					90					95	
Cys	Leu	Glu	Ala	Leu	Leu	Asp	Tyr	Leu	Asp	Ala	Arg	Ser	Pro	Asp	Ile
			100					105					110		

<210> 37

<211> 152

<212> PRT

<213> Homo sapiens

<400> 37

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

<210> 38

<211> 81

<212> PRT

<213> Homo sapiens

<400> 38

Val	Arg	Ser	Leu	Val	Ile	Gly	Leu	Gln	Asn	Leu	Leu	Val	Gln	Lys	Asp
1				5					10					15	
Pro	Leu	Leu	Ser	Gln	Ala	Cys	Val	Gly	Cys	Leu	Glu	Ala	Leu	Leu	Asp
			20					25					30		
Tyr	Leu	Asp	Ala	Arg	Ser	Pro	Asp	Ile	Ala	Leu	His	Val	Ala	Ser	Gln
			35				40					45			

Pro Trp Asn Arg Phe Leu Leu Phe Thr Leu Leu Asp Ala Gly Glu Asn
 50 55 60
 Ser Phe Leu Arg Pro Glu Ile Leu Arg Leu Met Thr Leu Phe Met Arg
 65 70 75 80
 Tyr

<210> 39
 <211> 41
 <212> PRT
 <213> Homo sapiens

<400> 39
 Val Arg Ser Leu Val Ile Gly Leu Gln Asn Leu Leu Val Gln Lys Asp
 1 5 10 15
 Pro Leu Leu Ser Gln Ala Cys Val Gly Cys Leu Glu Ala Leu Leu Asp
 20 25 30
 Tyr Leu Asp Ala Arg Ser Pro Asp Ile
 35 40

<210> 40
 <211> 55
 <212> PRT
 <213> Homo sapiens

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

<210> 41
 <211> 32
 <212> PRT
 <213> Homo sapiens

<400> 41
 Ala Gln Gln His Lys Gly Ser Leu Gln Lys Asp Pro Leu Leu Ser Gln
 1 5 10 15
 Ala Cys Val Gly Cys Leu Glu Ala Leu Leu Asp Tyr Leu Asp Ala Arg
 20 25 30

<210> 42
 <211> 31
 <212> PRT
 <213> Homo sapiens

<400> 42
 Pro Leu Pro Ala Thr Lys Asp Thr Val Leu Ala Pro Leu Arg Met Ser
 1 5 10 15
 Gln Val Arg Ser Leu Val Ile Gly Leu Gln Asn Leu Leu Val Cys
 20 25 30

<210> 43
 <211> 33
 <212> PRT
 <213> Homo sapiens

<400> 43
 Cys Glu Gly Leu Pro Pro Ser Thr Ser Ser Gly Gln Pro Pro Leu Gln
 1 5 10 15
 Asp Met Leu Cys Leu Gly Gly Val Ala Val Ser Leu Ser His Ile Arg
 20 25 30
 Asn

<210> 44
 <211> 15
 <212> PRT
 <213> Homo sapiens

<400> 44
 Phe Met Arg Tyr Arg Ser Ser Ser Val Leu Ser His Glu Glu Cys
 1 5 10 15

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/01015

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C07K14/52 C12N15/19 C07K16/24 A61K38/19 A61P43/00				
According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED				
Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12N A61K				
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched				
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.		
X	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 14 May 1997 (1997-05-14), XP002138914 HINXTON, GB AC = AA419437. zv01g08.r1 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGE:746270 5', mRNA sequence. EST.	3,4		
A	WO 95 13393 A (CELL THERAPEUTICS INC) 18 May 1995 (1995-05-18) the whole document	1		
A	WO 93 21308 A (UNIV HOSPITAL) 28 October 1993 (1993-10-28) the whole document	1		
--- -/--				
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.				
<input checked="" type="checkbox"/> Patent family members are listed in annex.				
° Special categories of cited documents :				
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none; vertical-align: top;"> *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed </td> <td style="width: 50%; border: none; vertical-align: top;"> *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family </td> </tr> </table>			*A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family
A document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family			
Date of the actual completion of the international search	Date of mailing of the international search report			
29 May 2000	14/06/2000			
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Mateo Rosell, A.M.			

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/01015

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 99 29720 A (ZYMOGENETICS INC) 17 June 1999 (1999-06-17) the whole document	1,6-12
P,X	----- DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 1 May 1999 (1999-05-01), XP002138913 HINXTON, GB AC = 095505. TrEMBLrel. DJ821D11.1 (PUTATIVE PROTEIN) (FRAGMENT). From nt 1-51. abstract	1-4
P,X	----- DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 30 November 1999 (1999-11-30), XP002138915 HINXTON, GB AC = AW195720. xn85b08.x1 Soares_NFL_T_GBC_S1 Homo sapiens cDNA clone IMAGE:2701239 3', mRNA sequence. EST. abstract	3,4
P,X	----- DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 2 August 1999 (1999-08-02), XP002138916 HINXTON, GB AC = AI928166. wp11h06.x1 NCI_CGAP_Kid12 Homo sapiens cDNA clone IMAGE:2464571 3', mRNA sequence. EST. abstract	3,4

INTERNATIONAL SEARCH REPORT

International application No.

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 9,11

Present claims 9 and 11 relating to the use of an antagonist to the polypeptides comprised in the SEQ.ID.Nos. 2,4,6,8,9 and 10, could not be searched as its subject-matter was insufficiently disclosed.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Internat'l Application No PCT/US 00/01015

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9513393 A	18-05-1995	AU 1091995 A EP 0738329 A	29-05-1995 23-10-1996
WO 9321308 A	28-10-1993	AU 4289193 A	18-11-1993
WO 9929720 A	17-06-1999	AU 1908599 A	28-06-1999