



(86) Date de dépôt PCT/PCT Filing Date: 2003/01/21
(87) Date publication PCT/PCT Publication Date: 2004/01/08
(45) Date de délivrance/Issue Date: 2014/09/09
(85) Entrée phase nationale/National Entry: 2004/07/16
(86) N° demande PCT/PCT Application No.: US 2003/001983
(87) N° publication PCT/PCT Publication No.: 2004/003140
(30) Priorités/Priorities: 2002/01/18 (US60/350,325);
2002/06/14 (US60/389,108); 2002/12/19 (US60/435,361)

(51) Cl.Int./Int.Cl. *C12N 15/19* (2006.01),
A61K 38/17 (2006.01), *A61K 38/19* (2006.01),
A61P 29/00 (2006.01), *A61P 35/00* (2006.01),
C07K 14/715 (2006.01), *C07K 16/28* (2006.01),
C12N 15/63 (2006.01), *C12P 21/02* (2006.01),
G01N 33/566 (2006.01)

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(54) Titre : RECEPTEURS MULTIMERES DES CYTOKINES CONTENANT ZCYTOR17
(54) Title: CYTOKINE RECEPTOR ZCYTOR17 MULTIMERS

(57) **Abrégé/Abstract:**

Novel polypeptide combinations, polynucleotides encoding the polypeptides, and related compositions and methods are disclosed for zcytor17-containing multimeric or heterodimer cytokine receptors that may be used as novel cytokine antagonists, and within methods for detecting ligands that stimulate the proliferation and/or development of hematopoietic, lymphoid and myeloid cells in vitro and in vivo. The present invention also includes methods for producing the multimeric or heterodimeric cytokine receptor, uses therefor and antibodies thereto.



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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
8 January 2004 (08.01.2004)

PCT

(10) International Publication Number
WO 2004/003140 A2

(51) International Patent Classification⁷: **C12N**

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(21) International Application Number:
PCT/US2003/001983

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(22) International Filing Date: 21 January 2003 (21.01.2003)

(25) Filing Language: English

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW.

(26) Publication Language: English

(30) Priority Data:
60/350,325 18 January 2002 (18.01.2002) US
60/389,108 14 June 2002 (14.06.2002) US
60/435,361 19 December 2002 (19.12.2002) US

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(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CYTOKINE RECEPTOR ZCYTOR17 MULTIMERS

(57) Abstract: Novel polypeptide combinations, polynucleotides encoding the polypeptides, and related compositions and methods are disclosed for zcytor17-containing multimeric or heterodimer cytokine receptors that may be used as novel cytokine antagonists, and within methods for detecting ligands that stimulate the proliferation and/or development of hematopoietic, lymphoid and myeloid cells in vitro and in vivo. The present invention also includes methods for producing the multimeric or heterodimeric cytokine receptor, uses therefor and antibodies thereto.

WO 2004/003140 A2

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CYTOKINE RECEPTOR ZCYTOR17 MULTIMERS

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BACKGROUND OF THE INVENTION

Proliferation and differentiation of cells of multicellular organisms are controlled by hormones and polypeptide growth factors. These diffusable molecules allow cells to communicate with each other and act in concert to form cells, tissues and
10 organs, and to repair damaged tissue. Examples of hormones and growth factors include the steroid hormones (e.g., estrogen, testosterone), parathyroid hormone, follicle stimulating hormone, the interleukins, platelet derived growth factor (PDGF), epidermal growth factor (EGF), granulocyte-macrophage colony stimulating factor (GM-CSF), erythropoietin (EPO) and calcitonin.

15 Hormones and growth factors influence cellular metabolism by binding to receptors. Receptors may be integral membrane proteins that are linked to signaling pathways within the cell, such as second messenger systems. Other classes of receptors are soluble molecules, such as the transcription factors.

Cytokines generally stimulate proliferation or differentiation of cells of
20 the hematopoietic lineage or participate in the immune and inflammatory response mechanisms of the body. Examples of cytokines which affect hematopoiesis are erythropoietin (EPO), which stimulates the development of red blood cells; thrombopoietin (TPO), which stimulates development of cells of the megakaryocyte lineage; and granulocyte-colony stimulating factor (G-CSF), which stimulates
25 development of neutrophils. These cytokines are useful in restoring normal blood cell levels in patients suffering from anemia, thrombocytopenia, and neutropenia or receiving chemotherapy for cancer.

The interleukins are a family of cytokines that mediate immunological responses, including inflammation. The interleukins mediate a variety of inflammatory
30 pathologies. Central to an immune response are T cells, which produce many cytokines and adaptive immunity to antigens. Cytokines produced by T cells have been classified as type 1 and type 2 (Kelso, A. *Immun. Cell Biol.* 76:300-317, 1998). Type 1 cytokines

include IL-2, IFN- γ , LT- α , and are involved in inflammatory responses, viral immunity, intracellular parasite immunity and allograft rejection. Type 2 cytokines include IL-4, IL-5, IL-6, IL-10 and IL-13, and are involved in humoral responses, helminth immunity and allergic response. Shared cytokines between Type 1 and 2 include IL-3, GM-CSF and TNF- α . There is some evidence to suggest that Type 1 and Type 2 producing T cell populations preferentially migrate into different types of inflamed tissue.

Mature T cells may be activated, i.e., by an antigen or other stimulus, to produce, for example, cytokines, biochemical signaling molecules, or receptors that further influence the fate of the T cell population.

10 B cells can be activated via receptors on their cell surface including B cell receptor and other accessory molecules to perform accessory cell functions, such as production of cytokines.

Monocytes/macrophages and T-cells can be activated by receptors on their cell surface and play a central role in the immune response by presenting antigen to lymphocytes and also act as accessory cells to lymphocytes by secreting numerous cytokines.

Natural killer (NK) cells have a common progenitor cell with T cells and B cells, and play a role in immune surveillance. NK cells, which comprise up to 15% of blood lymphocytes, do not express antigen receptors, and therefore do not use MHC recognition as requirement for binding to a target cell. NK cells are involved in the recognition and killing of certain tumor cells and virally infected cells. *In vivo*, NK cells are believed to require activation, however, *in vitro*, NK cells have been shown to kill some types of tumor cells without activation.

The demonstrated *in vivo* activities of these cytokines illustrate the enormous clinical potential of, and need for, other cytokines, cytokine agonists, and cytokine antagonists or binding partners. The present invention addresses these needs by providing a new hematopoietic multimeric cytokine receptor, as well as related compositions and methods.

The present invention provides such polypeptides for these and other uses that should be apparent to those skilled in the art from the teachings herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an illustration of a multiple alignment of human zcytor17lig (SEQ ID NO:2) (zcytor17lig), and mouse zcytor17lig (SEQ ID NO:11) (mzcytor17lig), mouse IL-3 (mIL-3) (SEQ ID NO:100), and human IL-3 (hIL-3) (SEQ ID NO:102).

5 Figure 2 is an illustration of a multiple alignment of human zcytor17lig (SEQ ID NO:2) (zcytor17lig), and mouse zcytor17lig (SEQ ID NO:11) (mzcytor17lig).

Figure 3 is a Hopp/Woods hydrophilicity plot of human zcytor17lig (SEQ ID NO:2).

Figure 4 is a multiple alignment of zcytor17 polynucleotide sequences
10 SEQ ID NO:109, SEQ ID NO:113, SEQ ID NO:5, SEQ ID NO:111, and SEQ ID NO:115.

Figure 5 is an alignment of human zcytor17 (ZCYTOR) (SEQ ID NO:5) and mouse zcytor17 (M17R-O) (SEQ ID NO:117). Between the two sequences, identical residues (:), Conserved residues (.) and gaps (-) are indicated.

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SUMMARY OF THE INVENTION

The present invention provides an isolated multimeric or heterodimeric cytokine receptor comprising at least one polypeptide having at least 90 percent sequence identity with SEQ ID NO:111 or SEQ ID NO:109; and wherein the
20 multimeric or heterodimeric cytokine receptor binds a ligand comprising SEQ ID NO:2. Optionally, the isolated multimeric or heterodimeric cytokine receptor may further comprise a cytokine-binding domain of a class I cytokine receptor. The cytokine-binding domain of the class I cytokine receptor may comprise amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 28 to amino acid residue
25 739 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 761 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 761 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 979 of SEQ ID NO:7, or amino acid residue 1 to amino acid residue 979 of SEQ ID NO:7. The
30 isolated multimeric or heterodimeric cytokine receptor may antagonize an activity of SEQ ID NO:2. The isolated multimeric or heterodimeric cytokine receptor may inhibit

proliferation of hematopoietic cells, inhibit proliferation of immune cells, inhibit proliferation of inflammatory cells, inhibit an immune response, inhibit an inflammatory response, or inhibit proliferation of tumor cells of epithelial origin. The isolated multimeric or heterodimeric cytokine receptor may be soluble. The isolated
5 multimeric or heterodimeric cytokine receptor may further comprises an affinity tag, such as, for instance, polyhistidine, protein A, glutathione S transferase, Glu-Glu, substance P, FlagTM peptide, streptavidin binding peptide, and immunoglobulin F_c polypeptide, or cytotoxic molecule, such as, for instance, a toxin or radionuclide. The isolated multimeric or heterodimeric cytokine receptor wherein the polypeptide having
10 at least 90 percent identity with SEQ ID NO:111 may comprise amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 519 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 543 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 732 of SEQ ID NO:111, amino acid residue 1 to amino acid residue 227, amino acid residue 1 to amino acid
15 residue 519, amino acid residue 1 to amino acid residue 543, or amino acid residue 1 to amino acid residue 732. The isolated multimeric or heterodimeric cytokine receptor wherein the polypeptide having at least 90 percent identity with SEQ ID NO:109 may comprise amino acid residue 1 to amino acid residue 649 of SEQ ID NO:109, or amino acid residue 20 to amino acid residue 649 of SEQ ID NO:109.

20 The present invention also provides an isolated multimeric or heterodimeric cytokine receptor comprising at least one polypeptide comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111. The at least one polypeptide may comprise amino acid residue 1 to amino acid residue 227 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 519 of SEQ ID NO:111, amino
25 acid residue 1 to amino acid residue 519 of SEQ ID NO:111, amino acid residue 1 to amino acid residue 543 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 543 of SEQ ID NO:111, amino acid residue 1 to amino acid residue 732 of SEQ ID NO:111, or amino acid residue 20 to amino acid residue 732 of SEQ ID NO:111. The isolated multimeric or heterodimeric cytokine receptor may further comprise a
30 cytokine-binding domain of a class I cytokine receptor, for instance, amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 1 to amino acid

residue 429 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 761 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 761 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 979 of SEQ ID NO:7, or amino acid residue 1 to amino acid residue 979 of SEQ ID NO:7. The isolated multimeric or heterodimeric cytokine receptor may antagonize an activity of a ligand comprising SEQ ID NO:2. The isolated multimeric or heterodimeric cytokine receptor may inhibit proliferation of hematopoietic cells, inhibit proliferation of immune cells, inhibit proliferation of inflammatory cells, inhibit an immune response, inhibit an inflammatory response, or inhibit proliferation of tumor cells of epithelial origin. Optionally, the isolated multimeric or heterodimeric cytokine receptor may be is soluble. The isolated multimeric or heterodimeric cytokine receptor may further comprise an affinity tag, such as, for instance, polyhistidine, protein A, glutathione S transferase, Glu-Glu, substance P, Flag™ peptide, streptavidin binding peptide, and immunoglobulin F_c polypeptide, or cytotoxic molecule, such as, for instance, a toxin or radionuclide.

The present invention also provides a soluble multimeric or heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7.

The present invention also provides an isolated polynucleotide that encodes a cytokine receptor polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with SEQ ID NO:111 or SEQ ID NO:109, wherein the cytokine receptor polypeptide forms a multimeric or heterodimeric cytokine receptor, and wherein the multimeric or heterodimeric cytokine receptor binds a ligand comprising SEQ ID NO:2. The multimeric or heterodimeric cytokine receptor may further comprise a cytokine-binding domain of a class I cytokine receptor, such as, for instance, amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 761 of SEQ ID NO:7, amino

acid residue 28 to amino acid residue 761 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 979 of SEQ ID NO:7, or amino acid residue 1 to amino acid residue 979 of SEQ ID NO:7. The multimeric or heterodimeric cytokine receptor may antagonize an activity of SEQ ID NO:2. The multimeric or heterodimeric cytokine receptor may inhibit proliferation of hematopoietic cells, inhibit proliferation of immune cells, inhibit proliferation of inflammatory cells, inhibit an immune response, inhibit an inflammatory response, or inhibit proliferation of tumor cells of epithelial origin. Optionally, the multimeric or heterodimeric cytokine receptor may be soluble. The multimeric or heterodimeric cytokine receptor may further comprise an affinity tag, such as, for instance, polyhistidine, protein A, glutathione S transferase, Glu-Glu, substance P, FlagTM peptide, streptavidin binding peptide, and immunoglobulin F_c polypeptide, or cytotoxic molecule, such as, for instance, a toxin or radionuclide. The encoded cytokine receptor polypeptide having at least 90 percent identity with SEQ ID NO:111 may comprise amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 519 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 543 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 732 of SEQ ID NO:111, amino acid residue 1 to amino acid residue 227 of SEQ ID NO:111, amino acid residue 1 to amino acid residue 519 of SEQ ID NO:111, amino acid residue 1 to amino acid residue 543 of SEQ ID NO:111, or amino acid residue 1 to amino acid residue 732 of SEQ ID NO:111. The encoded cytokine receptor polypeptide having at least 90 percent identity with SEQ ID NO:109 may comprise amino acid residue 1 to amino acid residue 649 of SEQ ID NO:109, or amino acid residue 20 to amino acid residue 649 of SEQ ID NO:109.

The present invention also provides an isolated polynucleotide that encodes a cytokine receptor polypeptide comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111, wherein the cytokine receptor polypeptide forms a multimeric or heterodimeric cytokine receptor. The cytokine receptor polypeptide may comprise amino acid residue 1 to amino acid residue 227 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 519 of SEQ ID NO:111, amino acid residue 1 to amino acid residue 519 of SEQ ID NO:111, amino acid residue 1 to amino acid residue 543 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 543 of SEQ ID

NO:111, amino acid residue 1 to amino acid residue 732 of SEQ ID NO:111, or amino acid residue 20 to amino acid residue 732 of SEQ ID NO:111. The multimeric or heterodimeric cytokine receptor may further comprise a cytokine-binding domain of a class I cytokine receptor, such as, for instance, amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 761 of SEQ ID NO:7, amino acid residue 1 to amino acid residue 761 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 979 of SEQ ID NO:7, or amino acid residue 1 to amino acid residue 979 of SEQ ID NO:7. The multimeric or heterodimeric cytokine receptor may antagonize an activity of a ligand comprising SEQ ID NO:2. The multimeric or heterodimeric cytokine receptor may inhibit proliferation of hematopoietic cells, inhibit proliferation of immune cells, inhibit proliferation of inflammatory cells, inhibit an immune response, inhibit an inflammatory response, or inhibit proliferation of tumor cells of epithelial origin. Optionally, the multimeric or heterodimeric cytokine receptor may be soluble. The multimeric or heterodimeric cytokine receptor may further comprise an affinity tag or cytotoxic molecule as described herein.

The present invention also provides an expression vector that comprises the the following operably linked elements: a transcription promoter; a DNA segment encoding a cytokine receptor polypeptide having at least 90 percent sequence identity with SEQ ID NO:111; and a transcription terminator; wherein the cytokine receptor polypeptide forms a multimeric or heterodimeric cytokine receptor, and wherein the multimeric or heterodimeric cytokine receptor binds a ligand comprising SEQ ID NO:2.

Alternatively, the present invention also provides an expression vector that comprises the following operably linked elements: a) a first transcription promoter; a first DNA segment encoding a cytokine receptor polypeptide having at least 90 percent sequence identity with SEQ ID NO:111; and a first transcription terminator; and b) a second transcription promoter; a second DNA segment encoding a cytokine-binding domain of a class I cytokine receptor; and a second transcription terminator; wherein the cytokine receptor polypeptide and the class I cytokine receptor form a

multimeric or heterodimeric cytokine receptor; and wherein the multimeric or heterodimeric cytokine receptor binds to a ligand comprising SEQ ID NO:2.

Alternatively, the present invention also provides an expression vector that comprises the following operably linked elements: a) a first transcription
5 promoter; a first DNA segment encoding a polypeptide having at least 90 percent sequence identity with SEQ ID NO:111; and a first transcription terminator; and b) a second transcription promoter; a second DNA segment encoding at least a portion of a class I cytokine receptor; and a second transcription terminator; wherein the polypeptide and the class I cytokine receptor form a multimeric cytokine receptor; and wherein the
10 multimeric cytokine receptor binds to at least a portion of SEQ ID NO:2.

The expression vectors of the present invention may further include a secretory signal sequence linked to the first and second DNA segments. The multimeric or heterodimeric cytokine receptor may be soluble, membrane-bound, or attached to a solid support. The multimeric or heterodimeric cytokine receptor may
15 antagonize an activity of a ligand comprising SEQ ID NO:2. The multimeric or heterodimeric cytokine receptor may inhibit proliferation of hematopoietic cells, inhibit proliferation of immune cells, inhibit proliferation of inflammatory cells, inhibit an immune response, inhibit an inflammatory response, or inhibit proliferation of tumor cells of epithelial origin. Optionally, the multimeric or heterodimeric cytokine receptor
20 may be soluble. The multimeric or heterodimeric cytokine receptor may further comprise an affinity tag or cytotoxic molecule as described herein.

The present invention also provides a cultured cell including an expression vector as described herein, wherein the cell expresses the polypeptide or polypeptides encoded by the DNA segment or segments. The cell may secrete the
25 multimeric or heterodimeric cytokine receptor. The multimeric cytokine receptor may bind and/or antagonize an activity of SEQ ID NO:2 as further described herein.

The present invention also provides a cultured cell which includes a first expression vector comprising: a) a transcription promoter; b) a DNA segment encoding a cytokine receptor polypeptide having at least 90 percent sequence identity with SEQ
30 ID NO:111; and c) a transcription terminator; and a second expression vector comprising: a) a transcription promoter; b) a DNA segment encoding a cytokine-

binding domain of a class I cytokine receptor; and c) a transcription terminator; wherein the cytokine receptor polypeptide and the class I cytokine receptor form a multimeric or heterodimeric cytokine receptor, and wherein the multimeric or heterodimeric cytokine receptor binds to a ligand that comprises SEQ ID NO:2. The first and second
5 expression vectors may include a secretory signal sequence operably linked to the first and second DNA segments. The cultured cell may further comprise a third expression vector which includes a) a transcription promoter; b) a DNA segment encoding a cytokine-binding domain of a second class I cytokine receptor; and c) a transcription terminator; wherein the cytokine receptor polypeptide, the first class I cytokine
10 receptor, and the second class I cytokine receptor form a multimeric cytokine receptor. The cytokine-binding domain of a class I cytokine receptor may be of SEQ ID NO:7 and/or SEQ ID NO:9. Optionally, the multimeric or heterodimeric cytokine receptor may be soluble. The multimeric or heterodimeric cytokine receptor may further include an affinity tag as described herein. The multimeric or heterodimeric cytokine receptor
15 may bind to at least a portion of SEQ ID NO:2 and/or antagonize an activity of SEQ ID NO:2 as described herein.

The present invention also provides a method of producing an antibody to a multimeric or heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I
20 cytokine receptor. The method includes inoculating an animal with the multimeric or heterodimeric cytokine receptor, wherein the multimeric or heterodimeric cytokine receptor elicits an immune response in the animal to produce an antibody that specifically binds the multimeric or heterodimeric cytokine receptor; and isolating the antibody from the animal. The antibody may optionally be a monoclonal antibody. The
25 antibody may optionally be a neutralizing antibody. The antibody may specifically bind to a multimeric or heterodimeric cytokine receptor as described herein.

The present invention also provides a composition which includes an effective amount of a soluble multimeric or heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-
30 binding domain of a class I cytokine receptor; and a pharmaceutically acceptable vehicle. The binding domain of the class I cytokine receptor may include amino acid

residue 28 to amino acid residue 429 of SEQ ID NO:7. The soluble multimeric or heterodimeric cytokine receptor may bind to a ligand comprising SEQ ID NO:2. The soluble multimeric or heterodimeric cytokine receptor may further include an affinity tag or cytotoxic molecule as described herein. The composition may antagonize an activity of a ligand comprising SEQ ID NO:2. The composition may inhibit proliferation of hematopoietic cells, inhibit proliferation of immune cells, inhibit proliferation of inflammatory cells, inhibit an immune response, inhibit an inflammatory response, or inhibit proliferation of tumor cells of epithelial origin

The present invention also provides a method of producing a multimeric or heterodimeric cytokine receptor comprising culturing a cell as described herein, and isolating the multimeric or heterodimeric cytokine receptor produced by the cell.

The present invention also provides an immune cell inhibiting composition which includes an effective amount of a soluble multimeric or heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor; and a pharmaceutically acceptable vehicle; wherein the soluble multimeric or heterodimeric cytokine receptor inhibits the proliferation of immune cells.

The present invention also provides an immune response inhibiting composition which includes an effective amount of a soluble multimeric or heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor; and a pharmaceutically acceptable vehicle; wherein the soluble multimeric or heterodimeric cytokine receptor inhibits an immune response.

The present invention also provides an inflammatory cell inhibiting composition which includes an effective amount of a soluble multimeric or heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor; and a pharmaceutically acceptable vehicle; wherein the soluble multimeric or heterodimeric cytokine receptor inhibits the proliferation of inflammatory cells.

The present invention also provides an inflammatory response inhibiting composition which includes an effective amount of a soluble multimeric or

heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor; and a pharmaceutically acceptable vehicle; wherein the soluble multimeric or heterodimeric cytokine receptor inhibits an inflammatory response.

5 The present invention also provides a method of inhibiting an immune response in a mammal exposed to an antigen or pathogen. The method includes (a) determining directly or indirectly the level of antigen or pathogen present in the mammal; (b) administering a composition comprising a soluble multimeric or heterodimeric cytokine receptor in a pharmaceutically acceptable vehicle; (c)
10 determining directly or indirectly the level of antigen or pathogen in the mammal; and (d) comparing the level of the antigen or pathogen in step (a) to the antigen or pathogen level in step (c), wherein a change in the level is indicative of inhibiting an immune response. The method may further comprise (e) re-administering a composition comprising a multimeric cytokine receptor in a pharmaceutically acceptable vehicle; (f)
15 determining directly or indirectly the level of antigen or pathogen in the mammal; and (g) comparing the number of the antigen or pathogen level in step (a) to the antigen level in step (f), wherein a change in the level is indicative of inhibiting an immune response.

 The present invention also provides a method for reducing
20 hematopoietic cells and/or hematopoietic progenitors cells in a mammal. The method includes culturing bone marrow or peripheral blood cells with a composition comprising an effective amount of a soluble multimeric or heterodimeric cytokine receptor to produce a decrease in the number of lymphoid cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in
25 the absence of the multimeric or heterodimeric cytokine receptor. The hematopoietic cells and hematopoietic cell progenitors may be lymphoid, which can be monocytic cells, macrophages, or T cells.

 The present invention also provides a method of detecting the presence of a multimeric or heterodimeric cytokine receptor in a biological sample. The method includes contacting the biological sample with an antibody, or an antibody fragment, as described herein, wherein the contacting is performed under conditions that allow the

binding of the antibody or antibody fragment to the biological sample; and detecting any of the bound antibody or bound antibody fragment.

The present invention also provides a method of a method of killing cancer cells. The method includes obtaining *ex vivo* a tissue or biological sample
5 containing cancer cells from a patient, or identifying cancer cells *in vivo*; producing a multimeric or heterodimeric cytokine receptor by a method as described herein; formulating the multimeric or heterodimeric cytokine receptor in a pharmaceutically acceptable vehicle; and administering to the patient or exposing the cancer cells to the multimeric or heterodimeric cytokine receptor formulation; wherein the multimeric or
10 heterodimeric cytokine receptor kills the cells. The multimeric or heterodimeric cytokine receptor may be further conjugated to a toxin.

The present invention also provides an antibody that specifically binds to a multimeric or heterodimeric cytokine receptor as described herein. The antibody may be a polyclonal antibody, a murine monoclonal antibody, a humanized antibody derived
15 from a murine monoclonal antibody, an antibody fragment, a neutralizing antibody, or a human monoclonal antibody. The antibody or antibody fragment may specifically bind to a multimeric or heterodimeric cytokine receptor of the present invention which may comprise a cytokine receptor polypeptide comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I
20 cytokine receptor. The antibody may further include a radionuclide, enzyme, substrate, cofactor, fluorescent marker, chemiluminescent marker, peptide tag, magnetic particle, drug, or toxin.

The present invention also provides a method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic
25 progenitor cells. The method includes culturing bone marrow or peripheral blood cells with a composition comprising an amount of a soluble multimeric or heterodimeric cytokine receptor comprising a cytokine receptor polypeptide comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor sufficient to reduce proliferation or differentiation
30 of the hematopoietic cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of the soluble multimeric

or heterodimeric cytokine receptor. The hematopoietic cells and hematopoietic progenitor cells may be lymphoid cells, such as macrophages or T cells.

The present invention also provides a method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic progenitor cells comprising culturing bone marrow or peripheral blood cells with a composition comprising an amount of a soluble multimeric or heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO : 111 and a cytokine-binding domain of a class I cytokine receptor sufficient to reduce proliferation or differentiation of the hematopoietic cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of the soluble multimeric or heterodimeric cytokine receptor.

The present invention also provides a method of reducing zcytor17lig-induced induced inflammation. The method includes administering to a mammal with inflammation an amount of a composition comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor sufficient to reduce inflammation.

The present invention also provides a method of suppressing an inflammatory response in a mammal with inflammation. The method includes (1) determining a level of an inflammatory molecule; (2) administering a composition comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor in a pharmaceutically acceptable vehicle; (3) determining a post administration level of the inflammatory molecule; (4) comparing the level of the inflammatory molecule in step (1) to the level of the inflammatory molecule in step (3), wherein a lack of increase or a decrease the inflammatory molecule level is indicative of suppressing an inflammatory response.

The present invention also provides a method for inhibiting zcytor17lig-induced proliferation or differentiation of hematopoietic cells and hematopoietic progenitor cells. The method includes culturing bone marrow or peripheral blood cells with a composition comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor in a pharmaceutically acceptable vehicle sufficient to reduce proliferation or differentiation of the hematopoietic cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of soluble multimeric or

heterodimeric cytokine receptor. The hematopoietic cells and hematopoietic progenitor cells may be lymphoid cells, such as macrophages or T cells.

The present invention also provides a method of reducing zcytor17lig-induced induced inflammation. The method includes administering to a mammal with inflammation an amount of a composition comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor in a pharmaceutically acceptable vehicle sufficient to reduce

inflammation.

The present invention also provides a method of suppressing an inflammatory response in a mammal with inflammation. The method includes (1) determining a level of an inflammatory molecule; (2) administering a composition
5 comprising a multimeric or heterodimeric cytokine receptor which comprises amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 in a pharmaceutically acceptable vehicle; (3) determining a post administration level of the inflammatory molecule; (4) comparing the level of the inflammatory molecule in step (1) to the level of the inflammatory molecule in step (3), wherein a lack of increase or a decrease in the
10 inflammatory molecule level is indicative of suppressing an inflammatory response.

The present invention also provides a method of treating a mammal afflicted with an inflammatory disease in which zcytor17lig plays a role. The method includes administering an antagonist of zcytor17lig to the mammal such that the inflammation is reduced, wherein the antagonist is a soluble multimeric or
15 heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor in a pharmaceutically acceptable vehicle. The inflammatory disease may be a chronic inflammatory disease, such as, for instance, inflammatory bowel disease, ulcerative colitis, Crohn's disease, atopic dermatitis, eczema, or psoriasis. The
20 inflammatory disease may be an acute inflammatory disease, such as, for instance, endotoxemia, septicemia, toxic shock syndrome, or infectious disease. Optionally, the soluble multimeric or heterodimeric cytokine receptor may further comprise a radionuclide, enzyme, substrate, cofactor, fluorescent marker, chemiluminescent marker, peptide tag, magnetic particle, drug, or toxin.

25 The present invention also provides a method for detecting inflammation in a patient. The method includes obtaining a tissue or biological sample from a patient; incubating the tissue or biological sample with a soluble multimeric or heterodimeric cytokine receptor comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine
30 receptor under conditions wherein the soluble multimeric or heterodimeric cytokine receptor binds to its complementary polypeptide in the tissue or biological sample;

visualizing the soluble multimeric or heterodimeric cytokine receptor bound in the tissue or biological sample; and comparing levels of soluble multimeric or heterodimeric cytokine receptor bound in the tissue or biological sample from the patient to a normal control tissue or biological sample, wherein an increase in the level
5 of soluble multimeric or heterodimeric cytokine receptor bound to the patient tissue or biological sample relative to the normal control tissue or biological sample is indicative of inflammation in the patient.

The present invention also provides a method for detecting a multiple cytokine receptor ligand from a test sample. The method includes contacting the test
10 sample with a multimeric or heterodimeric cytokine receptor comprising a cytokine receptor polypeptide comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and a cytokine-binding domain of a class I cytokine receptor; and detecting the binding of the multimeric or heterodimeric cytokine receptor to the ligand in the test sample.

15

DETAILED DESCRIPTION OF THE INVENTION

Definitions

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

20 Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a
25 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. 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* 82:7952-4, 1985), substance P, Flag™ peptide (Hopp et al., *Biotechnology* 6:1204-10, 1988),
30 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., 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 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 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 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 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 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 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" denotes a polynucleotide molecule having a 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 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, 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
5 nucleotides that includes one or 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 DNA molecule, linear
10 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 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
15 contain elements of both.

The term "isolated", when applied to a 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
20 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,
25 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
30 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 "neoplastic", when referring to cells, indicates cells undergoing new and abnormal proliferation, particularly in a tissue where the proliferation is uncontrolled and progressive, resulting in a neoplasm. The neoplastic cells can be either malignant, i.e., invasive and metastatic, or benign.

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, α -globin, β -globin, and myoglobin are paralogs of each other.

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 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-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 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.

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".

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' non-coding regions of genes.

5 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 are defined herein in terms of their amino acid backbone structures;
10 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 ligand) and mediates the effect of the ligand on the cell. Membrane-bound receptors are characterized by a multi-peptide structure comprising
15 an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of 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 to receptor-ligand
20 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 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.,
25 PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, 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 of a cell in
30 which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

A "soluble receptor" is a receptor polypeptide that is not bound to a cell membrane. Soluble receptors are most commonly ligand-binding receptor polypeptides that lack transmembrane and cytoplasmic domains. Soluble receptors can comprise additional amino acid residues, such as affinity tags that provide for purification of the polypeptide or provide sites for attachment of the polypeptide to a substrate, or immunoglobulin constant region sequences. Many cell-surface receptors have naturally occurring, soluble counterparts that are produced by proteolysis. Soluble receptor polypeptides are said to be substantially free of transmembrane and intracellular polypeptide segments when they lack sufficient portions of these segments to provide membrane anchoring or signal transduction, respectively.

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 $\pm 10\%$.

The present invention is based in part upon the discovery of a novel multimeric cytokine receptor protein having the structure of a class I cytokine receptor, referred to herein as "multimeric cytokine receptor," or "zcytor17 multimeric cytokine receptor."

Another receptor subunit polypeptide that may be included in the multimeric cytokine receptor of the present invention includes at least a portion of at least one polypeptide of a class I cytokine receptor, such as OSMRbeta and/or WSX-1. For example, the deduced amino acid sequence indicated that zcytor17 belongs to the

receptor subfamily that includes gp130, LIF, IL-12, oncostatinM receptor beta (OSMRbeta) (SEQ ID NO:7), WSX-1 receptors (SEQ ID NO:9) (Sprecher, CA et al., *Biochem. Biophys. Res. Comm.*, 246:81-90 (1998); and U.S. Patent No. 5,925,735), DCRS2 (WIPO Publication No. WO 00/73451), the IL-2 receptor β -subunit and the β -common receptor (i.e., IL-3, IL-5, and GM-CSF receptor subunits). A further example of class I cytokine receptor subunit polypeptides that may be included in the multimeric cytokine receptor are the receptors for IL-2, IL-4, IL-7, Lif, IL-12, IL-15, EPO, TPO, GM-CSF and G-CSF (Cosman, *Cytokine*, 5(2):95-106 (1993)).

Cytokine receptor subunits are characterized by a multi-domain structure comprising an extracellular domain, a transmembrane domain that anchors the polypeptide in the cell membrane, and an intracellular domain. The extracellular domain may be a ligand-binding domain, and the intracellular domain may be an effector domain involved in signal transduction, although ligand-binding and effector functions may reside on separate subunits of a multimeric receptor. The ligand-binding domain may itself be a multi-domain structure. Multimeric receptors include homodimers (e.g., PDGF receptor $\alpha\alpha$ and $\beta\beta$ isoforms, erythropoietin receptor, MPL, and G-CSF receptor), heterodimers whose subunits each have ligand-binding and effector domains (e.g., PDGF receptor $\alpha\beta$ isoform), and multimers having component subunits with disparate functions (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, and GM-CSF receptors). Some receptor subunits are common to a plurality of receptors. For example, the AIC2B subunit, which cannot bind ligand on its own but includes an intracellular signal transduction domain, is a component of IL-3 and GM-CSF receptors. Many cytokine receptors can be placed into one of four related families on the basis of the structure and function. Hematopoietic receptors, for example, are characterized by the presence of a domain containing conserved cysteine residues and the WSXWS motif (SEQ ID NO:3). Cytokine receptor structure has been reviewed by Urdal, *Ann. Reports Med. Chem.* 26:221-228, 1991 and Cosman, *Cytokine* 5:95-106, 1993. Under selective pressure for organisms to acquire new biological functions, new receptor family members likely arise from duplication of existing receptor genes leading to the existence of multi-gene families. Family members thus contain vestiges of the ancestral gene, and these characteristic features can be exploited in the isolation

and identification of additional family members. Thus, the cytokine receptor superfamily is subdivided into several families, for example, the immunoglobulin family (including CSF-1, MGF, IL-1, and PDGF receptors); the hematopoietin family (including IL-2 receptor β -subunit, GM-CSF receptor α -subunit, GM-CSF receptor β -subunit; and G-CSF, EPO, IL-3, IL-4, IL-5, IL-6, IL-7, and IL-9 receptors); TNF receptor family (including TNF (p80) TNF (p60) receptors, CD27, CD30, CD40, Fas, and NGF receptor).

Analysis of the zcytor17 sequence suggests that it is a member of the same receptor subfamily as the gp130, LIF, IL-12, WSX-1, IL-2 receptor β -subunit, IL-3, IL-4, and IL-6 receptors. Certain receptors in this subfamily (e.g., G-CSF) associate to form homodimers that transduce a signal. Other members of the subfamily (e.g., gp130, IL-6, IL-11, and LIF receptors) combine with a second subunit (termed a β -subunit) to bind ligand and transduce a signal. Specific β -subunits associate with a plurality of specific cytokine receptor subunits. For example, the β -subunit gp130 (Hibi et al., *Cell* 63:1149-1157, 1990) associates with receptor subunits specific for IL-6, IL-11, and LIF (Gearing et al., *EMBO J.* 10:2839-2848, 1991; Gearing et al., U.S. Patent No. 5,284,755). Oncostatin M binds to a heterodimer of LIF receptor and gp130. CNTF binds to trimeric receptors comprising CNTF receptor, LIF receptor, and gp130 subunits.

A multimeric cytokine receptor of the present invention can be a heterodimer, trimer, tetramer, pentamer, and the like, comprising at least a portion of zcytor17 and at least a portion of a class I cytokine receptor. In addition, a multimeric cytokine receptor can be soluble, membrane-bound, or attached to a solid support. Analysis of the tissue distribution of the mRNA of the zcytor17 receptor revealed expression in activated CD4+ and CD8+ T-cell subsets, CD14+ monocytes, and weaker expression in CD19+ B-cells. Moreover, the mRNA was present in both resting or activated monocytic cell lines THP-1 (ATCC No. TIB-202), U937 (ATCC No. CRL-1593.2) and HL60 (ATCC No. CCL-240).

Nucleotide sequences of representative zcytor17-encoding DNA are described in SEQ ID NO:110 (from nucleotide 171 to 2366), with its deduced 732 amino acid sequence described in SEQ ID NO:111; SEQ ID NO:108 (from nucleotide

162 to 2108), with its deduced 649 amino acid sequence described in SEQ ID NO:109; and in SEQ ID NO:4 (from nucleotide 497 to 2482), with its deduced 662 amino acid sequence described in SEQ ID NO:5. In its entirety, the zcytor17 polypeptide (SEQ ID NO:111, SEQ ID NO:109 or SEQ ID NO:5) represents a full-length polypeptide segment (residue 1 (Met) to residue 732 (Val) of SEQ ID NO:111; residue 1 (Met) to residue 649 (Ile) of SEQ ID NO:109; residue 1 (Met) to residue 662 (Ile) of SEQ ID NO:5). The domains and structural features of the zcytor17 polypeptides are further described below.

Analysis of the zcytor17 polypeptide encoded by the DNA sequence of SEQ ID NO:110 revealed an open reading frame encoding 732 amino acids (SEQ ID NO:111) comprising a predicted secretory signal peptide of 19 amino acid residues (residue 1 (Met) to residue 19 (Ala) of SEQ ID NO:111), and a mature polypeptide of 713 amino acids (residue 20 (Ala) to residue 732 (Val) of SEQ ID NO:111). Analysis of the zcytor17 polypeptide encoded by the DNA sequence of SEQ ID NO:108 revealed an open reading frame encoding 649 amino acids (SEQ ID NO:109) comprising a predicted secretory signal peptide of 19 amino acid residues (residue 1 (Met) to residue 19 (Ala) of SEQ ID NO:109), and a mature polypeptide of 630 amino acids (residue 20 (Ala) to residue 649 (Ile) of SEQ ID NO:109). Analysis of the zcytor17 polypeptide encoded by the DNA sequence of SEQ ID NO:4 revealed an open reading frame encoding 662 amino acids (SEQ ID NO:5) comprising a predicted secretory signal peptide of 32 amino acid residues (residue 1 (Met) to residue 32 (Ala) of SEQ ID NO:5), and a mature polypeptide of 630 amino acids (residue 33 (Ala) to residue 662 (Ile) of SEQ ID NO:5). In addition to the WSXWS motif (SEQ ID NO:3) (corresponding to residues 211 to 215 of SEQ ID NO:111 and SEQ ID NO:109; and residues 224 to 228 of SEQ ID NO:5), the receptor comprises an extracellular domain (residues 20 (Ala) to 519 (Glu) of SEQ ID NO:111 and SEQ ID NO:109; residues 33 (Ala) to 532 (Glu) of SEQ ID NO:5) which includes a cytokine-binding domain of approximately 200 amino acid residues (residues 20 (Ala) to 227 (Pro) of SEQ ID NO:111 and SEQ ID NO:109; residues 33 (Ala) to 240 (Pro) of SEQ ID NO:5); a domain linker (residues 122 (Thr) to 125 (Pro) of SEQ ID NO:111 and SEQ ID NO:109; residues 135 (Thr) to 138 (Pro) of SEQ ID NO:111); a penultimate strand

region (residues 194 (Phe) to 202 (Arg) of SEQ ID NO:111 and SEQ ID NO:109; residues 207 (Phe) to 215 (Arg) of SEQ ID NO:5); a fibronectin type III domain (residues 228 (Cys) to 519 (Glu) of SEQ ID NO:111 and SEQ ID NO:109; residues 241 (Cys) to 532 (Glu) of SEQ ID NO:5); a transmembrane domain (residues 520 (Ile) to 543 (Leu) of SEQ ID NO:111 and SEQ ID NO:109; residues 533 (Ile) to 556 (Leu) of SEQ ID NO:5); complete intracellular signaling domain (residues 544 (Lys) to 732 (Val) of SEQ ID NO:111; residues 544 (Lys) to 649 (Ile) of SEQ ID NO:109; and residues 557 (Lys) to 662 (Ile) of SEQ ID NO:5) which contains a "Box I" signaling site (residues 554 (Trp) to 560 (Pro) of SEQ ID NO:111 and SEQ ID NO:109; residues 567 (Trp) to 573 (Pro) of SEQ ID NO:5), and a "Box II" signaling site (residues 617 (Gln) to 620 (Phe) of SEQ ID NO:111 and SEQ ID NO:109; residues 630 (Gln) to 633 (Phe) of SEQ ID NO:5). Those skilled in the art will recognize that these domain boundaries are approximate, and are based on alignments with known proteins and predictions of protein folding. In addition to these domains, conserved receptor features in the encoded receptor include (as shown in SEQ ID NO:111 and SEQ ID NO:109) a conserved Cys residue at position 30 (position 43 as shown in SEQ ID NO:5), CXW motif (wherein X is any amino acid) at positions 40-42 (positions 53-55 as shown in SEQ ID NO:5), Trp residue at position 170 (position 183 as shown in SEQ ID NO:5), and a conserved Arg residue at position 202 (position 215 as shown in SEQ ID NO:5). The corresponding polynucleotides encoding the zcytor17 polypeptide regions, domains, motifs, residues and sequences described above are as shown in SEQ ID NO:110, SEQ ID NO:108, and SEQ ID NO:4.

Moreover, truncated forms of the zcytor17 polypeptide appear to be naturally expressed. Both forms encode soluble zcytor17 receptors. A polynucleotide encoding a "long-form" of the soluble zcytor17 receptor, truncated within the fibronectin type III domain, is shown in SEQ ID NO:112 and the corresponding polypeptide is shown in SEQ ID NO:113. This truncated form encodes residues 1 (Met) through 324 (Lys) of SEQ ID NO:111 and SEQ ID NO:109), and thus comprises an intact signal sequence, WSXWS (SEQ ID NO:3) motif, linker, cytokine binding domain, penultimate strand, and conserved, Cys, CXW motif, Trp and Arg residues as described above. A polynucleotide encoding a "short-form" of the soluble zcytor17

receptor, truncated at the end of the cytokine binding domain is shown in SEQ ID NO:114 and the corresponding polypeptide is shown in SEQ ID NO:115. This truncated form encodes a 239 residue polypeptide that is identical to residues 1 (Met) through 225 (Glu) of SEQ ID NO:111 and SEQ ID NO:109 and then diverges, and thus
5 comprises an intact signal sequence, WSXWS (SEQ ID NO:3) motif, linker, cytokine binding domain, penultimate strand, and conserved, Cys, CXW motif, Trp and Arg residues as described above. A multiple alignment of the truncated forms compared to the full-length forms of zcytor17 is shown in Figure 1.

Moreover, the zcytor17 cDNA of SEQ ID NO:110, SEQ ID NO:108,
10 SEQ ID NO:112, and SEQ ID NO:114 encode polypeptides that may use an alternative initiating methionine (at nucleotide 75 of SEQ ID NO:110, at nucleotide 66 of SEQ ID NO:108, at nucleotide 66 of SEQ ID NO:112, and at nucleotide 66 of SEQ ID NO:114) that would encode a polypeptide in the same open reading frame (ORF) as the zcytor17 polypeptides of SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:113, and SEQ ID
15 NO:115. Use of the alternative initiating methionine would add 32 amino acids (shown in SEQ ID NO:48) in-frame to the N-terminus of SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:113, and SEQ ID NO:111. In addition, nucleotide 536 of SEQ ID NO:4 may serve as an alternative initiating methionine, thus generating the same N-terminus (starting at amino acid 14 (Met) of SEQ ID NO:5) and signal polypeptide sequence, as
20 SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:113, and SEQ ID NO:115. Moreover, the second Met at amino acid number 2 in the SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:113, and SEQ ID NO:115 sequences (similarly at amino acid number 15 (Met) in SEQ ID NO:5) may also serve as an alternative starting methionine for the polypeptides.

25 Nucleotide sequences of representative OSMRbeta-encoding DNA are described in SEQ ID NO:6 (from nucleotide 368 to 3304), with its deduced 979 amino acid sequence described in SEQ ID NO:7. In its entirety, the OSMRbeta polypeptide (SEQ ID NO:7) represents a full-length polypeptide segment (residue 1 (Met) to residue 979 (Cys) of SEQ ID NO:7. The domains and structural features of the OSMRbeta
30 polypeptides are further described below.

Analysis of the OSMRbeta polypeptide encoded by the DNA sequence

of SEQ ID NO:6 revealed an open reading frame encoding 979 amino acids (SEQ ID NO:7) comprising a predicted secretory signal peptide of 27 amino acid residues (residue 1 (Met) to residue 27 (Ala) of SEQ ID NO:7), and a mature polypeptide of 952 amino acids (residue 28 (Glu) to residue 979 (Cys) of SEQ ID NO:7). In addition to the two WSXWS motifs (SEQ ID NO:3) (corresponding to residues 129 to 133 and residues 415 to 419 of SEQ ID NO:7), the receptor comprises an extracellular domain (residues 28 (Glu) to 739 (Ser) of SEQ ID NO:7); which includes a cytokine-binding domain of approximately 400 amino acid residues (residues 28 (Glu) to 429 (Ala) of SEQ ID NO:7, which includes two linker domains (residues 31 (Pro) to 34 (Pro) and residues 343 (Asn) to 347 (Thr)), three regions of cytokine binding (residues 35 (Val) to 137 (Glu), residues 240 (Pro) to 342 (Glu), and residues 348 (Asn) to 429 (Ala), an immunoglobulin domain (residues 138 (Val) to 239 (Glu), two penultimate strand regions (residues 106 (His) to 115 (Lys) and residues 398 (Thr) to 405 (Arg) of SEQ ID NO:7), and a fibronectin type III domain (residues 430 (Pro) to 739 (Ser) of SEQ ID NO:7); a transmembrane domain (residues 740 (Met) to 761 (Leu) of SEQ ID NO:7); complete intracellular signaling domain (residues 762 (Lys) to 979 (Cys) of SEQ ID NO:7) which contains a "Box I" signaling site (residues 771 (Tyr) to 777 (Pro) of SEQ ID NO:7), and a "Box II" signaling site (residues 829 (Glu) to 832 (Leu) of SEQ ID NO:7). Those skilled in the art will recognize that these domain boundaries are approximate, and are based on alignments with known proteins and predictions of protein folding. In addition to these domains, conserved receptor features in the encoded receptor include (as shown in SEQ ID NO:7) conserved Trp residues at positions 52 and 353, a conserved Cys residue at position 288, CXW motif (wherein X is any amino acid) at positions 294-296, and a conserved Arg residue at position 405. The corresponding polynucleotides encoding the OSMRbeta polypeptide regions, domains, motifs, residues and sequences described above are as shown in SEQ ID NO:6.

The presence of transmembrane regions, and conserved and low variance motifs generally correlates with or defines important structural regions in proteins. Regions of low variance (e.g., hydrophobic clusters) are generally present in regions of structural importance (Sheppard, P. et al., *supra.*). Such regions of low variance often contain rare or infrequent amino acids, such as Tryptophan. The regions

flanking and between such conserved and low variance motifs may be more variable, but are often functionally significant because they may relate to or define important structures and activities such as binding domains, biological and enzymatic activity, signal transduction, cell-cell interaction, tissue localization domains and the like.

5 The regions of conserved amino acid residues in zcytor17, described above, can be used as tools to identify new family members. For instance, reverse transcription-polymerase chain reaction (RT-PCR) can be used to amplify sequences encoding the conserved regions from RNA obtained from a variety of tissue sources or cell lines. In particular, highly degenerate primers designed from the zcytor17
10 sequences are useful for this purpose. Designing and using such degenerate primers may be readily performed by one of skill in the art.

 The present invention also contemplates a multimeric zcytor17 receptor, as detailed herein, which is capable of intracellular signaling. Such receptors may include at least a portion of at least one extracellular domain of a zcytor17 receptor, and
15 an intracellular domain from a zcytor17 receptor or another class I cytokine receptor. In addition to the extracellular domain of zcytor17, the multimeric cytokine receptor can also include the extracellular domain of at least a portion of class I cytokine receptor, for instance, the ligand binding domains of OSMRbeta receptor and/or WSX-1 receptor. Alternatively, the multimeric cytokine receptor may include the extracellular
20 domain of another receptor, such as another class I cytokine receptor, and the intracellular domain of zcytor17 to effect intracellular signaling.

 The present invention further contemplates a multimeric cytokine receptor that is soluble. For example, a multimeric cytokine receptor may be, for instance, a heterodimer which includes, for example, a portion of the extracellular
25 domain of zcytor17 and a portion of the extracellular domain of a class I cytokine receptor, such as OSMRbeta (SEQ ID NO:7) and/or WSX-1 (SEQ ID NO:9). Additionally, a soluble multimeric cytokine receptor may also include an affinity tag, such as an immunoglobulin F_c polypeptide. The soluble multimeric cytokine receptor can be expressed as a fusion with an immunoglobulin heavy chain constant region, such as
30 an F_c fragment, which contains two constant region domains and lacks the variable region. Such fusions are typically secreted as multimeric molecules wherein the F_c

residues 72, 133, and 147 of SEQ ID NO:2; and 74, 137, and 151 of SEQ ID NO:11 described herein. Consistent cysteine placement is further confirmation of the four-helical-bundle structure. Also highly conserved in the zcytor17lig is the Glu residue as shown in SEQ ID NO:2 at residue 43.

5 Moreover, the predicted amino acid sequence of murine zcytor17lig shows 31% identity to the predicted human protein over the entire length of the sequences (SEQ ID NO:2 and SEQ ID NO:11). Based on comparison between sequences of human and murine zcytor17lig conserved residues were found in the regions predicted to encode alpha helices C and D. The corresponding polynucleotides
10 encoding the human zcytor17lig polypeptide regions, domains, motifs, residues and sequences described herein are as shown in SEQ ID NO:1.

While helix D is relatively conserved between human and murine zcytor17lig, helix C is the most conserved. While both species have predominant acidic amino acids in this region, the differences may account for species specificity in interaction
15 between zcytor17lig and its receptor, zcytor17, comprising monomeric, heterodimeric (e.g., zcytor17/OSMRbeta, WSX-1/OSMRbeta, zcytor17/WSX-1) or multimeric (e.g., zcytor17/OSMRbeta/WSX-1) receptors. Loop A/B and helix B of zcytor17lig are marginally conserved, and helix C through Loop C/D into helix D is most conserved between species; conservation through this region suggests that it is functionally
20 significant. The D helices of human and murine zcytor17lig are also conserved. Zcytor17 receptor antagonists may be designed through mutations within zcytor17lig helix D. These may include truncation of the protein from residue Thr156 (SEQ ID NO:2), or conservation of residues that confer binding of the ligand to the receptor, but diminish signaling activity.

25 Four-helical bundle cytokines are also grouped by the length of their component helices. "Long-helix" form cytokines generally consist of between 24-30 residue helices, and include IL-6, ciliary neutrotrophic factor (CNTF), leukemia inhibitory factor (LIF) and human growth hormone (hGH). "Short-helix" form cytokines generally consist of between 18-21 residue helices and include IL-2, IL-4 and
30 GM-CSF. Zcytor17lig is believed to be a new member of the short-helix form cytokine group. Studies using CNTF and IL-6 demonstrated that a CNTF helix can be

exchanged for the equivalent helix in IL-6, conferring CTNF-binding properties to the chimera. Thus, it appears that functional domains of four-helical cytokines are determined on the basis of structural homology, irrespective of sequence identity, and can maintain functional integrity in a chimera (Kallen et al., *J. Biol. Chem.* 274:11859-11867, 1999). Therefore, the helical domains of zcytor17lig may be useful for preparing chimeric fusion molecules, particularly with other short-helix form cytokines to determine and modulate receptor binding specificity. The present invention also envisions fusion proteins engineered with helix A and/or helix D, and fusion proteins that combine helical and loop domains from other short-form cytokines such as IL-2, IL-4, IL-15, Lif, IL-12, IL-3 and GM-CSF.

The polynucleotide sequence for human IL-2 is shown in SEQ ID NO:176 and the corresponding amino acid sequence is shown in SEQ ID NO:177. The secretory signal sequence is comprised of amino acid residues 1 (Met) to 20 (Ser) of SEQ ID NO:177; nucleotides 48 to 107 of SEQ ID NO:176. The mature polypeptide is comprised of amino acid residues 21 (Ala) to 156 (Thr) of SEQ ID NO:177; nucleotides 108 to 515 of SEQ ID NO:176. Helix A of human IL-2 is comprised of amino acid residues 27 (Thr) to 48 (Leu) of SEQ ID NO:177; nucleotides 126 to 191 of SEQ ID NO:176. Helix B of human IL-2 comprises Helix B1 and Helix B2. Helix B1 of human IL-2 is comprised of amino acid residues 73 (Ala) to 80 (Gln) of SEQ ID NO:177; nucleotides 264 to 287 of SEQ ID NO:176. Helix B2 of human IL-2 is comprised of amino acid residues 83 (Glu) to 92 (Val) of SEQ ID NO:177; nucleotides 294 to 323 of SEQ ID NO:176. Thus, Helix B (comprising Helices B1 and B2) of IL-2 is represented by the amino acid sequence of SEQ ID NO:183 (nucleotide sequence of SEQ ID NO:182) wherein amino acid residues 9 and 10 can be any amino acid. SEQ ID NO:183 is identical to amino acids 73 (Ala) to 92 (Val) of SEQ ID NO:177 wherein amino acids 81 and 82 are any amino acid. In a preferred form, Helix B of IL-2 comprises amino acids 73 (Ala) to 92 (Val) of SEQ ID NO:177; nucleotides 264 to 323 of SEQ ID NO:176. Helix C of human IL-2 is comprised of amino acid residues 102 (His) to 116 (Val) of SEQ ID NO:177 nucleotides 351 to 395 of SEQ ID NO:176. Helix D of human IL-2 is comprised of amino acid residues 134 (Thr) to 149 (Gln) of SEQ ID NO:177; nucleotides 447 to 494 of SEQ ID NO:176.

The polynucleotide sequence for human IL-4 is shown in SEQ ID NO:178 and the corresponding amino acid sequence is shown in SEQ ID NO:179. The secretory signal sequence is comprised of amino acid residues 1 (Met) to 24 (Gly) of SEQ ID NO:179; nucleotides 64 to 135 of SEQ ID NO:178. The mature polypeptide is comprised of amino acid residues 25 (His) to 153 (Ser) of SEQ ID NO:179; nucleotides 136 to 522 of SEQ ID NO:178. Helix A of human IL-4 is comprised of amino acid residues 30 (Thr) to 42 (Thr) of SEQ ID NO:179; nucleotides 151 to 189 of SEQ ID NO:178. Helix B of human IL-4 is comprised of amino acid residues 65 (Glu) to 83 (His) of SEQ ID NO:179; nucleotides 256 to 312 of SEQ ID NO:178. Helix C of human IL-4 is comprised of amino acid residues 94 (Ala) to 118 (Ala) of SEQ ID NO:179; nucleotides 343 to 417 of SEQ ID NO:178. Helix D of human IL-4 is comprised of amino acid residues 133 (Leu) to 151 (Cys) of SEQ ID NO:179; nucleotides 460 to 516 of SEQ ID NO:178.

The polynucleotide sequence for human GM-CSF is shown in SEQ ID NO:180 and the corresponding amino acid sequence is shown in SEQ ID NO:181. The secretory signal sequence is comprised of amino acid residues 1 (Met) to 17 (Ser) of SEQ ID NO:181; nucleotides 9 to 59 of SEQ ID NO:180. The mature polypeptide is comprised of amino acid residues 18 (Ala) to 144 (Glu) of SEQ ID NO:181; nucleotides 60 to 440 of SEQ ID NO:180. Helix A of human GM-CSF is comprised of amino acid residues 30 (Trp) to 44 (Asn) of SEQ ID NO:181; nucleotides 96 to 140 of SEQ ID NO:180. Helix B of human GM-CSF is comprised of amino acid residues 72 (Leu) to 81 (Gln) of SEQ ID NO:181; nucleotides 222 to 251 of SEQ ID NO:180. Helix C of human GM-CSF is comprised of amino acid residues 85 (Gly) to 103 (Gln) of SEQ ID NO:181; nucleotides 261 to 317 of SEQ ID NO:180. Helix D of human GM-CSF is comprised of amino acid residues 120 (Phe) to 131 (Leu) of SEQ ID NO:181; nucleotides 366 to 401 of SEQ ID NO:180.

The amino acid residues comprising helices A, B, C, and D, for human zcytor17lig, IL-3, IL-2, IL-4, and GM-CSF are shown in Table 1.

Table 1

	Helix A	Helix B	Helix C	Helix D	
zcytor17lig	38-52	83-98	104-117	137-152	of SEQ ID NO:2

IL-3	35-45	73-86	91-103	123-141	of SEQ ID NO:102
IL-2	27-48	73-92	102-116	134-149	of SEQ ID NO:177 or Helix B as described in SEQ ID NO:183
IL-4	30-42	65-83	94-118	133-151	of SEQ ID NO:179
GM-CSF	30-44	72-81	85-103	120-131	of SEQ ID NO:181

The present invention provides polynucleotide molecules, including DNA and RNA molecules that encode the zcytor17 polypeptides disclosed herein that can be included in the multimeric cytokine receptor. Those skilled in the art will recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:120, SEQ ID NO:121 and SEQ ID NO:122 are degenerate DNA sequences that encompass all DNAs that encode the zcytor17 polypeptide of SEQ ID NO:111, SEQ ID NO:109 and SEQ ID NO:5, respectively, and fragments thereof. Those skilled in the art will recognize that the degenerate sequences of SEQ ID NO:120, SEQ ID NO:121 and SEQ ID NO:122 also provide all RNA sequences encoding SEQ ID NO:111, SEQ ID NO:109 and SEQ ID NO:5 by substituting U for T. Thus, zcytor17 polypeptide-encoding polynucleotides comprising nucleotide 1 to nucleotide 2196 of SEQ ID NO:120, nucleotide 1 to nucleotide 1947 of SEQ ID NO:121, and nucleotide 1 to nucleotide 1986 of SEQ ID NO:122 and their RNA equivalents are contemplated by the present invention. Table 2 sets forth the one-letter codes used within SEQ ID NO:120, SEQ ID NO:121 and SEQ ID NO:122 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

TABLE 2

Nucleotide	Resolution	Complement	Resolution
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A G	Y	C T
Y	C T	R	A G
M	A C	K	G T
K	G T	M	A C
S	C G	S	C G
W	A T	W	A T
H	A C T	D	A G T
B	C G T	V	A C G
V	A C G	B	C G T
D	A G T	H	A C T
N	A C G T	N	A C G T

The degenerate codons used in SEQ ID NO:120, SEQ ID NO:121 and
5 SEQ ID NO:122, encompassing all possible codons for a given amino acid, are set forth
in Table 3.

TABLE 3

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn Asp	B		RAY
Glu Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding each amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine
5 (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequences of SEQ ID NO:111, SEQ ID NO:109 and SEQ ID NO:5; or
10 SEQ ID NO:117 and SEQ ID NO:119. Variant sequences can be readily tested for functionality as described herein.

One of ordinary skill in the art will also appreciate that different species can exhibit “preferential codon usage.” In general, see, Grantham, et al., *Nuc. Acids Res.* 8:1893-912, 1980; Haas, et al. *Curr. Biol.* 6:315-24, 1996; Wain-Hobson et al.,
15 *Gene* 13:355-64, 1981; Grosjean and Fiers, *Gene* 18:199-209, 1982; Holm, *Nuc. Acids Res.* 14:3075-87, 1986; Ikemura, *J. Mol. Biol.* 158:573-97, 1982. As used herein, the term “preferential codon usage” or “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
20 acid (See Table 3). For example, the amino acid Threonine (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 introduced into the polynucleotides of the present invention by a variety of methods
25 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 within a particular cell type or species. Therefore, the degenerate codon sequences disclosed in SEQ ID NO:120, SEQ ID NO:121 and SEQ ID NO:122 serve as templates for optimizing expression of zcytor17 polynucleotides in various cell types
30 and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and

tested for functionality as disclosed herein.

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 cell that produces large amounts of zcytor17 RNA. Such tissues and cells are identified by Northern blotting (Thomas, *Proc. Natl. Acad. Sci. USA* 77:5201, 1980), and include PBLs, spleen, thymus, bone marrow, prostate, and lymph tissues, human erythroleukemia cell lines, acute monocytic leukemia cell lines, other lymphoid and hematopoietic cell lines, and the like. Total RNA can be prepared using guanidinium isothiocyanate extraction followed by isolation by centrifugation in 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* 69:1408-12, 1972). Complementary DNA (cDNA) is prepared from poly(A)⁺ RNA using known methods. In the alternative, genomic DNA can be isolated. Polynucleotides encoding zcytor17 polypeptides are then identified and isolated by, for example, hybridization or polymerase chain reaction (PCR) (Mullis, U.S. Patent No. 4,683,202).

A full-length clone encoding zcytor17 can be obtained by conventional cloning procedures. 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 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 zcytor17, receptor fragments, or other specific binding partners.

The polynucleotides of the present invention can also be synthesized using DNA synthesis machines. Currently the method of choice is the phosphoramidite method. If chemically synthesized double stranded DNA is required for an application such as the synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short polynucleotides (60 to 80 bp) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. However, for producing longer polynucleotides (>300 bp),

special strategies are usually employed, 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 100 nucleotides in length.

5 An alternative way to prepare a full-length gene is to synthesize a specified set of overlapping oligonucleotides (40 to 100 nucleotides). After the 3' and 5' short overlapping complementary regions (6 to 10 nucleotides) are annealed, large gaps still remain, but the short base-paired regions are both long enough and stable enough to hold the structure together. The gaps are filled and the DNA duplex is
10 completed via enzymatic DNA synthesis by *E. coli* DNA polymerase I. After the enzymatic synthesis is completed, the nicks are sealed with T4 DNA ligase. Double-stranded constructs are sequentially linked to one another to form the entire gene sequence which is verified by DNA sequence analysis. See Glick and Pasternak, *Molecular Biotechnology, Principles & Applications of Recombinant DNA*, (ASM
15 Press, Washington, D.C. 1994); Itakura et al., *Annu. Rev. Biochem.* 53: 323-56, 1984 and Climie et al., *Proc. Natl. Acad. Sci. USA* 87:633-7, 1990. Moreover, other sequences are generally added that contain signals for proper initiation and termination of transcription and translation.

 The present invention also provides reagents which will find use in
20 diagnostic applications. For example, the zcytor17lig gene, a probe comprising zcytor17lig DNA or RNA or a subsequence thereof, can be used to determine if the zcytor17lig gene is present on a human chromosome, such as chromosome 12, or if a gene mutation has occurred. Zcytor17lig is located at the 12q24.31 region of chromosome 12 (Example 13). Detectable chromosomal aberrations at the zcytor17lig
25 gene locus include, but are not limited to, aneuploidy, gene copy number changes, loss of heterozygosity (LOH), translocations, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis
30 employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., *ibid.*; Ausubel et. al., *ibid.*; Marian, *Chest* 108:255-65, 1995).

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 additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable
5 disease which shows linkage to the same chromosomal region; and 3) cross-referencing model organisms, such as mouse, which may aid in determining what function a particular gene might have.

One of skill in the art would recognize that the 12q24 region is frequently involved in gross genomic rearrangements, including translocations,
10 deletions, inversions, and duplications, that are associated with various cancers. The Mitelman Database of Chromosomal Aberrations in Cancer, at the Cancer Genome Anatomy Project, National Institutes of Health, Bethesda, Md located on the Internet lists 199 cases of cancers with genomic rearrangements involving 12q24. Of these, most are part of complex karyotypes with other rearrangements; however, in some cases
15 the rearrangement involving 12q24 is the only genomic alteration. Given the expression of the receptor for zcytor17lig on cells of lymphoid and myeloid lineages, it is particularly significant to note that there are at least 4 cases of myeloid leukemia reported in the literature in which either translocation (2 cases: Yamagata et al, *Cancer Genet Cytogenet* 97:90-93, 1997; Dunphy and Batanian, *Cancer Genet Cytogenet*
20 114:51-57, 1999) or duplication (2 cases: Bonomi et al, *Cancer Genet Cytogenet* 108:75-78, 1999) are the sole genomic alteration. This suggests that a gene or genes residing within 12q24 could be directly involved in the malignant transformation of these patients' cells. Inappropriate over expression of zcytor17lig could contribute to malignant transformation by promoting aberrant proliferation of receptor-bearing cells,
25 through either autocrine or paracrine mechanisms. Inhibition of zcytor17lig activity could thus inhibit growth of such cells. Alternatively, a genomic rearrangement resulting in inactivation of the zcytor17lig gene may promote malignant transformation and/or metastasis by removing zcytor17lig immunoregulatory functions. Indeed, a gene suppressing metastasis in prostate cancer has been mapped to 12q24-qter (Ichikawa et
30 al, *Asian J Androl* 2:167-171, 2000). If zcytor17lig is the gene within this region responsible for the suppression of metastasis, then zcytor17lig itself may have

therapeutic value in the treatment of cancer.

A diagnostic could assist physicians in determining the type of disease and appropriate associated therapy, or assistance in genetic counseling. As such, the inventive anti-zcytor17lig antibodies, polynucleotides, and polypeptides can be used for the detection of zcytor17lig polypeptide, mRNA or anti-zcytor17lig antibodies, thus serving as markers and be directly used for detecting or genetic diseases or cancers, as described herein, using methods known in the art and described herein. Further, zcytor17lig polynucleotide probes can be used to detect abnormalities or genotypes associated with chromosome 12q24.3 deletions and translocations associated with human diseases, or other translocations involved with malignant progression of tumors or other 12q24.3 mutations, which are expected to be involved in chromosome rearrangements in malignancy; or in other cancers. Similarly, zcytor17lig polynucleotide probes can be used to detect abnormalities or genotypes associated with chromosome 12 trisomy and chromosome loss associated with human diseases or spontaneous abortion. Thus, zcytor17lig polynucleotide probes can be used to detect abnormalities or genotypes associated with these defects.

One of skill in the art would recognize that zcytor17lig polynucleotide probes are particularly useful for diagnosis of gross chromosomal abnormalities associated with loss of heterogeneity (LOH), chromosome gain (e.g., trisomy), translocation, DNA amplification, and the like. Translocations within chromosomal locus 12q24.3 wherein the zcytor17lig gene is located are known to be associated with human disease. For example, 12q24 deletions and translocations, duplications and trisomy are associated with cancers as discussed above. Thus, since the zcytor17lig gene maps to this critical region, zcytor17lig polynucleotide probes of the present invention can be used to detect abnormalities or genotypes associated with 12q24 translocation, deletion and trisomy, and the like, described above.

As discussed above, defects in the zcytor17lig gene itself may result in a heritable human disease state. Molecules of the present invention, such as the polypeptides, antagonists, agonists, polynucleotides and antibodies of the present invention would aid in the detection, diagnosis prevention, and treatment associated with a zcytor17lig genetic defect. In addition, zcytor17lig polynucleotide probes can be

used to detect allelic differences between diseased or non-diseased individuals at the zcytor17lig chromosomal locus. As such, the zcytor17lig sequences can be used as diagnostics in forensic DNA profiling.

In general, the diagnostic methods used in genetic linkage analysis, to
5 detect a genetic abnormality or aberration in a patient, are known in the art. Analytical probes will be generally at least 20 nt in length, although somewhat shorter probes can be used (e.g., 14-17 nt). PCR primers are at least 5 nt in length, preferably 15 or more, more preferably 20-30 nt. For gross analysis of genes, or chromosomal DNA, a zcytor17lig polynucleotide probe may comprise an entire exon or more. Exons are
10 readily determined by one of skill in the art by comparing zcytor17lig sequences (SEQ ID NO:1) with the genomic DNA for mouse zcytor17lig (SEQ ID NO:76). In general, the diagnostic methods used in genetic linkage analysis, to detect a genetic abnormality or aberration in a patient, are known in the art. Most diagnostic methods comprise the steps of (a) obtaining a genetic sample from a potentially diseased patient, diseased
15 patient or potential non-diseased carrier of a recessive disease allele; (b) producing a first reaction product by incubating the genetic sample with a zcytor17lig polynucleotide probe wherein the polynucleotide will hybridize to complementary polynucleotide sequence, such as in RFLP analysis or by incubating the genetic sample with sense and antisense primers in a PCR reaction under appropriate PCR reaction
20 conditions; (iii) visualizing the first reaction product by gel electrophoresis and/or other known methods such as visualizing the first reaction product with a zcytor17lig polynucleotide probe wherein the polynucleotide will hybridize to the complementary polynucleotide sequence of the first reaction; and (iv) comparing the visualized first reaction product to a second control reaction product of a genetic sample from wild type
25 patient, or a normal or control individual. A difference between the first reaction product and the control reaction product is indicative of a genetic abnormality in the diseased or potentially diseased patient, or the presence of a heterozygous recessive carrier phenotype for a non-diseased patient, or the presence of a genetic defect in a tumor from a diseased patient, or the presence of a genetic abnormality in a fetus or pre-
30 implantation embryo. For example, a difference in restriction fragment pattern, length of PCR products, length of repetitive sequences at the zcytor17lig genetic locus, and the

like, are indicative of a genetic abnormality, genetic aberration, or allelic difference in comparison to the normal wild type control. Controls can be from unaffected family members, or unrelated individuals, depending on the test and availability of samples. Genetic samples for use within the present invention include genomic DNA, mRNA, and cDNA isolated from any tissue or other biological sample from a patient, which includes, but is not limited to, blood, saliva, semen, embryonic cells, amniotic fluid, and the like. The polynucleotide probe or primer can be RNA or DNA, and will comprise a portion of SEQ ID NO:1, the complement of SEQ ID NO:1, or an RNA equivalent thereof. Such methods of showing genetic linkage analysis to human disease phenotypes are well known in the art. For reference to PCR based methods in diagnostics see generally, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), White (ed.), *PCR Protocols: Current Methods and Applications* (Humana Press, Inc. 1993), Cotter (ed.), *Molecular Diagnosis of Cancer* (Humana Press, Inc. 1996), Hanausek and Walaszek (eds.), *Tumor Marker Protocols* (Humana Press, Inc. 1998), Lo (ed.), *Clinical Applications of PCR* (Humana Press, Inc. 1998), and Meltzer (ed.), *PCR in Bioanalysis* (Humana Press, Inc. 1998).

Mutations associated with the *zcytor17lig* locus can be detected using nucleic acid molecules of the present invention by employing standard methods for direct mutation analysis, such as restriction fragment length polymorphism analysis, short tandem repeat analysis employing PCR techniques, amplification-refractory mutation system analysis, single-strand conformation polymorphism detection, RNase cleavage methods, denaturing gradient gel electrophoresis, fluorescence-assisted mismatch analysis, and other genetic analysis techniques known in the art (see, for example, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), Marian, *Chest* 108:255 (1995), Coleman and Tsongalis, *Molecular Diagnostics* (Human Press, Inc. 1996), Elles (ed.) *Molecular Diagnosis of Genetic Diseases* (Humana Press, Inc. 1996), Landegren (ed.), *Laboratory Protocols for Mutation Detection* (Oxford University Press 1996), Birren *et al.* (eds.), *Genome Analysis, Vol. 2: Detecting Genes* (Cold Spring Harbor Laboratory Press 1998), Dracopoli *et al.* (eds.), *Current Protocols in Human Genetics* (John Wiley & Sons 1998), and Richards and Ward, "Molecular Diagnostic Testing," in *Principles of Molecular Medicine*, pages 83-

88 (Humana Press, Inc. 1998). Direct analysis of an zcytor17lig gene for a mutation can be performed using a subject's genomic DNA. Methods for amplifying genomic DNA, obtained for example from peripheral blood lymphocytes, are well-known to those of skill in the art (see, for example, Dracopoli *et al.* (eds.), *Current Protocols in*
5 *Human Genetics*, at pages 7.1.6 to 7.1.7 (John Wiley & Sons 1998)).

The present invention further provides counterpart polypeptides and polynucleotides from other species (orthologs). These species include, but are not
10 limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are zcytor17 polypeptides from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides. Orthologs of human zcytor17 can be cloned using information and compositions provided by the present invention in combination with
15 conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses zcytor17 as disclosed herein. Suitable sources of mRNA 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 zcytor17-encoding cDNA can then be isolated by a
20 variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using PCR (Mullis, *supra.*), using primers designed from the representative human zcytor17 sequence disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and
25 expression of the cDNA of interest can be detected with an antibody to zcytor17 polypeptide. Similar techniques can also be applied to the isolation of genomic clones.

A polynucleotide sequence for the mouse ortholog of human zcytor17 has been identified and is shown in SEQ ID NO:116 and the corresponding amino acid sequence shown in SEQ ID NO:117. Analysis of the mouse zcytor17 polypeptide
30 encoded by the DNA sequence of SEQ ID NO:116 revealed an open reading frame encoding 662 amino acids (SEQ ID NO:117) comprising a predicted secretory signal

peptide of 45 amino acid residues (residue 1 (Met) to residue 45 (Ala) of SEQ ID NO:117), and a mature polypeptide of 617 amino acids (residue 46 (Val) to residue 662 (Cys) of SEQ ID NO:117). Moreover, an additional Met residue, Met (28) can be used as a starting methionine; comprising a second predicted secretory signal peptide of 18 amino acid residues (residue 28 (Met) to residue 45 (Ala) of SEQ ID NO:117), and the same mature polypeptide of 617 amino acids (residue 46 (Val) to residue 662 (Cys) of SEQ ID NO:117. In addition to the WSXWS motif (SEQ ID NO:3) corresponding to residues 224-228 of SEQ ID NO:117, the receptor comprises an extracellular domain from residues 46 (Val) to 533 (Glu) of SEQ ID NO:117) that includes a cytokine-binding domain of approximately 200 amino acid residues (residues 46 (Val) to 240 (Pro) of SEQ ID NO:117) and a fibronectin III domain (residues 241 (His) to 533 (Glu) of SEQ ID NO:117); a CXW motif (residues 66 (Cys) to 68 (Trp) of SEQ ID NO:117); a domain linker (residues 142 (Thr) to 145 (Pro) of SEQ ID NO:117); a penultimate strand region (residues 207 (Phe) to 215 (Arg) of SEQ ID NO:117); a transmembrane domain (residues 534 (Ile) to 550 (Ile) of SEQ ID NO:117); complete intracellular signaling domain (residues 551 (Lys) to 662 (Cys) of SEQ ID NO:117) which contains a "Box I" signaling site (residues 568 (Cys) to 574 (Pro) of SEQ ID NO:117), and a "Box II" signaling site (residues 628 (Glu) to 631 (Ile) of SEQ ID NO:117). Conserved residues common to class I cytokine receptors, are at residues 56 (Cys), 187 (Trp), and 215 (Arg). A comparison of the human and mouse amino acid sequences reveals that both the human and orthologous polypeptides contain corresponding structural features described above (and, see, Figure 2). The mature sequence for the mouse zcytor17 begins at Val₄₆ (as shown in SEQ ID NO:117), which corresponds to Ala₃₃ (as shown in SEQ ID NO:5) in the human sequence. There is about 61% identity between the mouse and human sequences over the entire amino acid sequence corresponding to SEQ ID NO:5 and SEQ ID NO:117. The above percent identity was determined using a FASTA program with ktup=1, gap opening penalty=12, gap extension penalty=2, and substitution matrix=BLOSUM62, with other parameters set as default. The corresponding polynucleotides encoding the mouse zcytor17 polypeptide regions, domains, motifs, residues and sequences described above are as shown in SEQ ID NO:116.

Moreover, a truncated soluble form of the mouse zcytor17 receptor polypeptide appears to be naturally expressed. A polynucleotide sequence for a truncated soluble form of the mouse zcytor17 receptor has been identified and is shown in SEQ ID NO:118 and the corresponding amino acid sequence shown in SEQ ID NO:119. Analysis of the truncated soluble mouse zcytor17 polypeptide encoded by the DNA sequence of SEQ ID NO:118 revealed an open reading frame encoding 547 amino acids (SEQ ID NO:119) comprising a predicted secretory signal peptide of 45 amino acid residues (residue 1 (Met) to residue 45 (Ala) of SEQ ID NO:119), and a mature polypeptide of 502 amino acids (residue 46 (Val) to residue 547 (Val) of SEQ ID NO:119). Moreover, an additional Met residue, Met (28) can be used as a starting methionine; comprising a second predicted secretory signal peptide of 18 amino acid residues (residue 28 (Met) to residue 45 (Ala) of SEQ ID NO:119), and the same mature polypeptide of 502 amino acids (residue 46 (Val) to residue 547 (Val) of SEQ ID NO:119). In addition to the WSXWS motif (SEQ ID NO:3) corresponding to residues 224-228 of SEQ ID NO:119, the receptor comprises an extracellular domain from residues 46 (Val) to 533 (Trp) of SEQ ID NO:119) that includes a cytokine-binding domain of approximately 200 amino acid residues (residues 46 (Val) to 240 (Pro) of SEQ ID NO:119) and a fibronectin III domain (residues 241 (His) to 533 (Trp) of SEQ ID NO:119); a CXW motif (residues 66 (Cys) to 68 (Trp) of SEQ ID NO:119); a domain linker (residues 142 (Thr) to 145 (Pro) of SEQ ID NO:119); a penultimate strand region (residues 207 (Phe) to 215 (Arg) of SEQ ID NO:119); and a C-terminal tail region (residues 534 (Leu) to 547 (Val)). Conserved residues common to class I cytokine receptors, are at residues 56 (Cys), 187 (Trp), and 215 (Arg). A comparison of the human and mouse amino acid sequences, including the truncated soluble mouse zcytor17, reveals that both the human and orthologous polypeptides contain corresponding structural features described above (and, see, Figure 2). The corresponding polynucleotides encoding the truncated soluble mouse zcytor17 polypeptide regions, domains, motifs, residues and sequences described above are as shown in SEQ ID NO:118.

Those skilled in the art will recognize that the sequences disclosed in SEQ ID NO:110, SEQ ID NO:108 and SEQ ID NO:4 represent alleles of human

zcytor17 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:110, SEQ ID NO:108 or SEQ ID NO:4, including those
5 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 NO:111, SEQ ID NO:109, SEQ ID NO:5 SEQ ID NO:117 or SEQ ID NO:119. cDNAs generated from alternatively spliced mRNAs, which retain the properties of the zcytor17 polypeptide are included within the scope of the present
10 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. For example, the short-form and long-form soluble zcytor17 receptors described above, and in SEQ ID NO:112 and SEQ ID NO:113 or SEQ ID NO:114 and
15 SEQ ID NO:115 can be considered allelic or splice variants of zcytor17.

The present invention also provides isolated zcytor17 polypeptides that are substantially similar to the polypeptides of SEQ ID NO:111, SEQ ID NO:109 or SEQ ID NO:5 and their orthologs, e.g., SEQ ID NO:117 and SEQ ID NO:119. The term "substantially similar" is used herein to denote polypeptides having at least 50%,
20 at least 60%, at least 70%, at least 80%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or greater than 99% sequence identity to the sequences shown in SEQ ID NO:111, SEQ ID NO:109 or SEQ ID NO:5 or their orthologs, e.g., SEQ ID NO:117 and SEQ ID NO:119. Such polypeptides will more preferably be at least 90% identical, and most
25 preferably 95% or more identical to SEQ ID NO:111, SEQ ID NO:109 and SEQ ID NO:5 or its 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 are aligned to optimize the alignment scores using a gap
30 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 4 (amino acids are indicated by the

standard one-letter codes). The percent identity is then calculated as:

$$5 \quad \frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

Table 4

A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

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

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity
5 search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative variant zcytor17. 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).

10 Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:5, SEQ ID NO:117 and SEQ ID NO:119) 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
15 ten regions with the highest density of identities are then rescored 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
20 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
25 insertions and deletions. Preferred parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62, with other parameters set as default. 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).

30 FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons,

the ktup value can range between one to six, preferably from three to six, most preferably three, with other FASTA program parameters set as default.

The BLOSUM62 table (Table 4) is an amino acid substitution matrix derived from about 2,000 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 conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. Although it is possible to design amino acid substitutions based solely upon chemical properties (as discussed below), the language "conservative amino acid substitution" preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (e.g., 1, 2 or 3), while more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (e.g., 2 or 3).

Variant zcytor17 polypeptides or substantially homologous zcytor17 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 5) 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 that comprise a sequence that is at least 80%, preferably at least 90%, and more preferably 95% or more identical to the corresponding region of SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:5, SEQ ID NO:117 or SEQ ID NO:119 excluding the tags, extension, linker sequences and the like. Polypeptides comprising affinity tags can further comprise a proteolytic cleavage site between the zcytor17 polypeptide and the affinity tag. Suitable sites include thrombin cleavage sites and factor Xa cleavage sites.

Table 5

Conservative amino acid substitutions

5	Basic:	arginine
		lysine
		histidine
10	Acidic:	glutamic acid
		aspartic acid
10	Polar:	glutamine
		asparagine
15	Hydrophobic:	leucine
		isoleucine
		valine
15	Aromatic:	phenylalanine
		tryptophan
		tyrosine
20	Small:	glycine
		alanine
		serine
		threonine
		methionine

The present invention provides an isolated an isolated multimeric cytokine receptor comprising a polypeptide having at least 90 percent sequence identity with SEQ ID NO:111, SEQ ID NO:109, or SEQ ID NO:5; and at least a portion of at least one class I cytokine receptor, wherein the multimeric cytokine receptor binds to at least a portion of SEQ ID NO:2. The polypeptide having at least 90 percent sequence identity with SEQ ID NO:111 may include, for instance, amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 227 of SEQ ID NO:519, amino acid residue 20 to amino acid residue 543 of SEQ ID NO:111, amino acid residue 544 to amino acid residue 732 of SEQ ID NO:111, amino acid residue 544 to amino acid residue 649 of SEQ ID NO:109, amino acid residue 20 to amino acid residue 732 of SEQ ID NO:111, amino acid residue 20 to amino acid residue 649 of SEQ ID NO:109, and combinations thereof. The at least a portion of at least one class I cytokine receptor includes, for example, OSMRbeta (SEQ ID NO:7) and/or WSX-1 (SEQ ID NO:9). For example, the at least a portion of at least one class I cytokine receptor comprising at least a portion of SEQ ID NO:7 may comprise amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 35 to amino acid residue 137 of SEQ ID NO:7, amino acid residue 240 to amino acid residue 342 of SEQ ID NO:7, amino acid residue 348 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 761 of SEQ ID NO:7, amino acid residue 762 to amino acid residue 979 of SEQ ID NO:7, and/or combinations thereof. The multimeric cytokine receptor may be a heterodimer, trimer, tetramer, pentamer, or the like. In addition, the multimeric cytokine receptor may be soluble, immobilized on a solid support, or membrane-bound. Optionally, the multimeric cytokine receptor may antagonize an activity of SEQ ID NO:2, such as inhibit or reduce the proliferation of hematopoietic, immune, and/or inflammatory cells, or inhibit or reduce the enhancement of the hematopoietic, immune, and/or inflammatory process, or inhibit or reduce the differentiation of hematopoietic cells, for instance lymphoid cells such as monocytic cells, macrophages and/or T cells. The multimeric cytokine receptor of the present invention may also comprise an affinity tag selected from the group of polyhistidine, protein A, glutathione S transferase, Glu-Glu, substance P, Flag™

peptide, streptavidin binding peptide, and an immunoglobulin F_c polypeptide.

The present invention also provides for a ligand/receptor complex comprising a polypeptide which includes at least a portion of SEQ ID NO:2; and a soluble multimeric cytokine receptor comprising at least a portion of at least one
5 polypeptide selected from the group of SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:7, and SEQ ID NO:9, wherein the polypeptide is attached to the soluble multimeric cytokine receptor. The soluble multimeric cytokine receptor may comprise the extracellular domain and/or transmembrane domain of zcytor17 (SEQ ID NO:111), OSMRbeta (SEQ ID NO:7), and/or WSX-1 (SEQ ID NO:9). For example, the soluble
10 multimeric cytokine receptor may comprise amino acid residue 20 to 227 to SEQ ID NO:111, amino acid residue 20 to 519 to SEQ ID NO:111, amino acid residue 20 to 543 to SEQ ID NO:111, amino acid residue 28 to 739 to SEQ ID NO:7, amino acid residue 28 to 429 to SEQ ID NO:7, amino acid residue 35 to 137 to SEQ ID NO:7, amino acid residue 240 to 342 to SEQ ID NO:7, amino acid residue 348 to 429 to SEQ
15 ID NO:7, or combinations thereof. The soluble multimeric cytokine receptor may be a heterodimer, trimer, tetramer, pentamer, or the like. The multimeric cytokine receptor of the present invention may also comprise an affinity tag as described herein. The polypeptide of the ligand/receptor complex may comprise amino acid residues of SEQ ID NO:2 selected from the group of 38 to 152, 27 to 164, 24 to 164, 1 to 164, 38 to 52,
20 83 to 98, 104 to 117, 137 to 152, and combinations thereof.

The ligand/receptor complex of the present may also comprise a fusion protein. The fusion protein may comprise at least four polypeptides, wherein the order of polypeptides from N-terminus to C-terminus are a first polypeptide comprising amino acid residues 38-52 of SEQ ID NO:2; a first spacer of 6-27 amino acid residues;
25 a second polypeptide comprising amino acid residues selected from the group of (a) IL-2 helix B residues of SEQ ID NO:183; (b) IL-4 helix B residues 65-83 of SEQ ID NO:179; (c) IL-3 helix B residues 73-86 of SEQ ID NO:102; (d) GM-CSF helix B residues 72-81 of SEQ ID NO:181; and (e) amino acid residues 83-98 of SEQ ID NO:2; a second spacer of 5-11 amino acid residues; a third polypeptide comprising amino acid
30 residues selected from the group of (a) IL-2 helix C residues 102-116 of SEQ ID NO:177; (b) IL-4 helix C residues 94-118 of SEQ ID NO:179; (c) IL-3 helix C residues

91-103 of SEQ ID NO:102; (d) GM-CSF helix C residues 85-103 of SEQ ID NO:181; and (e) amino acid residues 104-117 of SEQ ID NO:2; a third spacer of 3-29 amino acid residues; and a fourth polypeptide comprising amino acid residues selected from the group of (a) IL-2 helix D residues 134-149 of SEQ ID NO:177 ; (b) IL-3 helix D
5 residues 123-141 of SEQ ID NO:102; (c) IL-4 helix D residues 133-151 of SEQ ID NO:179; (d) GM-CSF helix D residues 120-131 of SEQ ID NO:181; and (e) amino acid residues 137-152 of SEQ ID NO:2; and a multimeric cytokine receptor comprising at least a portion of at least one polypeptide selected from the group of SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:7, and SEQ ID NO:9; wherein the fusion protein is
10 attached to the multimeric cytokine receptor.

Alternatively, the fusion protein may comprise at least four polypeptides, wherein the order of polypeptides from N-terminus to C-terminus are a first polypeptide comprising amino acid residues selected from a group of (a) IL-2 helix A residues 27-48 of SEQ ID NO:177; (b) IL-4 helix A residues 30-42 of SEQ ID NO:179; (c) IL-3
15 helix A residues 35-45 of SEQ ID NO:102; (d) GM-CSF helix A residues 30-44 of SEQ ID NO:181; and (e) amino acids residues 38-52 of SEQ ID NO:2; a first spacer of 6-27 amino acid residues; a second polypeptide comprising amino acid residues selected from the group of (a) IL-2 helix B residues of SEQ ID NO:183; (b); IL-4 helix B residues 65-83 of SEQ ID NO:179; (c) IL-3 helix B residues 73-86 of SEQ ID
20 NO:102; (d) GM-CSF helix B residues 72-81 of SEQ ID NO:181; and (e) amino acid residues 83-98 of SEQ ID NO:2; a second spacer of 5-11 amino acid residues; a third polypeptide comprising amino acid residues selected from the group of (a) IL-2 helix C residues 102-116 of SEQ ID NO:177; (b) IL-4 helix C residues 94-118 of SEQ ID NO:179; (c) IL-3 helix C residues 91-103 of SEQ ID NO:102; (d) GM-CSF helix C
25 residues 85-103 of SEQ ID NO:181; and (e) amino acid residues 104-117 of SEQ ID NO:2; a third spacer of 3-29 amino acid residues; and a fourth polypeptide comprising amino acid residues from 137-152 of SEQ ID NO:2; and a multimeric cytokine receptor comprising at least a portion of at least one polypeptide selected from the group of SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:7, and SEQ ID NO:9; wherein the fusion
30 protein is attached to the multimeric cytokine receptor. A multimeric cytokine receptor may comprise at least one of the following polypeptides of SEQ ID NO:111, SEQ ID

NO:109, SEQ ID NO:7, SEQ ID NO:9, or the extracellular domains thereof. The ligand/receptor complex can be soluble and may additionally include an affinity tag as described herein.

The present invention also provides an isolated and purified
5 polynucleotide that encodes a polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with SEQ ID NO:111, SEQ ID NO:109, or SEQ ID NO:5, wherein the polypeptide and at least a portion of at least one class I cytokine receptor form a multimeric cytokine receptor, and wherein the multimeric cytokine receptor binds to at least a portion of SEQ ID NO:2. The present invention also
10 provides an isolated and purified polynucleotide that encodes a polypeptide comprising at least a portion of at least one of SEQ ID NO:111, SEQ ID NO:109, or SEQ ID NO:5, wherein the polypeptide and at least a portion of a class I cytokine receptor form a multimeric cytokine receptor, and wherein the multimeric cytokine receptor binds to at least a portion of SEQ ID NO:2. The polynucleotide may encode a polypeptide that is
15 included in a soluble multimeric cytokine receptor and that may also include an affinity tag as described herein. The polypeptide may comprise, for example, amino acid residue 20 to 227 to SEQ ID NO:111, amino acid residue 20 to 519 to SEQ ID NO:111, amino acid residue 20 to 543 to SEQ ID NO:111, and/or combinations thereof. In addition, the at least a portion of at least one class I cytokine receptor may comprise, for
20 example, amino acid residue 28 to 739 to SEQ ID NO:7, amino acid residue 28 to 429 to SEQ ID NO:7, amino acid residue 35 to 137 to SEQ ID NO:7, amino acid residue 240 to 342 to SEQ ID NO:7, amino acid residue 348 to 429 to SEQ ID NO:7, and/or combinations thereof. The soluble multimeric cytokine receptor may be a heterodimer, trimer, tetramer, pentamer, or the like. The at least a portion of SEQ ID NO:2 may
25 include, for instance amino acid residues of SEQ ID NO:2 selected from the group of 38 to 152, 27 to 164, 24 to 164, 1 to 164, 38 to 52, 83 to 98, 104 to 117, 137 to 152, and combinations thereof. Optionally, the multimeric cytokine receptor may antagonize an activity of SEQ ID NO:2 as described herein.

The present invention further provides a variety of other polypeptide
30 fusions and related multimeric proteins comprising one or more polypeptide fusions. For example, a zcytor17 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 dimerizing proteins in this regard include immunoglobulin constant region domains. Immunoglobulin-zcytor17 polypeptide fusions can be expressed in genetically engineered cells to produce a variety of multimeric zcytor17 analogs. Auxiliary
5 domains can be fused to zcytor17 polypeptides to target them to specific cells, tissues, or macromolecules (e.g., collagen). A zcytor17 polypeptide can be fused to two or more moieties, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan et al., *Connective Tissue Research* 34:1-9, 1996. For example, one
10 or more domains from zcytor17 soluble receptor can be joined to a soluble cytokine receptor, such as OSMRbeta and/or WSX-1, which may enhance their biological properties or efficiency of production. Additionally, the soluble multimeric cytokine receptor may further include an affinity tag. An affinity tag can be, for example, a tag selected from the group of polyhistidine, protein A, glutathione S transferase, Glu-Glu,
15 substance P, Flag™ peptide, streptavidin binding peptide, and an immunoglobulin F_c polypeptide.

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-
20 hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic 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 methods are known in the art for
25 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. 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
30 an *E. coli* S30 extract and commercially available enzymes and other 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* 259:806-9, 1993; and Chung et al., *Proc. Natl. Acad. Sci. USA* 90:10145-9, 1993). In 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.* 271:19991-8, 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.

10 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. 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).

15 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 zcytor17 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: 1081-5, 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 (e.g. ligand binding and signal transduction) as disclosed below to identify amino acid residues that are critical

20 to the activity of the molecule. See also, Hilton et al., *J. Biol. Chem.* 271:4699-4708, 1996. Sites of ligand-receptor, protein-protein or other biological 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

25 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. The identities of essential

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amino acids can also be inferred from analysis of homologies with related receptors.

Determination of amino acid residues that are within regions or domains that are critical to maintaining structural integrity can be determined. Within these regions one can determine specific residues that will be more or less tolerant of change
5 and maintain the overall tertiary structure of the molecule. Methods for analyzing sequence structure include, but are not limited to, alignment of multiple sequences with high amino acid or nucleotide identity and computer analysis using available software (e.g., the Insight II® viewer and homology modeling tools; MSI, San Diego, CA), secondary structure propensities, binary patterns, complementary packing and buried
10 polar interactions (Barton, *Current Opin. Struct. Biol.* 5:372-376, 1995 and Cordes et al., *Current Opin. Struct. Biol.* 6:3-10, 1996). In general, when designing modifications to molecules or identifying specific fragments determination of structure will be accompanied by evaluating activity of modified molecules.

Amino acid sequence changes are made in zcytor17 polypeptides so as
15 to minimize disruption of higher order structure essential to biological activity. For example, when the zcytor17 polypeptide comprises one or more helices, changes in amino acid residues will be made so as not to disrupt the helix geometry and other components of the molecule where changes in conformation abate some critical function, for example, binding of the molecule to its binding partners. The effects of
20 amino acid sequence changes can be predicted by, for example, computer modeling as disclosed above or determined by analysis of crystal structure (see, e.g., Lapthorn et al., *Nat. Struct. Biol.* 2:266-268, 1995). Other techniques that are well known in the art compare folding of a variant protein to a standard molecule (e.g., the native protein). For example, comparison of the cysteine pattern in a variant and standard molecules
25 can be made. Mass spectrometry and chemical modification using reduction and alkylation provide methods for determining cysteine residues which are associated with disulfide bonds or are free of such associations (Bean et al., *Anal. Biochem.* 201:216-226, 1992; Gray, *Protein Sci.* 2:1732-1748, 1993; and Patterson et al., *Anal. Chem.* 66:3727-3732, 1994). It is generally believed that if a modified molecule does not have
30 the same disulfide bonding pattern as the standard molecule folding would be affected. Another well known and accepted method for measuring folding is circular dichroism

(CD). Measuring and comparing the CD spectra generated by a modified molecule and standard molecule is routine (Johnson, *Proteins* 7:205-214, 1990). Crystallography is another well known method for analyzing folding and structure. Nuclear magnetic resonance (NMR), digestive peptide mapping and epitope mapping are also known
5 methods for analyzing folding and structural similarities between proteins and polypeptides (Schaanan et al., *Science* 257:961-964, 1992).

A Hopp/Woods hydrophilicity profile of the zcytor17 protein sequence as shown in SEQ ID NO:2, SEQ ID NO:46, SEQ ID NO:54, SEQ ID NO:57 and SEQ ID NO:93 can be generated (Hopp et al., *Proc. Natl. Acad. Sci.* 78:3824-3828, 1981;
10 Hopp, *J. Immun. Meth.* 88:1-18, 1986 and Triquier et al., *Protein Engineering* 11:153-169, 1998). See, Figure 1. The profile is based on a sliding six-residue window. Buried G, S, and T residues and exposed H, Y, and W residues were ignored. For example, in zcytor17, hydrophilic regions include amino acid residues 43 through 48 of SEQ ID NO:2 and SEQ ID NO:46 (residues 56 through 61 of SEQ ID NO:54), amino
15 acid residues 157 through 162 of SEQ ID NO:2 and SEQ ID NO:46 (residues 170 through 175 of SEQ ID NO:54), amino acid residues 158 through 163 of SEQ ID NO:2 and SEQ ID NO:46 (residues 171 through 176 of SEQ ID NO:54), amino acid residues 221 through 226 of SEQ ID NO:2 and SEQ ID NO:46 (residues 234 through 239 of SEQ ID NO:54), and amino acid residues 426 through 431 of SEQ ID NO:2 and SEQ
20 ID NO:46 (residues 439 through 444 of SEQ ID NO:54).

Those skilled in the art will recognize that hydrophilicity or hydrophobicity will be taken into account when designing modifications in the amino acid sequence of a zcytor17 polypeptide, so as not to disrupt the overall structural and biological profile. Of particular interest for replacement are hydrophobic residues
25 selected from the group consisting of Val, Leu and Ile or the group consisting of Met, Gly, Ser, Ala, Tyr and Trp. For example, residues tolerant of substitution could include such residues as shown in SEQ ID NO:2, SEQ ID NO:46, SEQ ID NO:54, SEQ ID NO:57 and SEQ ID NO:93. However, Cysteine residues would be relatively intolerant of substitution.

30 The identities of essential amino acids can also be inferred from analysis of sequence similarity between class I cytokine receptor family members with zcytor17.

Using methods such as "FASTA" analysis described previously, regions of high similarity are identified within a family of proteins and used to analyze amino acid sequence for conserved regions. An alternative approach to identifying a variant zcytor17 polynucleotide on the basis of structure is to determine whether a nucleic acid molecule encoding a potential variant zcytor17 polynucleotide can hybridize to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:45 or SEQ ID NO:53, as discussed above.

Other methods of identifying essential amino acids in the polypeptides of the present invention are procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081 (1989), Bass et al., *Proc. Natl Acad. Sci. USA* 88:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in *Proteins: Analysis and Design*, Angeletti (ed.), pages 259-311 (Academic Press, Inc. 1998)). 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 (1996).

The present invention also includes a multimeric cytokine receptor which includes functional fragments of zcytor17 polypeptides and nucleic acid molecules encoding such functional fragments. A "functional" zcytor17 or fragment thereof defined herein is characterized by its ability to mediate proliferative or differentiating activity, by its ability to induce or inhibit specialized cell functions, or by its ability to bind specifically to an anti-zcytor17 antibody or zcytor17 ligand (either soluble or immobilized). Moreover, functional fragments also include the signal peptide, intracellular signaling domain, and the like. As previously described herein, zcytor17 is characterized by a class I cytokine receptor structure. Thus, the present invention further provides fusion proteins encompassing: (a) polypeptide molecules comprising an extracellular domain, cytokine-binding domain, or intracellular domain described herein; and (b) functional fragments comprising one or more of these domains. The other polypeptide portion of the fusion protein may comprise at least a portion of one or more of another class I cytokine receptor, for example, gp130, LIF,

IL-12, WSX-1, IL-2 receptor β -subunit and the β -common receptor (i.e., IL3, IL-5, and GM-CSF receptor β -subunits), or by a non-native and/or an unrelated secretory signal peptide that facilitates secretion of the fusion protein.

Routine deletion analyses of nucleic acid molecules can be performed to
5 obtain functional fragments of a nucleic acid molecule that encodes a zcytor17 polypeptide. As an illustration, DNA molecules having the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:45 or SEQ ID NO:53 or fragments thereof, can be digested with *Bal31* nuclease to obtain a series of nested deletions. These DNA fragments are then inserted into expression vectors in proper reading frame, and the expressed
10 polypeptides are isolated and tested for zcytor17 activity, or for the ability to bind anti-zcytor17 antibodies or zcytor17 ligand. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired zcytor17 fragment. Alternatively, particular fragments of a zcytor17 polynucleotide can be synthesized using the polymerase chain reaction.

15 Standard methods for identifying functional domains are well-known to those of skill in the art. For example, studies on the truncation at either or both termini of interferons have been summarized by Horisberger and Di Marco, *Pharmac. Ther.* 66:507 (1995). Moreover, standard techniques for functional analysis of proteins are described by, for example, Treuter et al., *Molec. Gen. Genet.* 240:113 (1993); Content
20 et al., "Expression and preliminary deletion analysis of the 42 kDa 2-5A synthetase induced by human interferon," in *Biological Interferon Systems, Proceedings of ISIR-TNO Meeting on Interferon Systems*, Cantell (ed.), pages 65-72 (Nijhoff 1987); Herschman, "The EGF Receptor," in *Control of Animal Cell Proliferation 1*, Boynton et al., (eds.) pages 169-199 (Academic Press 1985); Coumailleau et al., *J. Biol. Chem.*
25 270:29270 (1995); Fukunaga et al., *J. Biol. Chem.* 270:25291 (1995); Yamaguchi et al., *Biochem. Pharmacol.* 50:1295 (1995); and Meisel et al., *Plant Molec. Biol.* 30:1 (1996).

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and
30 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 92/062045) and region-directed mutagenesis (Derbyshire et al., *Gene* 46:145, 1986; Ner et al., *DNA* 7:127, 1988).

Variants of the disclosed zcytor17 DNA and polypeptide sequences can be generated through DNA shuffling as disclosed by Stemmer, *Nature* 370:389-91, 1994, Stemmer, *Proc. Natl. Acad. Sci. USA* 91:10747-51, 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 zcytor17 receptor polypeptides in host cells. Preferred assays in this regard include cell proliferation assays and biosensor-based ligand-binding assays, which are described below. Mutagenized DNA molecules that encode active receptors or portions thereof (e.g., ligand-binding fragments, signaling domains, and the like) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods 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.

The present invention also provides a novel multimeric cytokine receptor in which a segment comprising at least a portion of one or more of the domains of zcytor17, for instance, secretory, extracellular, transmembrane, and intracellular, is fused to another polypeptide, for example, an extracellular domain of a class I cytokine

receptor, such as OSMRbeta and/or WSX-1. Fusion is preferably done by splicing at the DNA level to allow expression of chimeric molecules in recombinant production systems. The resultant molecules are then assayed for such properties as improved solubility, improved stability, prolonged clearance half-life, improved expression and secretion levels, and pharmacodynamics. Such a multimeric cytokine receptor may further comprise additional amino acid residues (e.g., a polypeptide linker) between the component proteins or polypeptides. A domain linker may comprise a sequence of amino acids from about 3 to about 20 amino acids long, from about 5 to 15 about amino acids long, from about 8 to about 12 amino acids long, and about 10 amino acids long. One function of a linker is to separate the active protein regions to promote their independent bioactivity and permit each region to assume its bioactive conformation independent of interference from its neighboring structure.

Using the methods discussed herein, one of ordinary skill in the art can identify and/or prepare a variety of polypeptide fragments or variants of SEQ ID NO:111, SEQ ID NO:109, SEQ ID NO:5, SEQ ID NO:117 and SEQ ID NO:119 that retain the signal transduction or ligand binding activity. For example, one can make a zcytor17 "soluble receptor" by preparing a variety of polypeptides that are substantially homologous to the cytokine-binding domain (residues 20 (Ala) to 227 (Pro) of SEQ ID NO:111 and SEQ ID NO:109; residues 33 (Ala) to 240 (Pro) of SEQ ID NO:5), the extracellular domain (residues 20 (Ala) to 519 (Glu) of SEQ ID NO:111 and SEQ ID NO:109; residues 33 (Ala) to 532 (Glu) of SEQ ID NO:5), or allelic variants or species orthologs thereof (e.g., see SEQ ID NO:117 and SEQ ID NO:119 and functional fragments thereof as described herein)) and retain ligand-binding activity of the wild-type zcytor17 protein. Moreover, variant zcytor17 soluble receptors such as those shown in SEQ ID NO:113 and SEQ ID NO:115 can be isolated. Such polypeptides may include additional amino acids from, for example, part or all of the transmembrane and intracellular domains. Such polypeptides may also include additional polypeptide segments as generally disclosed herein such as labels, affinity tags, and the like.

For any zcytor17 polypeptide, including variants, soluble receptors, and fusion polypeptides or 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.

The zcytor17 multimeric cytokine receptors of the present invention, including full-length polypeptides, biologically active fragments, and fusion polypeptides, can be produced in genetically engineered host cells 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 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 Press, Cold Spring Harbor, NY, 1989, and Ausubel et al., eds., *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987.

The present invention also provides an expression vector comprising an isolated and purified DNA molecule including the following operably linked elements: a first transcription promoter, a first DNA segment encoding a polypeptide having at least 90 percent sequence identity with SEQ ID NO:111, and a first transcription terminator; and a second transcription promoter, a second DNA segment encoding at least a portion of a class I cytokine receptor, and a second transcription terminator; wherein the polypeptide and the class I cytokine receptor form a multimeric cytokine receptor; and wherein the multimeric cytokine receptor binds to at least a portion of SEQ ID NO:2. The DNA molecule may further comprise a secretory signal sequence operably linked to the first and second DNA segments. The multimeric cytokine receptor may be soluble and/or may further comprise an affinity tag as described herein. In addition, the multimeric cytokine receptor may antagonize an activity of SEQ ID NO:2 as described herein. The at least a portion of a class I cytokine receptor may comprise portions of SEQ ID NO:7 and/or SEQ ID NO:9, such as, for instance, amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 35 to amino acid residue 137 of SEQ ID NO:7, amino acid residue 240 to amino acid residue 342 of SEQ ID NO:7, amino acid residue 348 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 761 of SEQ ID NO:7, amino acid residue 762 to

amino acid residue 979 of SEQ ID NO:7, or combinations thereof. The present invention also provides a cultured cell containing the above-described expression vector.

In general, a DNA sequence, for example, encoding a zcytor17 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, for example, a zcytor17 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 zcytor17, 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 zcytor17 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. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from amino acid 1 (Met) to amino acid 19 (Ala) of SEQ ID NO:111 and SEQ ID

NO:109, or wherein a secretory signal sequence derived from amino acid 1 (Met) to amino acid 32 (Ala) of SEQ ID NO:5, or amino acid 1 (Met) to amino acid 45 (Ala) of SEQ ID NO:117 or SEQ ID NO:119), or amino acid 28 (Met) to residue 45 (Ala) of SEQ ID NO:117 or SEQ ID NO:119), is operably linked to another polypeptide using
5 methods known in the art and disclosed herein. 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
10 active component of a normally non-secreted protein. Such fusions may be used *in vivo* or *in vitro* to direct peptides through the secretory pathway.

The present invention also provides a cultured cell comprising a first expression vector comprising a DNA molecule containing the following operably linked elements: a transcription promoter, a DNA segment encoding a polypeptide
15 having at least 90 percent sequence identity with SEQ ID NO:111, and a transcription terminator; and a second expression vector comprising a transcription promoter, a DNA segment encoding at least a portion of a class I cytokine receptor, and a transcription terminator; wherein the polypeptide and the class I cytokine receptor form a multimeric cytokine receptor. The first and second expression vectors may further comprise a
20 secretory signal sequence operably linked to the first and second DNA segments. The multimeric cytokine receptor may be soluble, may be a heterodimer, and/or may further comprise an affinity tag as described herein. In addition, the multimeric cytokine receptor may antagonize an activity of SEQ ID NO:2 as described herein. The at least at portion of a class I cytokine receptor may comprise portions of SEQ ID NO:7 and/or
25 SEQ ID NO:9, such as, for instance, amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 35 to amino acid residue 137 of SEQ ID NO:7, amino acid residue 240 to amino acid residue 342 of SEQ ID NO:7, amino acid residue 348 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 739 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 761 of SEQ
30 ID NO:7, amino acid residue 762 to amino acid residue 979 of SEQ ID NO:7, or combinations thereof.

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, 5 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* 7:980-90, 1989; Wang and Finer, *Nature Med.* 2:714-716, 1996). The production of recombinant polypeptides in 10 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 15 1573; Graham et al., *J. Gen. Virol.* 36:59-72, 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. 20 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.

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 25 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 30 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
5 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
10 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)* 11:47-58, 1987. 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
15 baculovirus, commonly derived from *Autographa californica nuclear polyhedrosis virus* (AcNPV). See, King, L.A. and Possee, R.D., *The Baculovirus Expression System: A Laboratory Guide*, London, Chapman & Hall; O'Reilly, D.R. et al., *Baculovirus Expression Vectors: A Laboratory Manual*, New York, Oxford University Press., 1994; and, Richardson, C. D., Ed., *Baculovirus Expression Protocols. Methods*
20 *in Molecular Biology*, Totowa, NJ, Humana Press, 1995. A second method of making recombinant zcytor17 baculovirus utilizes a transposon-based system described by Luckow (Luckow, V.A, et al., *J Virol* 67:4566-79, 1993). This system, which utilizes transfer vectors, is sold in the Bac-to-Bac™ kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a
25 Tn7 transposon to move the DNA encoding the zcytor17 polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." See, Hill-Perkins, M.S. and Possee, R.D., *J Gen Virol* 71:971-6, 1990; Bonning, B.C. et al., *J Gen Virol* 75:1551-6, 1994; and, Chazenbalk, G.D., and Rapoport, B., *J Biol Chem* 270:1543-9, 1995. In addition, transfer vectors can include an in-frame fusion with DNA encoding
30 an epitope tag at the C- or N-terminus of the expressed zcytor17 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer, T. et al., *Proc. Natl. Acad. Sci.*

82:7952-4, 1985). Using a technique known in the art, a transfer vector containing zcytor17 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 zcytor17 is subsequently produced. Recombinant viral stocks are made by methods commonly used in the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *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 No. 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 Ex-cello405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. 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 zcytor17 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 5 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., *J. Gen. Microbiol.* 132:3459-3465, 1986 and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be 10 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 15 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* 20 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 25 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 30 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 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most
5 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. 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
10 zcytor17 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as 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 and dimerized by diluting the
15 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 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,
20 thereby obviating the need for denaturation and refolding.

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, are known in the art and generally
25 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 an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-
30 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 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% Bacto™ yeast extract (Difco Laboratories), 0.004% adenine and
5 0.006% L-leucine).

Within one aspect of the present invention, a zcytor17 multimeric cytokine receptor (including transmembrane and intracellular domains) is produced by a cultured cell, and the cell is used to screen for ligands for the receptor, including the natural ligand (SEQ ID NO:2), as well as agonists and antagonists of the natural ligand.
10 To summarize this approach, a cDNA or gene encoding the receptor is combined with other genetic elements required for its expression (e.g., a transcription promoter), and the resulting expression vector is inserted into a host cell. Cells that express the DNA and produce functional receptor are selected and used within a variety of screening systems.

15 Mammalian cells suitable for use in expressing the novel receptors of the present invention and transducing a receptor-mediated signal include cells that express a β -subunit, such as gp130, and cells that co-express gp130 and LIF receptor (Gearing et al., *EMBO J.* 10:2839-2848, 1991; Gearing et al., U.S. Patent No. 5,284,755). In this regard it is generally preferred to employ a cell that is responsive to other cytokines that
20 bind to receptors in the same subfamily, such as IL-6 or LIF, because such cells will contain the requisite signal transduction pathway(s). Preferred cells of this type include BaF3 cells (Palacios and Steinmetz, *Cell* 41: 727-734, 1985; Mathey-Prevot et al., *Mol. Cell. Biol.* 6: 4133-4135, 1986), the human TF-1 cell line (ATCC number CRL-2003) and the DA-1 cell line (Branch et al., *Blood* 69:1782, 1987; Broudy et al., *Blood*
25 75:1622-1626, 1990). In the alternative, suitable host cells can be engineered to produce a β -subunit or other cellular component needed for the desired cellular response. For example, the murine cell line BaF3 (Palacios and Steinmetz, *Cell* 41:727-734, 1985; Mathey-Prevot et al., *Mol. Cell. Biol.* 6: 4133-4135, 1986), a baby hamster kidney (BHK) cell line, or the CTLL-2 cell line (ATCC TIB-214) can be
30 transfected to express the mouse gp130 subunit, or mouse gp130 and LIF receptor, in addition to zcytor17. It is generally preferred to use a host cell and receptor(s) from the

same species, however this approach allows cell lines to be engineered to express multiple receptor subunits from any species, thereby overcoming potential limitations arising from species specificity. In the alternative, species homologs of the human receptor cDNA can be cloned and used within cell lines from the same species, such as
5 a mouse cDNA in the BaF3 cell line. Cell lines that are dependent upon one hematopoietic growth factor, such as IL-3, can thus be engineered to become dependent upon a zcytor17 ligand or anti-zcytor17 antibody.

Cells expressing functional zcytor17 are used within screening assays. A variety of suitable assays are known in the art. These assays are based on the
10 detection of a biological response in the target cell. One such assay is a cell proliferation assay. Cells are cultured in the presence or absence of a test compound, and cell proliferation is detected by, for example, measuring incorporation of tritiated thymidine or by colorimetric assay based on the reduction or metabolic breakdown of Alymar BlueTM (AccuMed, Chicago, IL) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl
15 tetrazolium bromide (MTT) (Mosman, *J. Immunol. Meth.* 65:55-63, 1983). An alternative assay format uses cells that are further engineered to express a reporter gene. The reporter gene is linked to a promoter element that is responsive to the receptor-linked pathway, and the assay detects activation of transcription of the reporter gene. A preferred promoter element in this regard is a serum response element, STAT or SRE
20 (see, for example, Shaw et al., *Cell* 56:563-572, 1989). A preferred such reporter gene is a luciferase gene (de Wet et al., *Mol. Cell. Biol.* 7:725, 1987). Expression of the luciferase gene is detected by luminescence using methods known in the art (e.g., Baumgartner et al., *J. Biol. Chem.* 269:19094-29101, 1994; Schenborn and Goiffin, *Promega Notes* 41:11, 1993). Luciferase assay kits are commercially available from,
25 for example, Promega Corp., Madison, WI. Target cell lines of this type can be used to screen libraries of chemicals, cell-conditioned culture media, fungal broths, soil samples, water samples, and the like. For example, a bank of cell- or tissue-conditioned media samples can be assayed on a target cell to identify cells that produce ligand. Positive cells are then used to produce a cDNA library in a mammalian cell expression
30 vector, which is divided into pools, transfected into host cells, and expressed. Media samples from the transfected cells are then assayed, with subsequent division of pools,

retransfection, subculturing, and re-assay of positive cells to isolate a clonal cell line expressing the ligand. Media samples conditioned by kidney, liver, spleen, thymus, other lymphoid tissues, or T-cells are preferred sources of ligand for use in screening procedures.

5 The present invention also provides a method of producing a multimeric cytokine receptor. The method includes culturing a cell as described herein, and isolating the multimeric cytokine receptor produced by the cell.

 The present invention also provides a method for detecting a multiple cytokine receptor ligand in a test sample. The method includes contacting the test
10 sample with a multimeric cytokine receptor comprising a polypeptide comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 or SEQ ID NO:109 (amino acid residue 33 to amino acid residue 240 of SEQ ID NO:5), and at least a portion of at least one class I cytokine receptor; and detecting the binding of the multimer cytokine receptor to the ligand in the test sample. The at least a portion of at
15 least one class I cytokine receptor can include, for example, a portion of SEQ ID NO:9 and/or a portion of SEQ ID NO:7, such as, for instance, amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 35 to amino acid residue 137 of SEQ ID NO:7, amino acid residue 240 to amino acid residue 342 of SEQ ID NO:7, amino acid residue 348 to amino acid residue 429 of SEQ ID NO:7, amino acid residue
20 28 to amino acid residue 739 of SEQ ID NO:7, and/or combinations thereof.

 A natural ligand for a zcytor17 multimeric cytokine receptor of the present invention can also be identified by mutagenizing a cytokine-dependent cell line expressing zcytor17 and culturing it under conditions that select for autocrine growth. See WIPO publication WO 95/21930. Within a typical procedure, cells expressing
25 zcytor17 are mutagenized, such as with EMS. The cells are then allowed to recover in the presence of the required cytokine, then transferred to a culture medium lacking the cytokine. Surviving cells are screened for the production of a ligand for zcytor17 multimeric cytokine receptor, such as by adding soluble receptor polypeptide comprising the zcytor17 cytokine-binding domain and at least a portion of a class I
30 cytokine receptor, such as the cytokine-binding domain of OSMRbeta (SEQ ID NO:7) and/or WSX-1 (SEQ ID NO:9), as described herein to the culture medium to compete

against the ligand or by assaying conditioned media on wild-type cells compared to transfected cells expressing the zcytor17 multimeric cytokine receptor. Preferred cell lines for use within this method include cells that are transfected to express gp130 or gp130 in combination with LIF receptor. Preferred such host cell lines include
5 transfected CTLL-2 cells (Gillis and Smith, *Nature* 268:154-156, 1977) and transfected BaF3 cells.

Moreover, a secretion trap method employing zcytor17 soluble multimeric cytokine receptor can be used to isolate a zcytor17 ligand, such as SEQ ID NO:2 (Aldrich, et al, *Cell* 87: 1161-1169, 1996). A cDNA expression library prepared
10 from a known or suspected ligand source is transfected into COS-7 cells. The cDNA library vector generally has an SV40 origin for amplification in COS-7 cells, and a CMV promoter for high expression. The transfected COS-7 cells are grown in a monolayer and then fixed and permeabilized. Tagged or biotin-labeled zcytor17 soluble multimeric cytokine receptor, described herein, is then placed in contact with
15 the cell layer and allowed to bind cells in the monolayer that express an anti-complementary molecule, i.e., a zcytor17 ligand. A cell expressing a ligand will thus be bound with receptor molecules. An anti-tag antibody (anti-Ig for Ig fusions, M2 or anti-FLAG for FLAG-tagged fusions, streptavidin, anti-Glu-Glu tag, and the like) which is conjugated with horseradish peroxidase (HRP) is used to visualize these cells
20 to which the tagged or biotin-labeled zcytor17 soluble multimeric cytokine receptor has bound. The HRP catalyzes deposition of a tyramide reagent, for example, tyramide-FITC. A commercially-available kit can be used for this detection (for example, Renaissance TSA-Direct™ Kit; NEN Life Science Products, Boston, MA). Cells which express zcytor17 multimeric cytokine receptor ligand will be identified under
25 fluorescence microscopy as green cells and picked for subsequent cloning of the ligand using procedures for plasmid rescue as outlined in Aldrich, et al, *supra.*, followed by subsequent rounds of secretion trap assay, or conventional screening of cDNA library pools, until single clones are identified.

As a multimeric receptor complex, the activity of zcytor17 polypeptide
30 can be measured by a silicon-based biosensor microphysiometer which measures the extracellular acidification rate or proton excretion associated with receptor binding and

subsequent physiologic cellular responses. An exemplary device is the Cytosensor™ Microphysiometer manufactured by Molecular Devices, Sunnyvale, CA. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method. See, for example, McConnell, H.M. et al., *Science* 257:1906-1912, 1992; Pitchford, S. et al., *Meth. Enzymol.* 228:84-108, 1997; Arimilli, S. et al., *J. Immunol. Meth.* 212:49-59, 1998; Van Liefde, I. Et al., *Eur. J. Pharmacol.* 346:87-95, 1998. The microphysiometer can be used for assaying eukaryotic, prokaryotic, adherent or non-adherent cells. By measuring extracellular acidification changes in cell media over time, the microphysiometer directly measures cellular responses to various stimuli, including agonists, ligands, or antagonists of the zcytor17 polypeptide. Preferably, the microphysiometer is used to measure responses of a zcytor17-expressing eukaryotic cell, compared to a control eukaryotic cell that does not express zcytor17 polypeptide. Zcytor17-expressing eukaryotic cells comprise cells into which zcytor17 has been transfected or infected via adenovirus vector, and the like, as described herein, creating a cell that is responsive to zcytor17-modulating stimuli, or are cells naturally expressing zcytor17, such as zcytor17-expressing cells derived from lymphoid, spleen, thymus tissue or PBLs. Differences, measured by an increase or decrease in extracellular acidification, in the response of cells expressing zcytor17, relative to a control, are a direct measurement of zcytor17-modulated cellular responses. Moreover, such zcytor17-modulated responses can be assayed under a variety of stimuli. Also, using the microphysiometer, there is provided a method of identifying agonists and antagonists of zcytor17 multimeric cytokine receptor, comprising providing cells expressing a zcytor17 multimeric cytokine receptor, culturing a first portion of the cells in the absence of a test compound, culturing a second portion of the cells in the presence of a test compound, and detecting an increase or a decrease in a cellular response of the second portion of the cells as compared to the first portion of the cells. Antagonists and agonists, including the natural ligand for zcytor17 multimeric cytokine receptor, can be rapidly identified using this method.

A zcytor17 multimeric cytokine receptor 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
5 proximity to each other. Fusions of this type can be used for example, for dimerization, increasing stability and *in vivo* half-life, to affinity purify ligand, as *in vitro* assay tool or antagonist. For use in assays, the chimeras are bound to a support via the F_c region and used in an ELISA format.

Additional assays provided by the present invention include the use of
10 hybrid receptor polypeptides. These hybrid polypeptides fall into two general classes. Within the first class, the intracellular domain of zcytor17, comprising approximately residues 544 (Lys) to 732 (Val) of SEQ ID NO:111, residues 544 (Lys) to 649 (Ile) of SEQ ID NO:109, or residues 557 (Lys) to 662 (Ile) of SEQ ID NO:5, or residues 551 (Lys) to 662 (Cys) of SEQ ID NO:117 is joined to the ligand-binding domain of a
15 second receptor. It is preferred that the second receptor be a hematopoietic cytokine receptor, such as, for instance, mpl receptor (Souyri et al., *Cell* 63:1137-1147, 1990). The hybrid receptor will further comprise a transmembrane domain, which may be derived from either receptor. A DNA construct encoding the hybrid receptor is then inserted into a host cell. Cells expressing the hybrid receptor are cultured in the
20 presence of a ligand for the binding domain and assayed for a response. This system provides a means for analyzing signal transduction mediated by zcytor17 while using readily available ligands. This system can also be used to determine if particular cell lines are capable of responding to signals transduced by zcytor17. A second class of hybrid receptor polypeptides comprise the extracellular (ligand-binding) domain
25 (approximately residues 20 (Ala) to 519 (Glu) of SEQ ID NO:111 and SEQ ID NO:109; approximately residues 33 (Ala) to 532 (Glu) of SEQ ID NO:5) or cytokine-binding domain of zcytor17 (approximately residues 20 (Ala) to 227 (Pro) of SEQ ID NO:111 and SEQ ID NO:109; or approximately residues 33 (Ala) to 240 (Pro) of SEQ ID NO:5; approximately residues 46 (Val) to 533 (Glu) of SEQ ID NO:117; or approximately
30 residues 46 (Val) to 533 (Trp) of SEQ ID NO:119) with a cytoplasmic domain of a second receptor, preferably a cytokine receptor, and a transmembrane domain. The

transmembrane domain may be derived from either receptor. Hybrid receptors of this second class are expressed in cells known to be capable of responding to signals transduced by the second receptor. Together, these two classes of hybrid receptors enable the use of a broad spectrum of cell types within receptor-based assay systems.

5 The expression of WSX-1 is strongest in thymus, spleen, PBL, and lymph node, as well as increased expression observed for activated T-cells. The tissue distribution for OSMRbeta is described as very broad. The tissue distribution of these three receptors suggests that a target for zcytor17lig is hematopoietic lineage cells, in particular T-cells, monocytes/macrophages and lymphoid progenitor cells and lymphoid
10 cells. Other known four-helical-bundle cytokines that act on lymphoid cells include IL-2, IL-4, IL-7, and IL-15. For a review of four-helical-bundle cytokines, see, Nicola et al., *Advances in Protein Chemistry* 52:1-65, 1999 and Kelso, A., *Immunol. Cell Biol.* 76:300-317, 1998.

 Conditioned media (CM) from CD3+ selected, PMA/Ionomycin-
15 stimulated human peripheral blood cells supported the growth of BaF3 cells that expressed the zcytor17 receptor, OSMRbeta and WSX-1 receptor and were otherwise dependent on IL-3. Conditioned medias from cells that were not: 1) PMA/Ionomycin-stimulated; or were not: 2) CD3 selected (with or without PMA/Ionomycin stimulation) did not support the growth of Baf3 cells expressing zcytor17, OSMRbeta and WSX-1
20 (BaF3/zcytor17/WSX-1/OSMRbeta) receptor-expressing cells. Control experiments demonstrated that this proliferative activity was not attributable to other known growth factors, and that the ability of such conditioned media to stimulate proliferation of zcytor17/WSX-1/OSMRbeta receptor-expressing cells could be neutralized by a soluble form of the zcytor17 receptor.

25 Conditioned-media from CD3+ selected cells activated with PMA/Ionomycin also supported growth of BaF3 cells that expressed the zcytor17 receptor and OSMRbeta receptor (zcytor17/OSMRbeta), while BaF3 cells expressing only zcytor17 receptor and WSX-1 receptor (zcytor17/WSX-1), or containing only the OSMRbeta receptor, were not stimulated by this conditioned-media.

30 Proliferation of zcytor17/WSX-1/OSMRbeta receptor-expressing BaF3 cells exposed to CM from CD3+ selected, PMA/Ionomycin-stimulated human

peripheral blood cells were identified by visual inspection of the cultures and/or by proliferation assay. Many suitable proliferation assays are known in the art, and include assays for reduction of a dye such as AlamarBlue™ (AccuMed International, Inc. Westlake, Ohio), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
5 (Mosman, *J. Immunol. Meth.* 65: 55-63, 1983); 3-(4,5 dimethyl thiazol-2yl)-5-3-carboxymethoxyphenyl-2H-tetrazolium; 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide; and cyanoditoly-tetrazolium chloride (which are commercially available from Polysciences, Inc., Warrington, PA); mitogenesis assays, such as measurement of incorporation of ³H-thymidine; dye
10 exclusion assays using, for example, naphthalene black or trypan blue; dye uptake using diacetyl fluorescein; and chromium release. See, in general, Freshney, *Culture of Animal Cells: A Manual of Basic Technique*, 3rd ed., Wiley-Liss, 1994.

A cDNA library was prepared from CD3+ selected, PMA- and
15 Ionomycin-stimulated primary human peripheral blood cells. The CD3+ selected, PMA- and Ionomycin-stimulated human peripheral blood cells cDNA library was divided into pools containing multiple cDNA molecules and was transfected into a host cell line, for example, BHK 570 cells (ATCC accession no. 10314). The transfected host cells were cultured in a medium that did not contain exogenous growth factors
20 (e.g., 5% FBS) and conditioned medium was collected. The conditioned media were assayed for the ability to stimulate proliferation of BaF3 cells transfected with the zcytor17, WSX-1, and OSMRbeta receptors. cDNA pools producing conditioned medium that stimulated BaF3/zcytor17/WSX-1/OSMRbeta receptor cells were identified. This pooled plasmid cDNA was electroporated into *E. coli*. cDNA was
25 isolated from single colonies and transfected individually into BHK 570 cells. Positive clones were identified by a positive result in the BaF3/zcytor17/WSX-1/OSMRbeta receptor proliferation assay, and the activity was confirmed by neutralization of proliferation using the soluble zcytor17 receptor.

In view of the tissue distribution observed for zcytor17 receptor agonists
30 (including the natural zcytor17lig/ substrate/ cofactor/ etc.) and/or antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as

zcytor17lig agonists are useful for expansion, proliferation, activation, differentiation, and/or induction or inhibition of specialized cell functions of cells involved in homeostasis of hematopoiesis and immune function. For example, zcytor17lig and agonist compounds are useful as components of defined cell culture media, and may be
5 used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Agonists are thus useful in specifically promoting the growth and/or development of T-cells, B-cells, monocytes/macrophages, NK cells, cytotoxic lymphocytes, and other cells of the lymphoid and myeloid lineages in culture.

Antagonists are also useful as research reagents for characterizing sites
10 of ligand-receptor interaction. Antagonists are useful to inhibit expansion, proliferation, activation, and/or differentiation of cells involved in regulating hematopoiesis. Inhibitors of zcytor17lig activity (zcytor17lig antagonists) include anti-zcytor17lig antibodies and soluble multimeric cytokine receptors, as well as other peptidic and non-peptidic agents (including ribozymes).

15 A zcytor17lig-binding protein, such as a multimeric cytokine receptor of the present invention, can also be used for purification of ligand. The multimeric cytokine receptor 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
20 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
25 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,
30 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* 145:229-40 (1991) and Cunningham and Wells, *J. Mol. Biol.* 234:554-63 (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
5 cell. A test sample is passed 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 change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates,
10 from which binding affinity can be calculated, and assessment of stoichiometry of binding. Alternatively, ligand/receptor binding can be analyzed using SELDI(TM) technology (CIPHERGEN, Inc., Palo Alto, CA).

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for
15 determination of binding affinity (see Scatchard, *Ann. NY Acad. Sci.* 51: 660-72 (1949)) and calorimetric assays (Cunningham et al., *Science* 253:545-48 (1991); and Cunningham et al., *Science* 245:821-25 (1991)).

The present invention also provides an antibody that specifically binds to a polypeptide or at least a portion of a multimeric cytokine receptor as described
20 herein.

Zcytor17 multimeric cytokine receptors can also be used to prepare antibodies that bind to epitopes, peptides or polypeptides thereof. The multimeric cytokine receptor or a fragment thereof serves as an antigen (immunogen) to inoculate an animal and elicit an immune response. One of skill in the art would recognize that
25 antigenic, epitope-bearing polypeptides may contain a sequence of at least 6, preferably at least 9, and more preferably at least 15 to about 30 contiguous amino acid residues of a polypeptide(s) of the multimeric cytokine receptor, such as zcytor17 (SEQ ID NO:111), OSMRbeta (SEQ ID NO:7), and/or WSX-1 (SEQ ID NO:9). Polypeptides comprising a larger portion of a multimeric cytokine receptor, i.e., from 30 to 100
30 residues up to the entire length of the amino acid sequence are included. Antigens or

immunogenic epitopes can also include attached tags, adjuvants, carriers and vehicles, as described herein.

Antibodies from an immune response generated by inoculation of an animal with these antigens can be isolated and purified as described herein. Methods
5 for preparing and isolating polyclonal and 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 Antibodies: Techniques and*
10 *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 horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats with a multimeric cytokine receptor or a fragment thereof. The immunogenicity of a multimeric cytokine
15 receptor may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Multimeric cytokine receptors useful for immunization also include fusion polypeptides, such as fusions of zcytor17, OSMRbeta, and/or WSX-1, or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a
20 full-length molecule or a portion thereof. If the polypeptide portion is "hapten-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 polyclonal antibodies,
25 affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as F(ab')₂ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human
30 CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" 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. Moreover, human antibodies can be produced in transgenic, non-human animals that have been engineered to contain human immunoglobulin genes as disclosed in WIPO Publication WO 98/24893. It is preferred that the endogenous immunoglobulin genes in these animals be inactivated or eliminated, such as by homologous recombination.

10 Antibodies are considered to be specifically binding if: 1) they exhibit a threshold level of binding activity, and 2) they do not significantly cross-react with related polypeptide molecules. A threshold level of binding is determined if anti-multimeric cytokine receptor antibodies herein bind to a multimeric cytokine receptor, peptide or epitope with an affinity at least 10-fold greater than the binding affinity to control (non-multimeric cytokine receptor) protein. It is preferred that the antibodies exhibit a binding affinity (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 (Scatchard, G., *Ann. NY Acad. Sci.* 51: 660-672 (1949)).

20 Whether anti-multimeric cytokine receptor antibodies do not significantly cross-react with related polypeptide molecules is shown, for example, by the antibody detecting zcytor17 multimeric cytokine receptor but not known related polypeptides using a standard Western blot analysis (Ausubel et al., *ibid.*). Examples of known related polypeptides are those disclosed in the prior art, such as known orthologs, and paralogs, and similar known members of a protein family. Screening can also be done using non-human multimeric cytokine receptor, and multimeric cytokine receptor mutant polypeptides. Moreover, antibodies can be “screened against” known related polypeptides, to isolate a population that specifically binds to the multimeric cytokine receptor. For example, antibodies raised to multimeric cytokine receptor are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to multimeric cytokine receptor will flow through the matrix under the proper buffer

conditions. Screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to known closely related polypeptides (*Antibodies: A Laboratory Manual*, Harlow and Lane (eds.), Cold Spring 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 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. Specifically binding anti-multimeric cytokine receptor antibodies can be detected by a number of methods in the art, and disclosed below.

A variety of assays known to those skilled in the art can be utilized to detect antibodies which bind to multimeric cytokine receptor proteins or polypeptides. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples 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 assay. In addition, antibodies can be screened for binding to wild-type versus mutant multimeric cytokine receptor protein or polypeptide.

Within another aspect the present invention provides an antibody produced by the method as disclosed above, wherein the antibody binds to at least a portion of a multimer cytokine receptor comprising at least a portion of SEQ ID NO:111, SEQ ID NO:109, or SEQ ID NO:5. In one embodiment, the antibody disclosed above specifically binds to a polypeptide shown in SEQ ID NO:111, SEQ ID NO:109, or SEQ ID NO:5. In another embodiment, the antibody can be a monoclonal antibody or a polyclonal antibody.

Antibodies to multimeric cytokine receptor may be used for tagging cells that express multimeric cytokine receptor; for isolating multimeric cytokine receptor by affinity purification; for diagnostic assays for determining circulating levels of multimeric cytokine receptor; for detecting or quantitating soluble multimeric cytokine receptor as a 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 multimeric cytokine receptor activity *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 multimeric cytokine receptor or fragments thereof may be used *in vitro* to detect denatured multimeric cytokine receptor or fragments thereof in assays, for example, Western Blots or other assays known in the art.

Suitable detectable molecules may be directly or indirectly attached to the multimeric cytokine receptor 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, saporin, *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). Multimeric cytokine receptors 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.

A soluble multimeric cytokine receptor can also act as zcytor17lig “antagonists” to block zcytor17lig binding and signal transduction *in vitro* and *in vivo*. These anti-zcytor17lig binding proteins would be useful for inhibiting zcytor17lig activity or protein-binding.

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 receptor 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.

Moreover, inflammation is a protective response by an organism to fend off an invading agent. Inflammation is a cascading event that involves many cellular and humoral mediators. On one hand, suppression of inflammatory responses can leave a host immunocompromised; however, if left unchecked, inflammation can lead to serious complications including chronic inflammatory diseases (e.g., rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease and the like), septic shock and multiple organ failure. Importantly, these diverse disease states share common inflammatory mediators. The collective diseases that are characterized by inflammation have a large impact on human morbidity and mortality. Therefore it is clear that anti-inflammatory antibodies and binding polypeptides, such as anti-zcytor17lig antibodies and binding polypeptides described herein, could have crucial therapeutic potential for a vast number of human and animal diseases, from asthma and allergy to autoimmunity and septic shock. As such, use of anti-inflammatory anti zcytor17lig antibodies and binding polypeptides described herein can be used therapeutically as zcytor17lig antagonists described herein, particularly in diseases such as arthritis, endotoxemia, inflammatory bowel disease, psoriasis, related disease and the like.

1. Arthritis

Arthritis, including osteoarthritis, rheumatoid arthritis, arthritic joints as a result of injury, and the like, are common inflammatory conditions which would benefit from the therapeutic use of anti-inflammatory antibodies and binding

polypeptides, such as anti-zcytor17lig antibodies and binding polypeptides of the present invention. For example, rheumatoid arthritis (RA) is a systemic disease that affects the entire body and is one of the most common forms of arthritis. It is characterized by the inflammation of the membrane lining the joint, which causes pain, stiffness, warmth, redness and swelling. Inflammatory cells release enzymes that may digest bone and cartilage. As a result of rheumatoid arthritis, the inflamed joint lining, the synovium, can invade and damage bone and cartilage leading to joint deterioration and severe pain amongst other physiologic effects. The involved joint can lose its shape and alignment, resulting in pain and loss of movement.

10 Rheumatoid arthritis (RA) is an immune-mediated disease particularly characterized by inflammation and subsequent tissue damage leading to severe disability and increased mortality. A variety of cytokines are produced locally in the rheumatoid joints. Numerous studies have demonstrated that IL-1 and TNF-alpha, two prototypic pro-inflammatory cytokines, play an important role in the mechanisms involved in synovial inflammation and in progressive joint destruction. Indeed, the administration of TNF-alpha and IL-1 inhibitors in patients with RA has led to a dramatic improvement of clinical and biological signs of inflammation and a reduction of radiological signs of bone erosion and cartilage destruction. However, despite these encouraging results, a significant percentage of patients do not respond to these agents, suggesting that other mediators are also involved in the pathophysiology of arthritis (Gabay, *Expert. Opin. Biol. Ther.* 2(2):135-149, 2002). One of those mediators could be zcytor17lig, and as such a molecule that binds or inhibits zcytor17lig, such as anti zcytor17lig antibodies or binding partners, could serve as a valuable therapeutic to reduce inflammation in rheumatoid arthritis, and other arthritic diseases.

25 There are several animal models for rheumatoid arthritis known in the art. For example, in the collagen-induced arthritis (CIA) model, mice develop chronic inflammatory arthritis that closely resembles human rheumatoid arthritis. Since CIA shares similar immunological and pathological features with RA, this makes it an ideal model for screening potential human anti-inflammatory compounds. The CIA model is a well-known model in mice that depends on both an immune response, and an inflammatory response, in order to occur. The immune response comprises the

interaction of B-cells and CD4+ T-cells in response to collagen, which is given as antigen, and leads to the production of anti-collagen antibodies. The inflammatory phase is the result of tissue responses from mediators of inflammation, as a consequence of some of these antibodies cross-reacting to the mouse's native collagen and activating the complement cascade. An advantage in using the CIA model is that the basic mechanisms of pathogenesis are known. The relevant T-cell and B-cell epitopes on type II collagen have been identified, and various immunological (e.g., delayed-type hypersensitivity and anti-collagen antibody) and inflammatory (e.g., cytokines, chemokines, and matrix-degrading enzymes) parameters relating to immune-mediated arthritis have been determined, and can thus be used to assess test compound efficacy in the CIA model (Wooley, *Curr. Opin. Rheum.* 3:407-20, 1999; Williams et al., *Immunol.* 89:9784-788, 1992; Myers et al., *Life Sci.* 61:1861-78, 1997; and Wang et al., *Immunol.* 92:8955-959, 1995).

The administration of soluble zcytor17 comprising polypeptides (including heterodimeric and multimeric receptors described herein), such as zcytor17-Fc4 or other zcytor17 soluble and fusion proteins to these CIA model mice was used to evaluate the use of zcytor17 to ameliorate symptoms and alter the course of disease. As a molecule that modulates immune and inflammatory response, zcytor17lig, may induce production of SAA, which is implicated in the pathogenesis of rheumatoid arthritis, zcytor17lig antagonists may reduce SAA activity *in vitro* and *in vivo*, the systemic or local administration of zcytor17lig antagonists such as anti-zcytor17lig antibodies or binding partners, zcytor17 comprising polypeptides (including heterodimeric and multimeric receptors described herein), such as zcytor17-Fc4 or other zcytor17 soluble and fusion proteins can potentially suppress the inflammatory response in RA. Other potential therapeutics include zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti zcytor17lig antibodies or binding partners of the present invention, and the like.

2. Endotoxemia

Endotoxemia is a severe condition commonly resulting from infectious agents such as bacteria and other infectious disease agents, sepsis, toxic shock syndrome, or in immunocompromised patients subjected to opportunistic infections,

and the like. Therapeutically useful of anti-inflammatory antibodies and binding polypeptides, such as anti-zcytor17lig antibodies and binding polypeptides of the present invention, could aid in preventing and treating endotoxemia in humans and animals. Other potential therapeutics include zcytor17 polypeptides, soluble
5 heterodimeric and multimeric receptor polypeptides, or anti zcytor17lig antibodies or binding partners of the present invention, and the like, could serve as a valuable therapeutic to reduce inflammation and pathological effects in endotoxemia.

Lipopolysaccharide (LPS) induced endotoxemia engages many of the proinflammatory mediators that produce pathological effects in the infectious diseases
10 and LPS induced endotoxemia in rodents is a widely used and acceptable model for studying the pharmacological effects of potential pro-inflammatory or immunomodulating agents. LPS, produced in gram-negative bacteria, is a major causative agent in the pathogenesis of septic shock (Glausner et al., Lancet 338:732, 1991). A shock-like state can indeed be induced experimentally by a single injection of
15 LPS into animals. Molecules produced by cells responding to LPS can target pathogens directly or indirectly. Although these biological responses protect the host against invading pathogens, they may also cause harm. Thus, massive stimulation of innate immunity, occurring as a result of severe Gram-negative bacterial infection, leads to excess production of cytokines and other molecules, and the development of a fatal
20 syndrome, septic shock syndrome, which is characterized by fever, hypotension, disseminated intravascular coagulation, and multiple organ failure (Dumitru et al. Cell 103:1071-1083, 2000).

These toxic effects of LPS are mostly related to macrophage activation leading to the release of multiple inflammatory mediators. Among these mediators,
25 TNF appears to play a crucial role, as indicated by the prevention of LPS toxicity by the administration of neutralizing anti-TNF antibodies (Beutler et al., *Science* 229:869, 1985). It is well established that 1ug injection of *E. coli* LPS into a C57Bl/6 mouse will result in significant increases in circulating IL-6, TNF-alpha, IL-1, and acute phase proteins (for example, SAA) approximately 2 hours post injection. The toxicity of LPS
30 appears to be mediated by these cytokines as passive immunization against these mediators can result in decreased mortality (Beutler et al., *Science* 229:869, 1985). The

potential immunointervention strategies for the prevention and/or treatment of septic shock include anti-TNF mAb, IL-1 receptor antagonist, LIF, IL-10, and G-CSF. Since LPS induces the production of pro-inflammatory factors possibly contributing to the pathology of endotoxemia, the neutralization of zcytor17lig activity, SAA or other pro-inflammatory factors by antagonizing zcytor17lig polypeptide can be used to reduce the symptoms of endotoxemia, such as seen in endotoxic shock. Other potential therapeutics include zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti-zcytor17lig antibodies or binding partners of the present invention, and the like.

10 3 *Inflammatory Bowel Disease. IBD*

In the United States approximately 500,000 people suffer from Inflammatory Bowel Disease (IBD) which can affect either colon and rectum (Ulcerative colitis) or both, small and large intestine (Crohn's Disease). The pathogenesis of these diseases is unclear, but they involve chronic inflammation of the affected tissues. Potential therapeutics include zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti-zcytor17lig antibodies or binding partners of the present invention, and the like, could serve as a valuable therapeutic to reduce inflammation and pathological effects in IBD and related diseases.

Ulcerative colitis (UC) is an inflammatory disease of the large intestine, commonly called the colon, characterized by inflammation and ulceration of the mucosa or innermost lining of the colon. This inflammation causes the colon to empty frequently, resulting in diarrhea. Symptoms include loosening of the stool and associated abdominal cramping, fever and weight loss. Although the exact cause of UC is unknown, recent research suggests that the body's natural defenses are operating against proteins in the body which the body thinks are foreign (an "autoimmune reaction"). Perhaps because they resemble bacterial proteins in the gut, these proteins may either instigate or stimulate the inflammatory process that begins to destroy the lining of the colon. As the lining of the colon is destroyed, ulcers form releasing mucus, pus and blood. The disease usually begins in the rectal area and may eventually extend through the entire large bowel. Repeated episodes of inflammation lead to thickening of the wall of the intestine and rectum with scar tissue. Death of colon

tissue or sepsis may occur with severe disease. The symptoms of ulcerative colitis vary in severity and their onset may be gradual or sudden. Attacks may be provoked by many factors, including respiratory infections or stress.

Although there is currently no cure for UC available, treatments are
5 focused on suppressing the abnormal inflammatory process in the colon lining. Treatments including corticosteroids immunosuppressives (eg. azathioprine, mercaptopurine, and methotrexate) and aminosalicicylates are available to treat the disease. However, the long-term use of immunosuppressives such as corticosteroids and azathioprine can result in serious side effects including thinning of bones, cataracts,
10 infection, and liver and bone marrow effects. In the patients in whom current therapies are not successful, surgery is an option. The surgery involves the removal of the entire colon and the rectum.

There are several animal models that can partially mimic chronic ulcerative colitis. The most widely used model is the 2,4,6-trinitrobenesulfonic
15 acid/ethanol (TNBS) induced colitis model, which induces chronic inflammation and ulceration in the colon. When TNBS is introduced into the colon of susceptible mice via intra-rectal instillation, it induces T-cell mediated immune response in the colonic mucosa, in this case leading to a massive mucosal inflammation characterized by the dense infiltration of T-cells and macrophages throughout the entire wall of the large
20 bowel. Moreover, this histopathologic picture is accompanied by the clinical picture of progressive weight loss (wasting), bloody diarrhea, rectal prolapse, and large bowel wall thickening (Neurath et al. *Intern. Rev. Immunol.* 19:51-62, 2000).

Another colitis model uses dextran sulfate sodium (DSS), which induces an acute colitis manifested by bloody diarrhea, weight loss, shortening of the colon and
25 mucosal ulceration with neutrophil infiltration. DSS-induced colitis is characterized histologically by infiltration of inflammatory cells into the lamina propria, with lymphoid hyperplasia, focal crypt damage, and epithelial ulceration. These changes are thought to develop due to a toxic effect of DSS on the epithelium and by phagocytosis of lamina propria cells and production of TNF-alpha and IFN-gamma. Despite its
30 common use, several issues regarding the mechanisms of DSS about the relevance to the human disease remain unresolved. DSS is regarded as a T cell-independent model

because it is observed in T cell-deficient animals such as SCID mice.

The administration of anti-zcytor17lig antibodies or binding partners, soluble zcytor17 comprising polypeptides (including heterodimeric and multimeric receptors), such as zcytor17-Fc4 or other zcytor17 soluble and fusion proteins to these
5 TNBS or DSS models can be used to evaluate the use of zcytor17lig antagonists to ameliorate symptoms and alter the course of gastrointestinal disease. Zcytor17lig may play a role in the inflammatory response in colitis, and the neutralization of zcytor17lig activity by administering zcytor17lig antagonists is a potential therapeutic approach for IBD. Other potential therapeutics include zcytor17 polypeptides, soluble heterodimeric
10 and multimeric receptor polypeptides, or anti-zcytor17lig antibodies or binding partners of the present invention, and the like.

4. Psoriasis

Psoriasis is a chronic skin condition that affects more than seven million Americans. Psoriasis occurs when new skin cells grow abnormally, resulting in
15 inflamed, swollen, and scaly patches of skin where the old skin has not shed quickly enough. Plaque psoriasis, the most common form, is characterized by inflamed patches of skin ("lesions") topped with silvery white scales. Psoriasis may be limited to a few plaques or involve moderate to extensive areas of skin, appearing most commonly on the scalp, knees, elbows and trunk. Although it is highly visible, psoriasis is not a
20 contagious disease. The pathogenesis of the diseases involves chronic inflammation of the affected tissues. Zcytor17 polypeptides, soluble heterodimeric and multimeric receptor polypeptides, or anti-zcytor17lig antibodies or binding partners of the present invention, and the like, could serve as a valuable therapeutic to reduce inflammation and pathological effects in psoriasis, other inflammatory skin diseases, skin and
25 mucosal allergies, and related diseases.

Psoriasis is a T-cell mediated inflammatory disorder of the skin that can cause considerable discomfort. It is a disease for which there is no cure and affects people of all ages. Psoriasis affects approximately two percent of the populations of European and North America. Although individuals with mild psoriasis can often
30 control their disease with topical agents, more than one million patients worldwide require ultraviolet or systemic immunosuppressive therapy. Unfortunately, the

inconvenience and risks of ultraviolet radiation and the toxicities of many therapies limit their long-term use. Moreover, patients usually have recurrence of psoriasis, and in some cases rebound, shortly after stopping immunosuppressive therapy.

Differentiation is a progressive and dynamic process, beginning with pluripotent stem cells and ending with terminally differentiated cells. Pluripotent stem cells that can regenerate without commitment to a lineage express a set of differentiation markers that are lost when commitment to a cell lineage is made. Progenitor cells express a set of differentiation markers that may or may not continue to be expressed as the cells progress down the cell lineage pathway toward maturation. Differentiation markers that are expressed exclusively by mature cells are usually functional properties such as cell products, enzymes to produce cell products, and receptors. The stage of a cell population's differentiation is monitored by identification of markers present in the cell population.

There is evidence to suggest that factors that stimulate specific cell types down a pathway towards terminal differentiation or dedifferentiation affect the entire cell population originating from a common precursor or stem cell.

A multimeric cytokine receptor of the present invention can be useful for stimulating proliferation, activation, differentiation and/or induction or inhibition of specialized cell function of cells of the involved homeostasis of the hematopoiesis and immune function. In particular, multimeric cytokine receptors as described herein are useful for stimulating proliferation, activation, differentiation, induction or inhibition of specialized cell functions of cells of the hematopoietic lineages, including, but not limited to, T cells, B cells, monocytes/macrophages, NK cells, neutrophils, endothelial cells, fibroblasts, eosinophils, chondrocytes, mast cells, langerhan cells, monocytes, and macrophages, as well as epithelial cells. Epithelial cells include, for example, ameloblasts, chief cells, chromatophores, enterochromaffin cells, enterochromaffin-like cells, goblet cells, granulosa cells, keratinocytes, dendritic cells, labyrinth supporting cells, melanocytes, merkel cells, paneth cells, parietal cells, sertoli cells, and the like.

The present invention also provides a method for reducing hematopoietic cells and hematopoietic cell progenitors of a mammal. The method includes culturing bone marrow or peripheral blood cells with a composition

comprising an effective amount of a soluble multimeric cytokine receptor to produce a decrease in the number of lymphoid cells in the bone marrow or peripheral blood cells as compared to bone marrow or peripheral blood cells cultured in the absence of the multimeric cytokine receptor. The hematopoietic cells and hematopoietic cell
5 progenitors can be lymphoid cells, such as monocytic cells, macrophages, or T cells.

The present invention also provides a method of inhibiting an immune response in a mammal exposed to an antigen or pathogen. The method includes (a) determining directly or indirectly the level of antigen or pathogen present in the mammal; (b) administering a composition comprising a soluble multimeric cytokine
10 receptor in an acceptable pharmaceutical vehicle; (c) determining directly or indirectly the level of antigen or pathogen in the mammal; and (d) comparing the level of the antigen or pathogen in step (a) to the antigen or pathogen level in step (c), wherein a change in the level is indicative of inhibiting an immune response. The method may further include (e) re-administering a composition comprising a multimeric cytokine
15 receptor in an acceptable pharmaceutical vehicle; (f) determining directly or indirectly the level of antigen or pathogen in the mammal; and (g) comparing the number of the antigen or pathogen level in step (a) to the antigen level in step (f), wherein a change in the level is indicative of inhibiting an immune response.

Alternatively, the method can include (a) determining a level of an
20 antigen- or pathogen-specific antibody; (b) administering a composition comprising a soluble multimeric cytokine receptor in an acceptable pharmaceutical vehicle; (c) determining a post administration level of antigen- or pathogen-specific antibody; (d) comparing the level of antibody in step (a) to the level of antibody in step (c), wherein a decrease in antibody level is indicative of inhibiting an immune response.

25 Zcytor17lig was isolated from tissue known to have important immunological function and which contain cells that play a role in the immune system. Zcytor17lig is expressed in CD3+ selected, activated peripheral blood cells, and it has been shown that zcytor17lig expression increases after T cell activation. Moreover, results of experiments described in the Examples section herein suggest that a
30 multimeric cytokine receptor of the present invention can have an effect on the growth/expansion of monocytes/macrophages, T-cells, B-cells, NK cells and/or

differentiated state of monocytes/macrophages, T-cells, B-cells, NK cells or these cells' progenitors. Factors that both stimulate proliferation of hematopoietic progenitors and activate mature cells are generally known, however, proliferation and activation can also require additional growth factors. For example, it has been shown that IL-7 and Steel Factor (c-kit ligand) were required for colony formation of NK progenitors. IL-15 plus IL-2 in combination with IL-7 and Steel Factor was more effective (Mrózek et al., *Blood* 87:2632-2640, 1996). However, unidentified cytokines may be necessary for proliferation of specific subsets of NK cells and/or NK progenitors (Robertson et. al., *Blood* 76:2451-2438, 1990). Similarly, zcytor17lig may act alone or in concert or synergy with other cytokines to enhance growth, proliferation expansion and modification of differentiation of monocytes/macrophages, T-cells, B-cells or NK cells.

Assays measuring differentiation include, for example, measuring cell markers associated with stage-specific expression of a tissue, enzymatic activity, functional activity or morphological changes (Watt, *FASEB*, 5:281-284 (1991); Francis, *Differentiation* 57:63-75 (1994); and Raes, *Adv. Anim. Cell Biol. Technol. Bioprocesses*, 161-171 (1989)). Alternatively, zcytor17lig polypeptide itself can serve as an additional cell-surface or secreted marker associated with stage-specific expression of a tissue. As such, direct measurement of zcytor17lig polypeptide, or its loss of expression in a tissue as it differentiates, can serve as a marker for differentiation of tissues.

Similarly, direct measurement of zcytor17lig polypeptide, or its loss of expression in a tissue can be determined in a tissue or in cells as they undergo tumor progression. Increases in invasiveness and motility of cells, or the gain or loss of expression of zcytor17lig in a pre-cancerous or cancerous condition, in comparison to normal tissue, can serve as a diagnostic for transformation, invasion and metastasis in tumor progression. As such, knowledge of a tumor's stage of progression or metastasis will aid the physician in choosing the most proper therapy, or aggressiveness of treatment, for a given individual cancer patient. Methods of measuring gain and loss of expression (of either mRNA or protein) are well known in the art and described herein and can be applied to zcytor17lig expression. For example, appearance or disappearance of polypeptides that regulate cell motility can be used to aid diagnosis

and prognosis of prostate cancer (Banyard, J. and Zetter, B.R., *Cancer and Metast. Rev.* 17:449-458, 1999). As an effector of cell motility, zcytor17lig gain or loss of expression may serve as a diagnostic for lymphoid, B-cell, epithelial, hematopoietic and other cancers.

5 Moreover, the activity and effect of zcytor17lig on tumor progression and metastasis can be measured *in vivo*. Several syngeneic mouse models have been developed to study the influence of polypeptides, compounds or other treatments on tumor progression. In these models, tumor cells passaged in culture are implanted into mice of the same strain as the tumor donor. The cells will develop into tumors having
10 similar characteristics in the recipient mice, and metastasis will also occur in some of the models. Appropriate tumor models for our studies include the Lewis lung carcinoma (ATCC No. CRL-1642) and B16 melanoma (ATCC No. CRL-6323), amongst others. These are both commonly used tumor lines, syngeneic to the C57BL6/J mouse, that are readily cultured and manipulated *in vitro*. Tumors resulting
15 from implantation of either of these cell lines are capable of metastasis to the lung in C57BL6/J mice. The Lewis lung carcinoma model has recently been used in mice to identify an inhibitor of angiogenesis (O'Reilly MS, et al. *Cell* 79: 315-328,1994). C57BL6/J mice are treated with an experimental agent either through daily injection of recombinant protein, agonist or antagonist or a one time injection of recombinant
20 adenovirus. Three days following this treatment, 10^5 to 10^6 cells are implanted under the dorsal skin. Alternatively, the cells themselves may be infected with recombinant adenovirus, such as one expressing zcytor17lig, before implantation so that the protein is synthesized at the tumor site or intracellularly, rather than systemically. The mice normally develop visible tumors within 5 days. The tumors are allowed to grow for a
25 period of up to 3 weeks, during which time they may reach a size of 1500 - 1800 mm³ in the control treated group. Tumor size and body weight are carefully monitored throughout the experiment. At the time of sacrifice, the tumor is removed and weighed along with the lungs and the liver. The lung weight has been shown to correlate well with metastatic tumor burden. As an additional measure, lung surface metastases are
30 counted. The resected tumor, lungs and liver are prepared for histopathological examination, immunohistochemistry, and *in situ* hybridization, using methods known in

the art and described herein. The influence of the expressed polypeptide in question, e.g., zcytor17lig, on the ability of the tumor to recruit vasculature and undergo metastasis can thus be assessed. In addition, aside from using adenovirus, the implanted cells can be transiently transfected with zcytor17lig. Use of stable
5 zcytor17lig transfectants as well as use of induceable promoters to activate zcytor17lig expression *in vivo* are known in the art and can be used in this system to assess zcytor17lig induction of metastasis. Moreover, purified zcytor17lig or zcytor17lig conditioned media can be directly injected in to this mouse model, and hence be used in this system. For general reference see, O'Reilly MS, et al. *Cell* 79:315-328, 1994; and
10 Rusciano D, et al. Murine Models of Liver Metastasis. *Invasion Metastasis* 14:349-361, 1995.

A soluble multimeric cytokine receptor of the present invention or antibodies thereto may be useful in treating tumorigenesis, and therefore would be useful in the treatment of cancer. Zcytor17lig is expressed in activated T-cells,
15 monocytes and macrophages, and is linked to a region of the human chromosome wherein translocations are common in leukemias. Moreover, the zcytor17lig is shown to act through a cytokine receptor, zcytor17 multimeric cytokine receptor, which is also expressed in activated T-cells, monocytes and macrophages. Over stimulation of activated T-cells, monocytes and macrophages by zcytor17lig could result in a human
20 disease state such as an immune cell cancer. As such, identifying zcytor17lig expression, polypeptides (e.g., by anti-zcytor17lig antibodies, zcytor17 soluble multimeric cytokine receptors (e.g., zcytor17 receptor, heterodimers (e.g., zcytor17/OSMRbeta, zcytor17/WSX-1), multimers (e.g., zcytor17/OSMRbeta/WSX-1)), or other zcytor17lig binding partners) can serve as a diagnostic, and can serve as
25 antagonists of zcytor17lig proliferative activity. The ligand could be administered in combination with other agents already in use including both conventional chemotherapeutic agents as well as immune modulators such as interferon alpha. Alpha/beta interferons have been shown to be effective in treating some leukemias and animal disease models, and the growth inhibitory effects of interferon-alpha and
30 zcytor17lig may be additive.

NK cells are thought to play a major role in elimination of metastatic tumor cells and patients with both metastases and solid tumors have decreased levels of NK cell activity (Whiteside et. al., *Curr. Top. Microbiol. Immunol.* 230:221-244, 1998). An agent that stimulates NK cells would be useful in the elimination of tumors.

5 The present invention provides a method of reducing proliferation of a neoplastic monocytes/macrophages comprising administering to a mammal with a monocyte/macrophage neoplasm an amount of a composition including a soluble multimeric cytokine receptor or antibody thereto sufficient to reduce proliferation of the neoplastic monocytes/macrophages.

10 The present invention provides a method for inhibiting activation or differentiation of monocytes/macrophages. Monocytes are incompletely differentiated cells that migrate to various tissues where they mature and become macrophages. Macrophages play a central role in the immune response by presenting antigen to lymphocytes and play a supportive role as accessory cells to lymphocytes by secreting
15 numerous cytokines. Macrophages can internalize extracellular molecules and upon activation have an increased ability to kill intracellular microorganisms and tumor cells. Activated macrophages are also involved in stimulating acute or local inflammation.

 In another aspect, the present invention provides a method of reducing proliferation of a neoplastic B or T-cells comprising administering to a mammal with a
20 B or T cell neoplasm an amount of a composition including a soluble multimeric cytokine receptor sufficient to reducing proliferation of the neoplastic monocytes/macrophages. Furthermore, the zcytor17lig antagonist can be a ligand/toxin fusion protein.

 A zcytor17 multimeric cytokine receptor-saporin fusion toxin may be
25 employed against a similar set of leukemias and lymphomas, extending the range of leukemias that can be treated with a cytokine antagonists. For example, such leukemias can be those that over-express zcytor17 receptors (e.g., zcytor17 receptor, heterodimers (e.g., zcytor17/OSMRbeta, zcytor17/WSX-1), multimers (e.g., zcytor17/OSMRbeta/WSX)). Fusion toxin mediated activation of the zcytor17
30 receptor, zcytor17 receptor heterodimers or multimers (e.g., zcytor17/OSMRbeta, zcytor17/WSX-1 or zcytor17/WSX-1/OSMR) provides two independent means to

inhibit the growth of the target cells, the first being identical to the effects seen by the ligand alone, and the second due to delivery of the toxin through receptor internalization. The lymphoid and monocyte restricted expression pattern of the zcytor17 receptor suggests that the ligand-saporin conjugate can be tolerated by
5 patients.

The tissue distribution of receptors for a given cytokine offers a strong indication of the potential sites of action of that cytokine. Expression of zcytor17 was seen in monocytes and B-cells, with a dramatic increase of expression upon activation for CD3+, CD4+, and CD8+ T-cells. In addition, two monocytic cell lines, THP-1
10 (Tsuchiya et al., *Int. J. Cancer* 26:171-176, 1980) and U937 (Sundstrom et al., *Int. J. Cancer* 17:565-577, 1976), were also positive for zcytor17 expression.

Northern analysis of WSX-1 receptor revealed transcripts in all tissues examined, with increased levels of expression in human spleen, thymus, lymph node, bone marrow, and peripheral blood leukocytes. Also, expression levels of WSX-1
15 increased upon activation of T-cells.

Expression of OSMR is reported to be very broad (Mosley et al, *JBC* 271:32635-32643, 1996). This distribution of zcytor17, WSX-1, and OSMRbeta receptors supports a role for zcytor17lig in immune responses, especially expansion of T-cells upon activation or a role in the monocyte/macrophage arm of the immune
20 system.

Thus, particular embodiments of the present invention are directed toward use of a soluble multimeric cytokine receptor, for instance zcytor17/WSX-1/OSMR, and zcytor17/OSMR heterodimers, as antagonists in inflammatory and immune diseases or conditions such as pancreatitis, type I diabetes (IDDM), pancreatic
25 cancer, pancreatitis, Graves Disease, inflammatory bowel disease (IBD), Crohn's Disease, colon and intestinal cancer, diverticulosis, autoimmune disease, sepsis, organ or bone marrow transplant; inflammation due to trauma, surgery or infection; amyloidosis; splenomegaly; graft versus host disease; and where inhibition of inflammation, immune suppression, reduction of proliferation of hematopoietic,
30 immune, inflammatory or lymphoid cells, macrophages, T-cells (including Th1 and Th2 cells, CD4+ and CD8+ cells), suppression of immune response to a pathogen or

antigen. Moreover the presence of zcytor17 expression in activated immune cells such as activated CD4+ and CD19+ cells showed that zcytor17 receptor may be involved in the body's immune defensive reactions against foreign invaders: such as microorganisms and cell debris, and could play a role in immune responses during
5 inflammation and cancer formation. As such, antibodies and binding partners of the present invention that are agonistic or antagonistic to zcytor17 receptor function, such as a soluble zcytor17 multimeric cytokine receptor, can be used to modify immune response and inflammation.

The zcytor17lig structure and tissue expression suggests a role in early
10 hematopoietic or thymocyte development and immune response regulation or inflammation. These processes involve stimulation of cell proliferation and differentiation in response to the binding of one or more cytokines to their cognate receptors. In view of the tissue distribution observed for this zcytor17lig, agonists (including the natural receptor(s)) and antagonists have enormous potential in both *in*
15 *vitro* and *in vivo* applications. Compounds identified as zcytor17lig agonists are useful for stimulating proliferation and development of target cells *in vitro* and *in vivo*. For example, agonist compounds, zcytor17lig, or anti-zcytor17lig antibodies, are useful as 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
20 culture. Agonists are thus useful in specifically promoting the growth and/or development or activation of monocytes, T-cells, B-cells, and other cells of the lymphoid and myeloid lineages, and hematopoietic cells in culture.

The molecules of the present invention have particular use in the monocyte/macrophage arm of the immune system. Methods are known that can assess
25 such activity. For example, interferon gamma (IFN γ) is a potent activator of mononuclear phagocytes. For example, an increase in expression of zcytor17 upon activation of THP-1 cells (ATCC No. TIB-202) with interferon gamma could suggest that this receptor is involved in monocyte activation. Monocytes are incompletely differentiated cells that migrate to various tissues where they mature and become
30 macrophages. Macrophages play a central role in the immune response by presenting antigen to lymphocytes and play a supportive role as accessory cells to lymphocytes by

secreting numerous cytokines. Macrophages can internalize extracellular molecules and upon activation have an increased ability to kill intracellular microorganisms and tumor cells. Activated macrophages are also involved in stimulating acute or local inflammation. Moreover, monocyte-macrophage function has been shown to be
5 abnormal in a variety of diseased states. For example see, Johnston, RB, *New Eng. J. Med.* 318:747-752, 1998.

One of skill in the art would recognize that agonists of zcytor17 multimeric cytokine receptor, such as zcytor17lig, are useful. For example, depressed migration of monocytes has been reported in populations with a predisposition to
10 infection, such as newborn infants, patients receiving corticosteroid or other immunosuppressive therapy, and patients with diabetes mellitus, burns, or AIDS. Agonists for zcytor17 multimeric cytokine receptor, such as zcytor17lig, could result in an increase in the ability of monocytes to migrate and possibly prevent infection in these populations. There is also a profound defect of phagocytic killing by
15 mononuclear phagocytes from patients with chronic granulomatous disease. This results in the formation of subcutaneous abscesses, as well as abscesses in the liver, lungs, spleen, and lymph nodes. An agonist of zcytor17 multimeric cytokine receptor, such as zcytor17lig, could correct or improve this phagocytic defect. In addition, defective monocyte cytotoxicity has been reported in patients with cancer and Wiskott-
20 Aldrich syndrome (eczema, thrombocytopenia, and recurrent infections). Activation of monocytes by agonists of zcytor17 multimeric cytokine receptor, such as zcytor17lig, could aid in treatment of these conditions. The monocyte-macrophage system is prominently involved in several lipid-storage diseases (sphingolipidoses) such as Gaucher's disease. Resistance to infection can be impaired because of a defect in
25 macrophage function, which could be treated by agonists to zcytor17 multimeric cytokine receptor such as zcytor17lig.

Moreover, one of skill in the art would recognize that antagonists of a zcytor17 multimeric cytokine receptor are useful. For example, in atherosclerotic lesions, one of the first abnormalities is localization of monocyte/macrophages to
30 endothelial cells. These lesions could be prevented by use of antagonists to zcytor17lig. Zcytor17 soluble multimeric cytokine receptors, such as, for instance, heterodimers and

trimers, can also be used as antagonists to the zcytor17lig. Moreover, monoclastic leukemia is associated with a variety of clinical abnormalities that reflect the release of the biologic products of the macrophage, examples include high levels of lysozyme in the serum and urine and high fevers. Moreover, such leukemias exhibit an abnormal
5 increase of monocytic cells. These effects could possibly be prevented by antagonists to zcytor17lig, such as described herein.

Using methods known in the art, and disclosed herein, one of skill could readily assess the activity of a zcytor17 multimeric cytokine receptor in the disease states disclosed herein, inflammation, cancer, or infection as well as other disease states
10 involving monocytic cells. In addition, as zcytor17lig is expressed in a T-cell, macrophage and monocyte-specific manner, and these diseases involve abnormalities in monocytic cells, such as cell proliferation, function, localization, and activation, the polynucleotides, polypeptides, and antibodies of the present invention can be used to as diagnostics to detect such monocytic cell abnormalities, and indicate the presence of
15 disease. Such methods involve taking a biological sample from a patient, such as blood, saliva, or biopsy, and comparing it to a normal control sample. Histological, cytological, flow cytometric, biochemical and other methods can be used to determine the relative levels or localization of zcytor17lig, or cells expressing zcytor17lig, i.e., monocytes, in the patient sample compared to the normal control. A change in the level
20 (increase or decrease) of zcytor17lig expression, or a change in number or localization of monocytes (e.g., increase or infiltration of monocytic cells in tissues where they are not normally present) compared to a control would be indicative of disease. Such diagnostic methods can also include using radiometric, fluorescent, and colorimetric tags attached to polynucleotides, polypeptides or antibodies of the present invention.
25 Such methods are well known in the art and disclosed herein.

Amino acid sequences having zcytor17lig activity can be used to modulate the immune system by binding soluble zcytor17 multimeric cytokine receptor, and thus, preventing the binding of zcytor17lig with endogenous zcytor17lig receptor. Zcytor17lig antagonists, such as a zcytor17 multimeric cytokine receptor, can also be
30 used to modulate the immune system by inhibiting the binding of Zcytor17 ligand with the endogenous zcytor17lig receptor. Accordingly, the present invention includes the

use of a multimeric cytokine receptor that can be also used to treat a subject which produces an excess of either zcytor17lig or Zcytor17 comprising receptor(s). Suitable subjects include mammals, such as humans or veterinary animals.

Zcytor17lig has been shown to be expressed in activated mononuclear
5 cells, and may be involved in regulating inflammation. As such, polypeptides of the present invention can be assayed and used for their ability to modify inflammation, or can be used as a marker for inflammation. Methods to determine proinflammatory and antiinflammatory qualities of zcytor17lig are known in the art and discussed herein. Moreover, it may be involved in up-regulating the production of acute phase reactants,
10 such as serum amyloid A (SAA), α 1-antichymotrypsin, and haptoglobin, and that expression of zcytor17 receptor ligand may be increased upon injection of lipopolysaccharide (LPS) *in vivo* that are involved in inflammatory response (Dumoutier, L. et al., *Proc. Nat'l. Acad. Sci.* 97:10144-10149 (2000)). Production of acute phase proteins, such as SAA, is considered a short-term survival mechanism
15 where inflammation is beneficial; however, maintenance of acute phase proteins for longer periods contributes to chronic inflammation and can be harmful to human health. For review, see Uhlar, CM and Whitehead, AS, *Eur. J. Biochem.* 265:501-523 (1999); and Baumann H. and Gauldie, J. *Immunology Today* 15:74-80 (1994). Moreover, the acute phase protein SAA is implicated in the pathogenesis of several chronic
20 inflammatory diseases, is implicated in atherosclerosis and rheumatoid arthritis, and is the precursor to the amyloid A protein deposited in amyloidosis (Uhlar, CM and Whitehead, *supra.*). Thus, where a ligand such as zcytor17lig that acts as a pro-inflammatory molecule and induces production of SAA, antagonists would be useful in treating inflammatory disease and other diseases associated with acute phase response
25 proteins induced by the ligand. Such antagonists are provided by the present invention. For example, a method of reducing inflammation comprises administering to a mammal with or at risk of developing inflammation an amount of a composition of a soluble multimeric cytokine receptor that is sufficient to reduce inflammation. Moreover, a method of suppressing an inflammatory response in a mammal with inflammation can
30 comprise: (1) determining a level of serum amyloid A protein; (2) administering a composition comprising a soluble multimeric cytokine receptor polypeptide as

described herein in an acceptable pharmaceutical vehicle; (3) determining a post administration level of serum amyloid A protein; (4) comparing the level of serum amyloid A protein in step (1) to the level of serum amyloid A protein in step (3), wherein a lack of increase or a decrease in serum amyloid A protein level is indicative
5 of suppressing an inflammatory response.

Like zcytor17lig, analysis of the tissue distribution of the mRNA corresponding to its zcytor17 receptor cDNA showed that mRNA level was highest in monocytes and prostate cells, and is elevated in activated monocytes, and activated CD4+, activated CD8+, and activated CD3+ cells. Hence, zcytor17 receptor is also
10 implicated in inducing inflammatory and immune response. Thus, particular embodiments of the present invention are directed toward use of zcytor17lig-antibodies, and zcytor17lig, as well as soluble zcytor17 receptor heterodimers as antagonists in inflammatory and immune diseases or conditions such as pancreatitis, type I diabetes (IDDM), pancreatic cancer, pancreatitis, Graves Disease, inflammatory bowel disease
15 (IBD), Crohn's Disease, colon and intestinal cancer, diverticulosis, autoimmune disease, sepsis, organ or bone marrow transplant; inflammation due to trauma, surgery or infection; amyloidosis; splenomegaly; graft versus host disease; and where inhibition of inflammation, immune suppression, reduction of proliferation of hematopoietic, immune, inflammatory or lymphoid cells, macrophages, T-cells (including Th1 and Th2
20 cells, CD4+ and CD8+ cells), suppression of immune response to a pathogen or antigen. Moreover the presence of zcytor17 receptor and zcytor17lig expression in activated immune cells such as activated CD3+, monocytes, CD4+ and CD19+ cells showed that zcytor17 receptor may be involved in the body's immune defensive reactions against foreign invaders: such as microorganisms and cell debris, and could
25 play a role in immune responses during inflammation and cancer formation. As such, zcytor17lig and zcytor17lig-antibodies of the present invention that are agonistic or antagonistic to zcytor17 receptor function, can be used to modify immune response and inflammation.

Moreover, zcytor17lig polypeptides that bind zcytor17 multimeric cytokine
30 receptors and antibodies thereto are useful to:

1) Antagonize or block signaling via a zcytor17 multimeric cytokine

receptor in the treatment of acute inflammation, inflammation as a result of trauma, tissue injury, surgery, sepsis or infection, and chronic inflammatory diseases such as asthma, inflammatory bowel disease (IBD), chronic colitis, splenomegaly, rheumatoid arthritis, recurrent acute inflammatory episodes (e.g., tuberculosis), and treatment of
5 amyloidosis, and atherosclerosis, Castleman's Disease, asthma, and other diseases associated with the induction of acute-phase response.

2) Antagonize or block signaling via the zcytor17 multimeric cytokine receptor in the treatment of autoimmune diseases such as IDDM, multiple sclerosis (MS), systemic Lupus erythematosus (SLE), myasthenia gravis, rheumatoid arthritis,
10 and IBD to prevent or inhibit signaling in immune cells (e.g. lymphocytes, monocytes, leukocytes) via zcytor17 receptor (Hughes C et al., *J. Immunol* 153: 3319-3325 (1994)). Asthma, allergy and other atopic disease may be treated with an MAb against, for example, soluble zcytor17 multimeric cytokine receptors or zcytor17/CRF2-4 heterodimers, to inhibit the immune response or to deplete offending cells. Blocking or
15 inhibiting signaling via zcytor17 multimeric cytokine receptor, using the polypeptides and antibodies of the present invention, may also benefit diseases of the pancreas, kidney, pituitary and neuronal cells. IDDM, NIDDM, pancreatitis, and pancreatic carcinoma may benefit. Zcytor17 multimeric cytokine receptor may serve as a target for MAb therapy of cancer where an antagonizing MAb inhibits cancer growth and
20 targets immune-mediated killing. (Holliger P, and Hoogenboom, *H Nature Biotech.* 16: 1015-1016 (1998)). Mabs to soluble zcytor17 receptor monomers, homodimers, heterodimers and multimers may also be useful to treat nephropathies such as glomerulosclerosis, membranous neuropathy, amyloidosis (which also affects the kidney among other tissues), renal arteriosclerosis, glomerulonephritis of various
25 origins, fibroproliferative diseases of the kidney, as well as kidney dysfunction associated with SLE, IDDM, type II diabetes (NIDDM), renal tumors and other diseases.

3) Agonize or initiate signaling via the zcytor17 multimeric cytokine receptor in the treatment of autoimmune diseases such as IDDM, MS, SLE, myasthenia
30 gravis, rheumatoid arthritis, and IBD. Zcytor17lig may signal lymphocytes or other immune cells to differentiate, alter proliferation, or change production of cytokines or

cell surface proteins that ameliorate autoimmunity. Specifically, modulation of a T-helper cell response to an alternate pattern of cytokine secretion may deviate an autoimmune response to ameliorate disease (Smith JA et al., *J. Immunol.* 160:4841-4849 (1998)). Similarly, zcytor17lig may be used to signal, deplete and deviate
5 immune cells involved in asthma, allergy and atopoid disease. Signaling via zcytor17 multimeric cytokine receptor may also benefit diseases of the pancreas, kidney, pituitary and neuronal cells. IDDM, NIDDM, pancreatitis, and pancreatic carcinoma may benefit. Zcytor17 multimeric cytokine receptor may serve as a target for MAb therapy of pancreatic cancer where a signaling MAb inhibits cancer growth and targets
10 immune-mediated killing (Tutt, AL et al., *J Immunol.* 161: 3175-3185 (1998)). Similarly T-cell specific leukemias, lymphomas, plasma cell dyscrasia (e.g., multiple myeloma), and carcinoma may be treated with monoclonal antibodies (e.g., neutralizing antibody) to zcytor17-comprising soluble receptors of the present invention.

Soluble zcytor17 multimeric cytokine receptors as described herein can
15 be used to neutralize/block zcytor17 receptor ligand activity in the treatment of autoimmune disease, atopic disease, NIDDM, pancreatitis and kidney dysfunction as described above. A soluble form of zcytor17 multimeric cytokine receptor may be used to promote an antibody response mediated by T cells and/or to promote the production of IL-4 or other cytokines by lymphocytes or other immune cells.

20 A soluble zcytor17 multimeric cytokine receptor may be useful as antagonists of zcytor17lig. Such antagonistic effects can be achieved by direct neutralization or binding of its natural ligand. In addition to antagonistic uses, the soluble receptors can bind zcytor17lig and act as carrier or vehicle proteins, in order to transport zcytor17lig to different tissues, organs, and cells within the body. As such,
25 the soluble receptors can be fused or coupled to molecules, polypeptides or chemical moieties that direct the soluble-receptor-ligand complex to a specific site, such as a tissue, specific immune cell, monocytes, or tumor. For example, in acute infection or some cancers, benefit may result from induction of inflammation and local acute phase response proteins. Thus, the soluble receptors described herein or antibodies thereto
30 can be used to specifically direct the action of a pro-inflammatory zcytor17lig ligand. See, Cosman, D. *Cytokine* 5: 95-106 (1993); and Fernandez-Botran, R. *Exp. Opin.*

Invest. Drugs 9:497-513 (2000).

Moreover, the soluble zcytor17 multimeric cytokine receptors can be used to stabilize the zcytor17lig, to increase the bioavailability, therapeutic longevity, and/or efficacy of the ligand by stabilizing the ligand from degradation or clearance, or by targeting the ligand to a site of action within the body. For example, the naturally occurring IL-6/soluble IL-6R complex stabilizes IL-6 and can signal through the gp130 receptor. See, Cosman, D. *supra.*, and Fernandez-Botran, R. *supra.* Moreover, Zcytor17 may be combined with a cognate ligand such as its ligand to comprise a ligand/soluble receptor complex. Such complexes may be used to stimulate responses from cells presenting a companion receptor subunit. The cell specificity of zcytor17 multimeric cytokine receptor/zcytor17lig complexes may differ from that seen for the ligand administered alone. Furthermore the complexes may have distinct pharmacokinetic properties such as affecting half-life, dose/response and organ or tissue specificity. Zcytor17 multimeric cytokine receptor/ligand complexes thus may have agonist activity to enhance an immune response or stimulate mesangial cells or to stimulate hepatic cells. Alternatively, only tissues expressing a signaling subunit the heterodimerizes with the complex may be affected analogous to the response to IL6/IL6R complexes (Hirota H. et al., *Proc. Nat'l. Acad. Sci.* 92:4862-4866 (1995); and Hirano, T. in Thomason, A. (Ed.) "The Cytokine Handbook", 3rd Ed., p. 208-209). Soluble receptor/cytokine complexes for IL12 and CNTF display similar activities.

Zcytor17lig may also be used within diagnostic systems for the detection of circulating levels of ligand, and in the detection of acute phase inflammatory response. Within a related embodiment, antibodies or other agents that specifically bind to zcytor17lig can be used to detect circulating zcytor17lig polypeptides; conversely, zcytor17lig itself can be used to detect circulating or locally-acting receptor polypeptides. Elevated or depressed levels of ligand or receptor polypeptides may be indicative of pathological conditions, including inflammation or cancer. Moreover, detection of acute phase proteins or molecules such as zcytor17lig can be indicative of a chronic inflammatory condition in certain disease states (e.g., rheumatoid arthritis). Detection of such conditions serves to aid in disease diagnosis as well as help a physician in choosing proper therapy.

Polynucleotides encoding a zcytor17 multimeric cytokine receptor are useful within gene therapy applications where it is desired to increase or inhibit zcytor17lig activity. If a mammal has a mutated or absent zcytor17 gene, the zcytor17 gene of the present invention can be introduced into the cells of the mammal. In one
5 embodiment, a gene encoding a zcytor17 multimeric cytokine receptor 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
10 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.* 2:320-30 (1991)); an attenuated adenovirus vector, such as the vector described by
15 Stratford-Perricaudet et al., *J. Clin. Invest.* 90:626-30 (1992); and a defective adeno-associated virus vector (Samulski et al., *J. Virol.* 61:3096-101 (1987); and Samulski et al., *J. Virol.* 63:3822-8 (1989)).

A zcytor17 gene of the present invention can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann
20 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.
25 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* 84:7413-7 (1987); Mackey et al., *Proc. Natl. Acad. Sci. USA* 85:8027-31 (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
30 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 immune system, 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
5 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,
10 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.* 267:963-7 (1992); and Wu et al., *J. Biol. Chem.* 263:14621-4 (1988).

Antisense methodology can be used to inhibit zcytor17 multimeric cytokine receptor gene transcription, such as to inhibit cell proliferation *in vivo*.
15 Polynucleotides that are complementary to a segment of a zcytor17-encoding polynucleotide (e.g., a polynucleotide as set forth in SEQ ID NO:110, SEQ ID NO:108, or SEQ ID NO:4) are designed to bind to zcytor17lig-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of zcytor17lig polypeptide-encoding genes in cell culture or in a subject.

20 Mice engineered to express the zcytor17lig gene, referred to as "transgenic mice," and mice that exhibit a complete absence of zcytor17lig gene function, referred to as "knockout mice," may also be generated (Snouwaert et al., *Science* 257:1083 (1992); Lowell et al., *Nature* 366:740-42 (1993); Capecchi, M.R., *Science* 244: 1288-1292 (1989); Palmiter, R.D. et al. *Annu Rev Genet.* 20: 465-499
25 (1986)). For example, transgenic mice that over-express zcytor17lig, either ubiquitously or under a tissue-specific or tissue-restricted promoter can be used to ask whether over-expression causes a phenotype. For example, over-expression of a wild-type zcytor17lig polypeptide, polypeptide fragment or a mutant thereof may alter normal cellular processes, resulting in a phenotype that identifies a tissue in which
30 zcytor17lig expression is functionally relevant and may indicate a therapeutic target for the zcytor17lig, its agonists or antagonists. For example, a preferred transgenic mouse

to engineer is one that over-expresses the zcytor17lig (amino acid residues 23-164 of SEQ ID NO:2; or 24-163 of SEQ ID NO:11). Moreover, such over-expression may result in a phenotype that shows similarity with human diseases. Similarly, knockout zcytor17lig mice can be used to determine where zcytor17lig is absolutely required *in vivo*. The phenotype of knockout mice is predictive of the *in vivo* effects of that a zcytor17lig antagonist, such as a soluble zcytor17 multimeric cytokine receptor, may have. The human or mouse zcytor17lig cDNA described herein can be used to generate knockout mice. These mice may be employed to study the zcytor17lig gene and the protein encoded thereby in an *in vivo* system, and can be used as *in vivo* models for corresponding human diseases. Moreover, transgenic mice expression of zcytor17lig antisense polynucleotides or ribozymes directed against zcytor17lig, described herein, can be used analogously to transgenic mice described above. Studies may be carried out by administration of purified zcytor17lig protein, as well.

The present invention also provides a composition which includes an effective amount of a soluble multimeric cytokine receptor comprising a polypeptide comprising amino acid residue 20 to amino acid residue 543 of SEQ ID NO:111 and at least a portion of at least one class I cytokine receptor; and a pharmaceutically acceptable vehicle. The polypeptide may be comprised of various fragment or portions of the extracellular domain of SEQ ID NO:111, SEQ ID NO:109, and/or SEQ ID NO:5, such as for instance, amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and amino acid residue 20 to amino acid residue 519 of SEQ ID NO:111. The at least a portion of at least one class I cytokine receptor can include, for example, a portion of SEQ ID NO:9 and/or a portion of SEQ ID NO:7, such as, for instance, amino acid residue 28 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 35 to amino acid residue 137 of SEQ ID NO:7, amino acid residue 240 to amino acid residue 342 of SEQ ID NO:7, amino acid residue 348 to amino acid residue 429 of SEQ ID NO:7, amino acid residue 28 to amino acid residue 739 of SEQ ID NO:7, and/or combinations thereof. The multimeric cytokine receptor may further include an affinity tag as described herein.

The present invention also provides an immune cell inhibiting composition which includes an effective amount of a soluble multimeric cytokine

receptor comprising a polypeptide comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and at least a portion of at least one class I cytokine receptor; and a pharmaceutically acceptable vehicle, wherein the soluble multimeric cytokine receptor inhibits the proliferation of immune cells.

5 The present invention also provides an inflammatory cell inhibiting composition which includes an effective amount of a soluble multimeric cytokine receptor comprising a polypeptide comprising amino acid residue 20 to amino acid residue 227 of SEQ ID NO:111 and at least a portion of at least one class I cytokine receptor; and a pharmaceutically acceptable vehicle, wherein the soluble multimeric
10 cytokine receptor inhibits the proliferation of inflammatory cells.

Experimental evidence suggests a role for zcytor17lig in the progression of diseases that involve the skin or epithelium of internal surfaces, such as, for instance, large intestine, small intestine, pancreas, lung, prostate, uterus, and the like. First, as disclosed herein, zcytor17 receptors, including both OSM receptor beta and zcytor17,
15 are expressed in several cell types located in epithelial surfaces including cell lines derived from lung epithelium, lung fibroblast, prostate, colon, breast, liver epithelium, bone and skin epithelium, bone fibroblast, and the like. Moreover, as disclosed herein, examples from each of these cell types also responded to zcytor17lig activation of a STAT reporter construct. In addition, several cell lines responded to zcytor17lig
20 stimulation by producing increased levels of IL-6, IL-8, MCP-1 (a chemotactic factor) as described herein. In whole, these data suggest a role for zcytor17lig in diseases that involve the epithelium such as, for instance, atopic dermatitis; dermatitis; psoriasis; psoriatic arthritis; eczema; gingivitis; periodontal disease; inflammatory bowel diseases (IBD) (e.g., ulcerative colitis, Crohn's disease); reproductive disorders, such as, for
25 instance, cervical dysplasia, cervical cancer; other skin diseases like cancers: sarcomas; carcinomas; melanoma, etc. i.e, not just inflammatory diseases, since immune system is involved in activating/curing cancers; diseases involving barrier dysfunction, such as, for instance, graft-versus-host disease (GVHD) and irritable bowel syndrome (IBS); and diseases that involve lung epithelium, such as asthma, emphysema, and the like. In
30 addition, the release of cytokines IL-6, IL-8, and MCP-1 by cells exposed to zcytor17lig suggests that zcytor17lig is involved in inflammation. Therefore, regulation of

zcytor17lig can be useful in the treatment of autoimmune, inflammatory, or cancerous diseases associated with the tissues that express receptor. These diseases include, for example, prostatitis, hepatitis, osteoarthritis, and the like. Zcytor17lig may positively or negatively directly or indirectly regulate these diseases. Therefore, the administration
5 of zcytor17lig can be used to treat diseases as described herein directly or with molecules that inhibit zcytor17lig activity including, for example, both monoclonal antibodies to zcytor17lig or monoclonal antibodies to zcytor17, or monoclonal antibodies that recognize the zcytor17 and OSM receptor beta complex.

Data also suggests that zcytor17lig may be involved in the regulation of
10 TH2 T cell mediated diseases. First, zcytor17lig is made by the TH2 subset of activated T cells. TH2 cells express more zcytor17lig as compared to TH1 cells. In addition, at least two lung epithelial cell lines (SK-LU-1, A549) were stimulated to increase IL13 receptor alpha-2 mRNA in response to zcyto17 ligand stimulation as described herein. There is an association of IL-13 receptor alpha2 chain and tumorigenicity of human
15 breast and pancreatic tumors. This suggests that zcytor17lig may play a role in regulating tumorigenicity of these types of cancers, as well as other cancers. Therefore, the administration of a zcytor17lig antagonist or direct use of zcytor17lig may be useful in treatment of these types of cancers, benign or malignant and at various grades (grades I-IV) and stages (e.g., TNM or AJC staging methods) of tumor development, in
20 mammals, preferably humans.

It is well-known in the art that IL13 is involved in the generation of activated TH2 cells and in TH2 mediated diseases, such as asthma, atopic dermatitis, and the like. Zcytor17lig or zcytor17lig antagonists may be useful in the treatment of diseases that involved TH2 T cells. This would include diseases such as, for instance,
25 atopic dermatitis, asthma, as well as other diseases that are exacerbated by activated TH2 cells. The involvement of zcytor17lig in diseases, such as, for instance, atopic dermatitis, is also supported by the phenotype of the transgenic mice that overexpress zcytor17lig and develop symptoms of atopic dermatitis as described herein.

Despite the preferential expression of zcytor17lig by TH2 cells, there is
30 still some expression of zcytor17lig in TH1 cells and in CD8+ T cells. Therefore, zcytor17lig or its antagonists may be useful in treating diseases that involve immune

modulation of activated T cells including, for example, viral infection, cancers, graft rejection, and the like.

Zcytor17lig may also be involved in the development of cancer. There is expression of the zcytor17 and OSM receptor beta receptors in human bone fibroblast
5 osteosarcomas, human skin fibroblast melanoma, colon epithelial carcinoma, adenocarcinoma, breast epithelial adenocarcinoma, prostate epithelial adenocarcinoma, and lung epithelial adenocarcinoma and carcinoma. Therefore, it may be useful to treat tumors of epithelial origin with either zcytor17lig, fragments thereof, or zcytor17lig antagonists which include, but are not limited to, carcinoma, adenocarcinoma, and
10 melanoma. Notwithstanding, zcytor17lig or a zcytor17lig antagonist may be used to treat a cancer, or reduce one or more symptoms of a cancer, from a cancer including but not limited to, squamous cell or epidermoid carcinoma, basal cell carcinoma, adenocarcinoma, papillary carcinoma, cystadenocarcinoma, bronchogenic carcinoma, bronchial adenoma, melanoma, renal cell carcinoma, hepatocellular carcinoma,
15 transitional cell carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, malignant mixed tumor of salivary gland origin, Wilms' tumor, immature teratoma, teratocarcinoma, and other tumors comprising at least some cells of epithelial origin.

Generally, the dosage of administered zcytor17lig polypeptide (or Zcytor16 analog or fusion protein) will vary depending upon such factors as the
20 patient's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of zcytor17lig polypeptide which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of patient), although a lower or higher dosage also may be administered as circumstances dictate. One skilled in the art can readily determine such
25 dosages, and adjustments thereto, using methods known in the art.

Administration of a zcytor17 multimeric receptor agonist or antagonist to a subject can be topical, inhalant, intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins
30 by injection, the administration may be by continuous infusion or by single or multiple boluses.

Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. Oral delivery is suitable for polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase and Morrel, "Oral Delivery of
5 Microencapsulated Proteins," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 255-288 (Plenum Press 1997)). The feasibility of an intranasal delivery is exemplified by such a mode of insulin administration (see, for example, Hinchcliffe and Illum, *Adv. Drug Deliv. Rev.* 35:199 (1999)). Dry or liquid particles comprising Zcytor17 multimeric receptor agonist or antagonist can be prepared and
10 inhaled with the aid of dry-powder dispersers, liquid aerosol generators, or nebulizers (e.g., Pettit and Gombotz, *TIBTECH* 16:343 (1998); Patton *et al.*, *Adv. Drug Deliv. Rev.* 35:235 (1999)). This approach is illustrated by the AERX diabetes management system, which is a hand-held electronic inhaler that delivers aerosolized insulin into the lungs. Studies have shown that proteins as large as 48,000 kDa have been delivered
15 across skin at therapeutic concentrations with the aid of low-frequency ultrasound, which illustrates the feasibility of transcutaneous administration (Mitrugotri *et al.*, *Science* 269:850 (1995)). Transdermal delivery using electroporation provides another means to administer a molecule having Zcytor17 multimeric receptor binding activity (Potts *et al.*, *Pharm. Biotechnol.* 10:213 (1997)).

20 A pharmaceutical composition comprising a protein, polypeptide, or peptide having Zcytor17 multimeric receptor binding activity can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable vehicle. A composition is said to be in a "pharmaceutically acceptable vehicle" if its
25 administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable vehicle. Other suitable vehicles are well-known to those in the art. See, for example, Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995).

For purposes of therapy, molecules having Zcytor17 multimeric receptor
30 binding activity and a pharmaceutically acceptable vehicle are administered to a patient in a therapeutically effective amount. A combination of a protein, polypeptide, or

peptide having Zcytor17 multimeric receptor binding activity and a pharmaceutically acceptable vehicle is said to be administered in a "therapeutically effective amount" or "effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates at least a portion of the inflammatory response.

A pharmaceutical composition comprising Zcytor17lig (or Zcytor17lig analog or fusion protein) can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions, aerosols, droplets, topological solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer *et al.*, *Pharm. Biotechnol.* 10:239 (1997); Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 95-123 (CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 239-254 (Plenum Press 1997); Yewey *et al.*, "Delivery of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 93-117 (Plenum Press 1997)). Other solid forms include creams, pastes, other topological applications, and the like.

Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg *et al.*, *Eur. J. Clin. Microbiol. Infect. Dis.* 12 (Suppl. 1):S61 (1993), Kim, *Drugs* 46:618 (1993), and Ranade, "Site-Specific Drug Delivery Using Liposomes as Carriers," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 3-24 (CRC Press 1995)). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging

from 0.02 μm to greater than 10 μm . A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s) (see, for example, Machy *et al.*, *Liposomes In Cell Biology And Pharmacology* (John Libbey 1987), and Ostro *et al.*, *American J. Hosp. Pharm.* 46:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof *et al.*, *Ann. N.Y. Acad. Sci.* 446:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0 μm) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0 μm are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen *et al.*, *Biochim. Biophys. Acta* 802:428 (1984)). In addition, incorporation of glycolipid- or polyethelene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen *et al.*, *Biochim. Biophys. Acta* 1068:133 (1991); Allen *et al.*, *Biochim. Biophys. Acta* 1150:9 (1993)).

Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used to target the liver (Hayakawa *et al.*, Japanese Patent 04-244,018; Kato *et al.*, *Biol. Pharm. Bull.* 16:960 (1993)). These formulations were prepared by mixing soybean phosphatidylcholine, α -tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under

vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu *et al.*, *Biol. Pharm. Bull.* 20:881 (1997)).

5 Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. For example, liposomes can be modified with branched type galactosyllipid derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato and Sugiyama, *Crit. Rev. Ther.*
10 *Drug Carrier Syst.* 14:287 (1997); Murahashi *et al.*, *Biol. Pharm. Bull.* 20:259 (1997)). Similarly, Wu *et al.*, *Hepatology* 27:772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be
15 inhibited by preinjection of asialofetuin (Murahashi *et al.*, *Biol. Pharm. Bull.* 20:259 (1997)). Polyacetylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps *et al.*, *Proc. Nat'l Acad. Sci. USA* 94:11681 (1997)). Moreover, Geho, *et al.* U.S. Patent No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for
20 hepatobiliary receptors associated with the specialized metabolic cells of the liver.

 In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym *et al.*, *Adv. Drug Deliv. Rev.* 32:99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting
25 antibodies are directly attached to liposomes (Harasym *et al.*, *Adv. Drug Deliv. Rev.* 32:99 (1998)).

 Polypeptides having Zcytor17 multimeric receptor binding activity can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson *et al.*, *Infect. Immun.* 31:1099 (1981),
30 Anderson *et al.*, *Cancer Res.* 50:1853 (1990), and Cohen *et al.*, *Biochim. Biophys. Acta* 1063:95 (1991), Alving *et al.* "Preparation and Use of Liposomes in Immunological

Studies,” in *Liposome Technology*, 2nd Edition, Vol. III, Gregoriadis (ed.), page 317 (CRC Press 1993), Wassef *et al.*, *Meth. Enzymol.* 149:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen *et al.*,
5 *Biochim. Biophys. Acta* 1150:9 (1993)).

Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the
10 polymer (Gombotz and Pettit, *Bioconjugate Chem.* 6:332 (1995); Ranade, “Role of Polymers in Drug Delivery,” in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 51-93 (CRC Press 1995); Roskos and Maskiewicz, “Degradable Controlled Release Systems Useful for Protein Delivery,” in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 45-92 (Plenum Press 1997); Bartus *et al.*, *Science*
15 *281*:1161 (1998); Putney and Burke, *Nature Biotechnology* 16:153 (1998); Putney, *Curr. Opin. Chem. Biol.* 2:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can also provide vehicles for intravenous administration of therapeutic proteins (see, for example, Gref *et al.*, *Pharm. Biotechnol.* 10:167 (1997)).

The present invention also contemplates chemically modified
20 polypeptides having binding Zcytor17 multimeric receptor activity such as zcytor17 multimeric receptor heterodimeric or multimeric soluble receptors, and Zcytor17 multimeric receptor antagonists, for example anti-zcytor17 multimeric receptor antibodies or binding polypeptides, which a polypeptide is linked with a polymer, as discussed above.

25 Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 5th Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

30 As an illustration, pharmaceutical compositions may be supplied as a kit comprising a container that comprises a polypeptide with a Zcytor17 multimeric

receptor extracellular domain, e.g., zcytor17 multimeric receptor heterodimeric or multimeric soluble receptors, or a Zcytor17 multimeric receptor antagonist (e.g., a neutralizing antibody or antibody fragment that binds a Zcytor17 multimeric receptor polypeptide). Therapeutic polypeptides can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic polypeptide. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition. Moreover, such information may include a statement that the Zcytor17 multimeric receptor composition is contraindicated in patients with known hypersensitivity to Zcytor17 multimeric receptor.

The foregoing detailed description and examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom. The invention is not limited to the exact details shown and described, for variations obvious to one skilled in the art will be included within the invention defined by the claims.

EXAMPLES

Example 1

Construction of MPL-zcytor17 Polypeptide Chimera: MPL Extracellular and TM Domain Fused to the zcytor17 Intracellular Signaling Domain

The 5' extracellular domain of the murine MPL receptor was isolated from a plasmid containing the murine MPL receptor (PHZ1/MPL plasmid) by digestion with EcoRI and BamHI generating a 1164 bp fragment. The digestion was run on a 1% agarose gel and the fragment was isolated using the Qiaquick gel extraction kit (Qiagen) as per manufacturer's instructions. The rest of the MPL extracellular domain and transmembrane domain were generated using PCR with primers ZC6,673 (SEQ ID

NO:13) and ZC29,082 (SEQ ID NO:14). The reaction conditions were as follows: 15 cycles at 94°C for 1 min., 55°C for 1 min., 72°C for 2 min.; followed by 72°C for 7 min.; then a 4°C soak. The PCR product was run on a 1% agarose gel and the approximately 400bp MPL receptor fragment was isolated using Qiaquick™ gel
5 extraction kit (Qiagen) as per manufacturer's instructions.

The intracellular domain of human zcytor17 was isolated from a plasmid containing zcytor17 receptor cDNA (#23/pCAP) using PCR with primers ZC29,083 (SEQ ID NO:15) and ZC29,145 (SEQ ID NO:16). The polynucleotide sequence that corresponds to the zcytor17 receptor coding sequence is shown in SEQ ID NO:5. The
10 reaction conditions were as per above. The PCR product was run on a 1% agarose gel and the approximately 320 bp zcytor17 fragment isolated using Qiaquick gel extraction kit as per manufacturer's instructions.

Each of the isolated PCR fragments described above were mixed at a 1:1 volumetric ratio and used in a PCR reaction using ZC6673 (SEQ ID NO:13) and
15 ZC29145 (SEQ ID NO:16) to create all but the 5' MPL portion of the MPL-zcytor17 chimera. The reaction conditions were as follows: 15 cycles at 94°C for 1 min., 55°C for 1 min., 72°C for 2 min.; followed by 72°C for 7 min.; then a 4°C soak. The entire PCR product was run on a 1% agarose gel and the approximately 700bp MPL-zcytor17 chimera fragment isolated using Qiaquick gel extraction kit (Qiagen) as per
20 manufacturer's instructions. The MPL-zcytor17 chimera fragment was digested with BamHI (BRL) and XbaI (Boehringer Mannheim) as per manufacturer's instructions. The entire digest was run on a 1% agarose gel and the cleaved MPL-zcytor17 chimera isolated using Qiaquick™ gel extraction kit (Qiagen) as per manufacturer's instructions. The resultant cleaved MPL-zvytor17 chimera plus 5' MPL EcoRI/BamHI
25 fragment described above were inserted into an expression vector to generate the full MPL-zcytor17 chimeric receptor as described below.

Recipient expression vector pZP-7 was digested with EcoRI (BRL) and XbaI (BRL) as per manufacturer's instructions, and gel purified as described above. This vector fragment was combined with the EcoRI and XbaI cleaved MPL-zcytor17
30 PCR chimera isolated above and the EcoRI and BamHI 5' MPL fragment isolated above in a ligation reaction. The ligation was run using T4 Ligase (Epicentre

Technologies), at room temperature for 1 hour as per manufacturer's instructions. A sample of the ligation was electroporated into DH10B ElectroMAX™ electrocompetent *E. coli* cells (25μF, 200Ω, 1.8V). Transformants were plated on LB+Ampicillin plates and single colonies screened by miniprep (Qiagen) and digestion with EcoRI to check
5 for the MPL-zcytor17 chimera. EcoRI digestion of correct clones yield about a 2kb fragment. Confirmation of the MPL-zcytor17 chimera sequence was made by sequence analyses. The insert was approximately 3.1 kb, and was full-length.

Example 2

MPL-zcytor17 Chimera Based Proliferation in BAF3 Assay Using Alamar Blue

A. Construction of BaF3 Cells Expressing MPL-zcytor17 chimera

BaF3, an interleukin-3 (IL-3) dependent pre-lymphoid cell line derived from murine bone marrow (Palacios and Steinmetz, *Cell* 41: 727-734, 1985; Mathey-Prevot et al., *Mol. Cell. Biol.* 6: 4133-4135, 1986), was maintained in complete media
15 (RPMI medium (JRH Bioscience Inc., Lenexa, KS) supplemented with 10% heat-inactivated fetal calf serum, 1-2 ng/ml murine IL-3 (mIL-3) (R & D, Minneapolis, MN), 2mM L-glutaMax-1™ (Gibco BRL), 1 mM Sodium Pyruvate (Gibco BRL), and PSN antibiotics (GIBCO BRL)). Prior to electroporation, pZP-7/MPL-zcytor17 plasmid DNA was prepared and purified using a Qiagen Maxi Prep kit (Qiagen) as per
20 manufacturer's instructions. BaF3 cells for electroporation were washed twice in RPMI media and then resuspended in RPMI media at a cell density of 10⁷ cells/ml. One ml of resuspended BaF3 cells was mixed with 30 μg of the pZP-7/MPL-zcytor17 plasmid DNA and transferred to separate disposable electroporation chambers (GIBCO BRL). At room temperature cells were given 5x.1msec shocks at 800 volts followed by 5x2ms
25 shocks at 600 volts delivered by an electroporation apparatus (Cyto-Pulse). Alternatively, cells were electroporated with two serial pulses (800 μFAD/300 V; followed by 1180 μFAD/300 V) delivered by a Cell-Porator (GibcoBRL) electroporation apparatus. The electroporated cells were transferred to 50 ml of complete media and placed in an incubator for 15-24 hours (37°C, 5% CO₂). Then
30 Geneticin™ (Gibco) selection (1mg/ml G418) was added to the cells in a T-162 flask to isolate the G418-resistant pool. Pools of the transfected BaF3 cells, hereinafter called

BaF3/MPL-zcytor17 cells, were assayed for signaling capability as described below.

B. Testing the signaling capability of the BaF3/MPL-zcytor17 cells using an Alamar Blue Proliferation Assay

5 BaF3/MPL-zcytor17 cells were spun down and washed in the complete media, described above, but without mIL-3 (hereinafter referred to as “mIL-3 free media”). The cells were spun and washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 µl per well using the mIL-3 free media.

10 Proliferation of the BaF3/MPL-zcytor17 cells was assessed using murine thrombopoietin (mTPO) diluted with mIL-3 free media to 200 ng/ml, 100ng/ml, 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.1ng/ml, 1.5 ng/ml concentrations. One hundred microliters of the diluted mTPO was added to the BaF3/MPL-zcytor17 cells. The total assay volume was 200 µl. Negative controls were run in parallel using mIL-3
15 free media only, without the addition of mTPO. The assay plates were incubated at 37°C, 5% CO₂ for 3 days at which time Alamar Blue (Accumed, Chicago, IL) was added at 20µl/well. Alamar Blue gives a fluorometric readout based on the metabolic activity of cells, and is thus a direct measurement of cell proliferation in comparison to a negative control. Plates were again incubated at 37°C, 5% CO₂ for 24 hours. Plates
20 were read on the Fmax™ plate reader (Molecular Devices Sunnyvale, CA) using the SoftMax™ Pro program, at wavelengths 544 (Excitation) and 590 (Emission), or a Wallac Victor 2 plate reader (PerkinElmer Life Sciences, Boston, MA).

Results confirmed the signaling capability of the intracellular portion of the zcytor17 receptor, as the thrombopoietin induced proliferation at approximately 9-
25 13 fold over background at mTPO concentrations of 50ng/ml and greater.

Example 3

Construction of Expression Vector Expressing Full-length zcytor17: pZp7pX/zcytor17

A. Cloning of full length zcytor17 cDNA for expression:

30 To obtain a full-length zcytor17 cDNA, 5' and 3' PCR products were isolated and joined using an internal PstI site. The PCR primers were designed using

the nucleotide sequence SEQ ID NO:4 and include BamHI and Xho I restriction sites for cloning purposes.

A 5' PCR product was generated using a WI-38 cDNA library as a template and oligonucleotides ZC29,359 (SEQ ID NO:18) and ZC27,899 (SEQ ID NO:19) as primers. WI-38 is an in-house cDNA library generated from a human embryonic lung cell line (ATCC CRL-2221). This 5' PCR reaction was run as follows: 30 cycles at 94°C for 1 minute, 65°C for 1 minute, 72°C for 2 minutes, then 72°C for 7 minutes; 10°C soak. The PCR reaction used approximately 3 µg of plasmid prepared from the cDNA library, 20 pmoles of each oligonucleotide, and five units of PWO DNA polymerase (Roche). About 90% of the 5' PCR product was ethanol precipitated, digested with BamHI and PstI and gel purified on a 1.0% agarose gel. The approximately 600 bp band was excised and used for ligation to the cloning vector pUC18 digested with BamHI and PstI. The resulting transformants were sequenced to confirm the zcytor17 cDNA sequence. For one of these transformants, plasmid DNA was prepared and digested with BamHI and PstI. The resulting approximately 600 bp band was gel purified and used for a ligation below to form a full-length cDNA.

A 3' PCR product was generated using a human testes in-house cDNA library as a template and oligonucleotides ZC27,895 (SEQ ID NO:20) and ZC29,122 (SEQ ID NO:21) as primers. This 3' PCR reaction was run as follows: 30 cycles at 94°C for 45 seconds, 65°C for 45 seconds, 72°C for 2 minutes, then 72°C for 7 minutes; 10°C soak. The entire 3' PCR reaction was gel purified on a 1.0% agarose gel and the major 1500 bp band excised. This band was cloned into the PCR Blunt II TOPO vector using the ZeroBlunt TOPO kit (Invitrogen). The resulting transformants were sequenced to confirm the zcytor17 cDNA sequence. For one of these transformants, plasmid DNA was prepared and digested with PstI and XhoI. The resulting approximately 1500 bp band was gel purified. A three-part ligation was performed with the 5' BamHI to Pst I fragment above, the 3' PstI to XhoI fragment, and the expression vector pZp7pX digested with BamHI and XhoI. This generated a pZp7pX plasmid containing a full-length cDNA for zcytor17 (SEQ ID NO:4), designated pZp7p/zcytor17. The full length zcytor17 cDNA in pZp7p/zcytor17 has a silent mutation that changes the T to G at position 1888 of SEQ ID NO:4 (encoding a Gly

residue at residue 464 of SEQ ID NO:5). As this mutation is silent, the zcytor17 cDNA in pZp7p/zcytor17 encodes the polypeptide as shown in SEQ ID NO:5. Plasmid pZp7pX is a mammalian expression vector containing an expression cassette having the CMV promoter, intron A, multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a puromycin resistance gene and the SV40 terminator.

10 *B. Construction of Expression Vector Expressing Full-Length WSX-1*

The entire WSX-1 receptor (SEQ ID NO:9) was isolated from a plasmid containing the WSX-1 receptor cDNA (SEQ ID NO:8) (United States Patent No. 5,925,735). hWSX-1/pBluescript SK(+) plasmid DNA (Stratagene, La Jolla, CA) was digested with EcoRI and XhoI to generate a 1075 bp fragment, and also digested with XhoI and XbaI to generate a 900 bp fragment. Both digests were run on a 1% agarose gel and the cleaved WSX-1 fragments isolated.

Recipient expression vector pZp7Z was digested with EcoRI and XbaI and gel purified as described above. This vector fragment was combined with the two cleaved zcytor17 fragments isolated above in a ligation reaction using T4 Ligase (BRL). The ligation was incubated at room temperature overnight. A sample of the ligation was electroporated in to DH10B electroMAX™ electrocompetent *E. coli* cells (25μF, 200Ω, 2.3V). Six colonies were grown in culture and miniprep DNA was prepared and digested to confirm the correct WSX-1 full-length insert of 2.0 kb. The resulting plasmid is pZPZ7Z/WSX-1.

25

Example 4

Zcytor17 based proliferation in BAF3 assay using Alamar Blue

A. Construction of BaF3 Cells Expressing zcytor17 receptor, WSX-1 receptor and OSMR

30 BaF3 cells expressing the full-length zcytor17 receptor were constructed as per Example 2A above, using 30μg of the zcytor17 expression vector, described in

Example 3A. One exception is that in place of Geneticin selection, 2 μ g/ml of Puromycin (ClonTech) was added to the transfected cells in a T-162 flask to isolate the puromycin-resistant pool. The BaF3 cells expressing the zcytor17 receptor mRNA were designated as BaF3/zcytor17. To obtain clones, Baf3/zcytor17 cells were counted
5 in a hemocytometer and plated at 1 cell/well, 0.5 cell/well, 0.1 cell/well, and 0.01 cell/well in 96-well dishes. Fifteen clones were scaled up to T75 flasks, and five clones were assayed for zcytor17 expression. Total RNA was isolated from cell pellets using a S.N.A.P.™ total RNA Isolation Kit (InVitrogen). First-strand cDNA was synthesized using the proSTAR™ First Strand RT-PCR kit, and then PCR with zcytor17 specific
10 primers ZC29,180 (SEQ ID NO:22) and ZC29,122 (SEQ ID NO:23) was performed to screen the clones for expression of zcytor17. One clone, BaF3/zcytor17#15 was chosen to expand and transfect with the WSX-1 expression vector.

BaF3 cells expressing zcytor17 and full-length WSX-1 were constructed as per Example 2A above, using 30 μ g of the WSX-1 expression vector WSX-1/pZp7Z
15 (Example 3B) to electroporate the BaF3/zcytor17#15 cells. One exception is that in place of Geneticin selection, 200 μ g/ml Zeocin (InVitrogen) was added to the transfected cells in a T-162 flask to isolate the zeocin-resistant pool. The BaF3 cells expressing zcytor17 and WSX-1 were designated BaF3/zcytor17/hWSX-1. To obtain clones, pools of Baf3/zcytor17/hWSX-1 cells were plated at limiting dilution in 96-well
20 plates. The resulting clones were expanded and total RNA was isolated using a S.N.A.P.™ total RNA Isolation Kit (InVitrogen). First-strand cDNA was synthesized using the proSTAR™ First Strand RT-PCR kit, and then PCR with WSX-1 specific primers ZC9791 (SEQ ID NO:24) and ZC9793 (SEQ ID NO:25) was used to screen the clones for expression of WSX-1. One clone, BaF3/zcytor17/hWSX-1#5 was chosen to
25 expand further and transfect with the OSMRbeta expression vector.

BaF3 cells expressing zcytor17, WSX-1 and full-length OSMRbeta were constructed as per Example 2A above, using 30 μ g of the OSMRbeta expression vector OSMR/pZp7NX described in Example 29 to electroporate the BaF3/zcytor17/hWSX-1#5 cells. The BaF3 cells expressing zcytor17, WSX-1, and OSMRbeta mRNA were
30 designated BaF3/zcytor17/WSX-1/OSMR. To obtain clones, pools of BaF3/zcytor17/WSX-1/OSMRbeta cells were plated at limiting dilution in 96-well

plates. Individual clones were expanded and total RNA was isolated using a S.N.A.P.[™] total RNA Isolation Kit (InVitrogen). First-strand cDNA was synthesized using the proSTAR[™] First Strand RT-PCR kit, and then PCR with OSMRbeta specific primers ZC40109 (SEQ ID NO:26) and ZC40112 (SEQ ID NO:27) was used to screen the
5 clones for expression of zcytor17, WSX-1, and OSMR. One clone, BaF3/zcytor17/WSX-1/OSMR#5 was selected and these cells were used to screen for zcytor17lig as described below in Examples 5 and 6.

B. Construction of BaF3 Cells Expressing zcytor17 receptor and OSMR

10 BaF3 cells expressing the full-length zcytor17 receptor were constructed as per Example 2A above, using 30µg of the zcytor17 expression vector, described in Example 3A. One exception is that in place of Geneticin selection, 2µg/ml of Puromycin (ClonTech) was added to the transfected cells in a T-162 flask to isolate the puromycin-resistant pool. The BaF3 cells expressing the zcytor17 receptor mRNA
15 were designated as BaF3/zcytor17. To obtain clones, pools of Baf3/zcytor17 cells were plated at limiting dilution in 96-well plates. These clones were expanded in culture and total RNA was isolated using a S.N.A.P.[™] total RNA Isolation Kit (InVitrogen). First-strand cDNA was synthesized using the proSTAR[™] First Strand RT-PCR kit, and then PCR was used to screen the clones for expression of zcytor17. One clone,
20 BaF3/zcytor17 #15 was chosen to expand and transfect with the OSMRbeta expression vector.

BaF3 cells expressing zcytor17 and full-length OSMRbeta were constructed as per Example 2A above, using 30 µg of the OSMRbeta expression vector OSMR/pZp7NX (example 29) to electroporate the BaF3/zcytor17#15 cells. The BaF3
25 cells expressing zcytor17 and OSMRbeta mRNA were designated BaF3/zcytor17/OSMR. These cells were used to screen for zcytor17lig as described below in Example 5.

Example 5

30 *Screening for zcytor17lig using BaF3/Zcytor17/WSX-1/OSMRbeta cells using an Alamar Blue Proliferation Assay*

A. Activation of CCRF-CEM and CCRF-HSB2 cells to test for presence of zcytor17lig

CCRF-CEM and CCRF-HSB2 cells were obtained from ATCC and stimulated in culture to produce conditioned media to test for the presence of zcytor17lig activity as described below. The suspension cells were seeded at 2×10^5 cells/ml or 5×10^5 cells/ml in RPMI-1640 media supplemented with 10% FBS, 2 mM L-glutamine (GibcoBRL), 1X PSN (GibcoBRL), and activated with 10 ng/ml Phorbol-12-myristate-13-acetate (PMA) (Calbiochem, San Diego, CA) and 0.5 μ g/ml IonomycinTM (Calbiochem) for 24 or 48hrs. The supernatant from the stimulated cells was used to assay proliferation of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells as described below.

B. Screening for zcytor17lig using BaF3/Zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells using an Alamar Blue Proliferation Assay

BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells were spun down and washed in mIL-3 free media. The cells were spun and washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 μ l per well using the mIL-3 free media.

Proliferation of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells was assessed using conditioned media from activated CCRFCEM and CCRF-HSB2 cells (see Example 5A). Conditioned media was diluted with mIL-3 free media to 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.75%, and 0.375% concentrations. 100 μ l of the diluted conditioned media was added to the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells. The total assay volume is 200 μ l. The assay plates were incubated at 37°C, 5% CO₂ for 3-5 days at which time Alamar Blue (Accumed, Chicago, IL) was added at 20 μ l/well. Plates were again incubated at 37°C, 5% CO₂ for 24 hours. Plates were read on the FmaxTM plate reader (Molecular devices) as described above (Example 2).

Results confirmed the proliferative response of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells to a factor present in the activated CCRF-CEM and CCRF-HSB2 conditioned media. The

response, as measured, was approximately 10-fold over background at the 25% concentration. The untransfected BaF3 cells did not proliferate in response to this factor, nor did BaF3 cells transfected with zcytor17 and WSX-1 (BaF3/zcytor17/WSX-1 cells), showing that this factor was specific for Zcytor17/OSMRbeta or zcytor17/OSMRbeta/WSX-1 receptors. Moreover soluble zcytor17 receptor diminished this proliferative activity of zcytor17lig in the BaF3/zcytor17/WSX-1/OSMRbeta cells (see, Example 11). Similar results are expected in BaF3/zcytor17/OSMRbeta cells.

10 *C. Human Primary Source used to isolate zcytor17lig*

One hundred milliliters blood draws were taken from each of six donors. The blood was drawn using 10X 10 ml vacutainer tubes containing heparin. Blood was pooled from six donors (600ml), diluted 1:1 in PBS, and separated using a Ficoll-Paque® PLUS (Pharmacia Biotech). The isolated primary human cell yield after separation on the ficoll gradient was 1.2×10^9 cells.

Cells were suspended in 9.6 ml MACS buffer (PBS, 0.5% EDTA, 2mM EDTA). 1.6 ml of cell suspension was removed and 0.4 ml CD3 microbeads (Miltenyi Biotec, Auburn, CA) added. The mixture was incubated for 15 min. at 4°C. These cells labeled with CD3 beads were washed with 30 ml MACS buffer, and then resuspended in 2 ml MACS buffer.

A VS+ column (Miltenyi) was prepared according to the manufacturer's instructions. The VS+ column was then placed in a VarioMACS™ magnetic field (Miltenyi). The column was equilibrated with 5 ml MACS buffer. The isolated primary human cells were then applied to the column. The CD3 negative cells were allowed to pass through. The column was rinsed with 9 ml (3 X 3 ml) MACS buffer. The column was then removed from the magnet and placed over a 15 ml falcon tube. CD3+ cells were eluted by adding 5 ml MACS buffer to the column and bound cells flushed out using the plunger provided by the manufacturer. The incubation of the cells with the CD3 magnetic beads, washes, and VS+ column steps (incubation through elution) above were repeated five more times. The resulting CD3+ fractions from the six column separations were pooled. The yield of CD3+ selected human cells were 3×10^8

total cells.

A sample of the pooled CD3+ selected human cells was removed for staining and sorting on a fluorescent antibody cell sorter (FACS) to assess their purity. The human CD3+ selected cells were 91% CD3+ cells.

5 The human CD3+ selected cells were activated by incubating in RPMI + 5% FBS + PMA 10 ng/ml and Ionomycin 0.5 µg/ml (Calbiochem) for 13 hours 37°C. The supernatant from these activated CD3+ selected human cells was tested for zcytor17lig activity as described below. Moreover, the activated CD3+ selected human cells were used to prepare a cDNA library, as described in Example 6, below.

10

D. Testing supernatant from activated CD3+ selected human cells for zcytor17lig using BaF3/Zcytor17/WSX-1/OSMRbeta cells and an Alamar Blue Proliferation Assay

BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells were spun down and washed in mIL-3 free media. The cells were spun and
15 washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 µl per well using the mIL-3 free media.

Proliferation of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells were assessed using conditioned media from activated
20 CD3+ selected human cells (see Example 5C) diluted with mIL-3 free media to 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.75%, 0.375% and 0.187% concentrations. 100 µl of the diluted conditioned media was added to the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells. The total assay volume was 200 µl. The assay plates were incubated and assayed as described in Example 5B.

25 Results confirmed the proliferative response of the BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells to a factor present in the activated CD3+ selected human Cell conditioned media. The response, as measured, was approximately 15-fold over background at the 25% concentration. The untransfected BaF3 cells did not proliferate in response to this factor, nor did BaF3
30 cells transfected with zcytor17 and WSX-1 (BaF3/zcytor17/WXS-1 cells), showing that this factor is specific for Zcytor17/OSMRbeta or zcytor17/OSMRbeta/WSX-1

receptors.

Example 6

Cloning of human zcytor17lig from a human CD3+ selected cell Library

5 Screening of a primary human activated CD3+ selected cell cDNA library revealed an isolated cDNA that is a novel member of the four-helix bundle cytokine family. This cDNA encoded the zcytor17lig. The cDNA was identified by screening for activity of the zcytor17lig using the zcytor17/WSX-1/OSM receptors.

10 *A. The vector for CD3+ selected library construction*

 The vector for CD3+ selected library construction was pZP7NX. The pZP7NX vector was constructed as follows: The coding region for the DHFR selective marker in vector pZP7 was removed by DNA digestion with NcoI and PstI restriction enzymes (Boehringer Mannheim). The digested DNA was run on 1% agarose gel, cut
15 out and gel purified using the Qiagen Gel Extraction Kit (Qiagen) as per manufacturer's instructions. A DNA fragment representing the coding region of Zeocin selective marker was amplified by PCR method with primers ZC13,946 (SEQ ID NO:28) and ZC13,945 (SEQ ID NO:29), and pZeoSV2(+) as a template. There are additional PstI and BclI restriction sites in primer ZC13,946 (SEQ ID NO:28), and additional NcoI
20 and SfuI sites in primer ZC13,945 (SEQ ID NO:29). The PCR fragment was cut with PstI and NcoI restriction enzymes and cloned into pZP7 vector prepared by cleaving with the same two enzymes and subsequent gel purification. This vector was named pZP7Z. Then the Zeocin coding region was removed by DNA digestion of vector pZP7Z with BclI and SfuI restriction enzymes. The digested DNA was run on 1%
25 agarose gel, cut out and gel purified, and then ligated with a DNA fragment of Neomycin coding region cut from pZem228 vector (deposited at the American Type Culture Collection (ATCC), Manassas, VA; ATCC Deposit No. 69446) with the same restriction enzymes (BclI and SfuI).

 This new vector was named pZP7N, in which the coding region for
30 DHFR selective marker was replaced by the coding region for a Neomycin selective marker from vector pZem228. A stuffer fragment including an XhoI site was added to

pZP7N to create a vector suitable for high efficiency directional cloning of cDNA; this new vector was called pZP7NX. To prepare the vector for cDNA, 20µg of pZP7NX was digested with 20 units of EcoR1 (Life Technologies Gaithersburg, MD) and 20 units of Xho1 (Boehringer Mannheim Indianapolis, IN) for 5 hours at 37°C, then 68°C for 15 minutes. The digest was then run on a 0.8% low melt agarose 1XTAE gel to separate the stuffer from the vector. The vector band was excised and digested with "beta-Agarase" (New England Biolabs, Beverly, MA) following the manufacturer's recommendations. After ethanol precipitation the digested vector was resuspended in water to 45ng/ml in preparation for ligation of CD3+ selected cDNA library described below.

B. Preparation of primary human activated CD3+ selected cell cDNA library

Approximately 1.5×10^8 primary human CD3+ selected cells stimulated in ionomycin/PMA were isolated by centrifugation after culturing at 37°C for 13 hours (Example 5C). Total RNA was isolated from the cell pellet using the "RNeasy Midi" kit from Qiagen, Inc. (Valencia, CA). mRNA was isolated from 225 micrograms of total RNA using the "MPG mRNA purification kit" from CPG Inc. (Lincoln Park, NJ). 3.4 micrograms of mRNA was isolated and converted to double stranded cDNA using the following procedure.

First strand cDNA from stimulated human CD3+ selected cells was synthesized as follows. Nine µl Oligo d(T)-selected poly(A) CD3+ RNA at a concentration of 0.34 µg/µl and 1.0 µl of 1 µg/µl first strand primer ZC18,698 (SEQ ID NO:30) containing an XhoI restriction site were mixed and heated at 65°C for 4 minutes and cooled by chilling on ice. First strand cDNA synthesis was initiated by the addition of 9 µl of first strand buffer (5x SUPERScript® buffer; (Life Technologies), 4 µl of 100 mM dithiothreitol and 2 µl of a deoxynucleotide triphosphate solution containing 10 mM each of dATP, dGTP, dTTP and 5-methyl-dCTP (Pharmacia Biotech Inc.) to the RNA-primer mixture. The reaction mixture was incubated at 45°C for 4 minutes followed by the addition of 8 µl of 200 U/µl SuperscriptII®, RNase H- reverse transcriptase (Life technologies). The reaction was incubated at 45°C for 45 minutes followed by an incubation ramp of 1°C every 2 minutes to 50°C where the reaction was

held for 10 minutes. To denature any secondary structure and allow for additional extension of the cDNA the reaction was then heated to 70°C for 2 minutes then dropped to 55°C for 4 minutes after which 2 µl of SuperscriptII® RT was added and incubated an additional 15 minutes followed by a ramp up to 70°C 1minute/1°C. Unincorporated
5 nucleotides were removed from the cDNA by twice precipitating in the presence of 2 µg of glycogen carrier, 2.0 M ammonium acetate and 2.5 volume ethanol, followed by a 100 µl wash with 70% ethanol. The cDNA was resuspended in 98 µl water for use in second strand synthesis.

Second strand synthesis was performed on the first strand cDNA under
10 conditions that promoted first strand priming of second strand synthesis resulting in DNA hairpin formation. The second strand reaction contained 98 µl of the first strand cDNA, 30 µl of 5x polymerase I buffer (100 mM Tris: HCl, pH 7.5, 500 mM KCl, 25 mM MgCl₂, 50 mM (NH₄)₂SO₄), 2 µl of 100 mM dithiothreitol, 6 µl of a solution containing 10 mM of each deoxynucleotide triphosphate, 5 µl of 5 mM b-NAD, 1 µl of
15 3 U/µl *E. coli* DNA ligase (New England Biolabs Inc.) and 4 µl of 10 U/µl *E. coli* DNA polymerase I (New England Biolabs Inc.). The reaction was assembled at room temperature and was incubated at room temperature for 2 minutes followed by the addition of 4 µl of 3.8 U/µl RNase H (Life Technologies). The reaction was incubated at 15°C for two hours followed by a 15 minute incubation at room temperature. 10 µl of
20 1M TRIS pH7.4 was added to the reaction and extracted twice with phenol/chloroform and once with chloroform, the organic phases were then back extracted with 50 µl of TE (10mM TRIS pH 7.4, 1mM EDTA), pooled with the other aqueous and ethanol precipitated in the presence of 0.3 M sodium acetate. The pellet was washed with 100 µl 70% ethanol air dried and resuspended in 40 µl water.

25 The single-stranded DNA of the hairpin structure was cleaved using mung bean nuclease. The reaction mixture contained 40 µl of second strand cDNA, 5 µl of 10x mung bean nuclease buffer (Life technologies), 5 µl of mung bean nuclease (Pharmacia Biotech Corp.) diluted to 1U/µl in 1X mung bean nuclease buffer. The reaction was incubated at 37°C for 45 minutes. The reaction was terminated by the
30 addition of 10 µl of 1 M Tris: HCl, pH 7.4 followed by sequential phenol/chloroform and chloroform extractions as described above. Following the extractions, the cDNA

was ethanol precipitated in the presence of 0.3 M sodium acetate. The pellet was washed with 100 µl 70% ethanol air dried and resuspended in 38 µl water.

The resuspended cDNA was blunt-ended with T4 DNA polymerase. The cDNA, which was resuspended in 38 µl of water, was mixed with 12 µl 5x T4 DNA polymerase buffer (250 mM Tris:HCl, pH 8.0, 250 mM KCl, 25 mM MgCl₂), 2
5 µl 0.1 M dithiothreitol, 6 µl of a solution containing 10 mM of each deoxynucleotide triphosphate and 2 µl of 1 U/µl T4 DNA polymerase (Boehringer Mannheim Corp.). After an incubation of 45 minutes at 15°C, the reaction was terminated by the addition of 30 µl TE followed by sequential phenol/chloroform and chloroform extractions and
10 back extracted with 20 µl TE as described above. The DNA was ethanol precipitated in the presence of 2 µl Pellet Paint™ (Novagen) carrier and 0.3 M sodium acetate and was resuspended 11 µl of water.

*Eco*RI adapters were ligated onto the 5' ends of the cDNA described above to enable cloning into an expression vector. 11 µl of cDNA and 4 µl of 65
15 pmole/µl of *Eco* RI hemiphosphorylated adaptor (Pharmacia Biotech Corp) were mixed with 5 µl 5x ligase buffer (Life Technologies), 2 µl of 10 mM ATP and 3 µl of 1 U/µl T4 DNA ligase (Life Technologies), 1 µl 10X ligation buffer (Promega Corp), 9 µl water. The extra dilution with 1X buffer was to prevent the pellet paint from precipitating. The reaction was incubated 9 hours in a water bath temperature ramp
20 from 10°C to 22°C over 9 hours, followed by 45 minutes at 25°C. The reaction was terminated by incubation at 68°C for 15 minutes.

To facilitate the directional cloning of the cDNA into an expression vector, the cDNA was digested with *Xho*I, resulting in a cDNA having a 5' *Eco* RI cohesive end and a 3' *Xho*I cohesive end. The *Xho*I restriction site at the 3' end of the
25 cDNA had been previously introduced using the ZC18698 (SEQ ID NO:31) primer. Restriction enzyme digestion was carried out in a reaction mixture containing 35 µl of the ligation mix described above, 6 µl of 10x H buffer (Boehringer Mannheim Corp.), 1 µl of 2mg/ml BSA (Biolabs Corp.), 17 µl water and 1.0 µl of 40 U/µl *Xho*I (Boehringer Mannheim). Digestion was carried out at 37°C for 1 hour. The reaction was
30 terminated by incubation at 68°C for 15 minutes followed by ethanol precipitation, washing drying as described above and resuspension in 30 µl water.

The resuspended cDNA was heated to 65°C for 5 minutes and cooled on ice, 4 µl of 5X gel loading dye (Research Genetics Corp.) was added, the cDNA was loaded onto a 0.8% low melt agarose 1X TAE gel (SEA PLAQUE GTG™ low melt agarose; FMC Corp.) and electrophoresed. The contaminating adapters and cDNA
5 below 0.6 Kb in length were excised from the gel. The electrodes were reversed, molten agarose was added to fill in the wells, the buffer was changed and the cDNA was electrophoresed until concentrated near the lane origin. The area of the gel containing the concentrated cDNA was excised and placed in a microfuge tube, and the agarose was melted by heating to 65°C for 15 minutes. Following equilibration of the
10 sample to 45°C, 2 µl of 1 U/µl Beta-agarase I (Biolabs, Inc.) was added, and the mixture was incubated for 90 min. at 45°C to digest the agarose. After incubation, 1 tenth volume of 3 M Na acetate was added to the sample, and the mixture was incubated on ice for 15 minutes. The sample was centrifuged at 14,000 x g for 15 minutes at room temperature to remove undigested agarose, the cDNA was ethanol
15 precipitated, washed in 70% ethanol, air-dried and resuspended in 40 µl water.

To determine the optimum ratio of cDNA to vector several ligations were assembled and electroporated. Briefly, 2 µl of 5X T4 ligase buffer (Life Technologies), 1 µl of 10mM ATP, 1 µl pZP7NX digested with EcoR1-Xho1, 1 µl T4 DNA ligase diluted to 0.25u/µl (Life Technologies) water to 10 µl and 0.5, 1,2 or 3 µl
20 of cDNA were mixed in 4 separate ligations, incubated at 22°C for 4 hours, 68°C for 20 minutes, sodium acetate-ethanol precipitated, washed, dried and resuspended in 10 µl. A single microliter of each ligation was electroporated into 40 µl DH10b ElectroMax™ electrocompetent bacteria (Life Technologies) using a 0.1cm cuvette (Biorad) and a Genepulser, pulse controller™ (Biorad) set to 2.5KV, 25IF, 200ohms. These cells were
25 immediately resuspended in 1 ml SOC broth (Manniatitis et al. *supra.*) followed by 500 µl of 50% glycerol-SOC as a preservative. These “glycerol stocks ” were frozen in several aliquots at -70°C. An aliquot of each was thawed and plated serially on LB-agar plates supplemented with ampicillin at 100 µg/ml. Colony numbers indicated that the optimum ratio of CD3+ cDNA to pZP7NX vector was 1 µl to 45 ng; such a ligation
30 yielded 4.5 million primary clones.

For the purpose of screening the library using a BaF3-based proliferation

assay (Example 5) glycerol stocks from above were diluted into liquid cultures of 100 or 250 clones per pool in deep well microtiter plates, grown 24 hours at 37°C with shaking and plasmid isolated using a Qiagen kit following the manufacturer's instructions. Such DNA was subsequently transfected into BHK cells, media
5 conditioned 72 hours, harvested and stored at -80°C, and subsequently placed on 5K BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells for 72 hours after which proliferation was assessed using an "Alamar blue" fluorescence assay (Example 5B and Example 2B).

10

Example 7

Expression Cloning of human zcytor17lig

The glycerol stocks from the activated human CD3+ selected cell library (Example 6) were added to Super Broth II™ (Becton Dickinson, Cockeysville, MD) + 0.1 mg/ml ampicillin (amp) at a concentration of 250 cells per 800 microliters. The *E.*
15 *coli* were allowed to equilibrate for 24 hours at room temperature. At the time of inoculation, 400 microliters was plated on LB + amp plates to determine the actual titer of the inoculation. After 24 hours the plates were counted and then the final concentration of the SuperBrothII™ + *E. coli* was adjusted so that the final concentration was 250 cells per 1.2 ml. Three times 2 liters were inoculated for a total
20 of 6 liters. The media were then plated into 96-well deep well blocks (Qiagen). Plating was done on the 8-channel Q-Fill2™ dispenser (Genetix, Christchurch, Dorset, UK). The *E. coli* were grown overnight at 37°C shaking at 250 rotations/min. on a New Brunswick Scientific Innova 4900 multi-tier environment shaker. The *E. coli* were spun out of solution at 3000 rpm, using a Beckman GS-6KR centrifuge. These *E. coli*
25 pellets were frozen at -20°C or used fresh before miniprepping the plasmid DNA. Each pellet contains approximately 250 cDNA clones from the human CD3+ selected cell library.

These pools of 250 cDNA clones were then mini-prepped using QIAprep™ 96 Turbo Miniprep kit (Qiagen). Plasmid DNA was eluted using 125 µl of
30 TE (10 mM Tris pH 8, 1 mM EDTA). This plasmid DNA was then used to transfect BHK cells.

BHK transfection

BHK cells were plated in 96-well tissue culture plates at a density of 12,000 cells per well in a volume of 100 μ l. per well. Culture media was DMEM (GibcoBRL), 5% heat-inactivated fetal bovine serum, 2 mM L-glutamine (GibcoBRL),
5 1X PSN (GibcoBRL), 1 mM NaPyruvate (GibcoBRL).

The following day, BHK cells were washed once with 100 μ l SFA. SFA is serum-free media which is DMEM/F12 or DMEM (Gibco/BRL), 2 mM GlutaMaxTM (Gibco/BRL), 1 mM NaPyruvate, 10 μ g/ml transferrin, 5 μ g/ml insulin, 10 μ g/ml
10 fetuin, 2 μ g/ml selenium, 25mM HEPES (Gibco/BRL), 100 μ M non-essential amino acids (Gibco/BRL).

A DNA/LipofectamineTM mix was made as follows: 2.2 μ l LipofectamineTM reagent (Gibco/BRL) was combined with 102.8 μ l SFA at room temperature; approximately 5 μ l of the plasmid DNA (200 ng/ μ l) was then added to the
15 LipofectamineTM/SFA to form the DNA/LipofectamineTM mixture, which was incubated at room temperature for 30 minutes. The SFA was removed from the BHK cells and the cells were incubated with 50 μ l of the DNA/lipofectamineTM mix for 5 hours at 37°C with 5% CO₂. Fifty μ l of the DNA/LipofectamineTM mixture was added to each of two wells of the BHK cells, so that transfections were done in duplicate.

20 After BHK cells were incubated with DNA/LipofectamineTM mix for 5 hours, the DNA/LipofectamineTM mix was removed and 100 μ l culture media was added. Cells were incubated overnight, the media was removed and replaced with 100 μ l. culture media. After culturing cells for 48-72 hours, conditioned media was removed, frozen at -80°C for a minimum of 20 minutes, thawed, and then 50 μ l was
25 assayed in the Baf3 proliferation assay, described in Example 5, to identify pools of 250 clones with ligand activity.

Twenty 96-well plates were screened in a single assay. This represented approximately 250 cDNAs/well or 480,000 cDNAs total. Of these, conditioned media from approximately 60 wells (representing 250 cDNAs per well) tested positive in the
30 proliferation assay. One of these positive pools was chosen to break down and isolate a single cDNA that would encode the zcytor17lig. This was pool 62A12.

For pool 62A12, 1 μ l. of DNA was used to transform ElectroMax™ DH10B cells (Gibco/BRL) by electroporation. The transformants were plated on LB + amp (100 μ g/ml) plates to give single colonies. From the electroporated pool, 672 individual colonies were selected by toothpick into seven 96-well plates containing 1.2 ml of SuperBrothII™ per well. These plates were numbered #62.1 through #62.7. These were cultured overnight and the plasmid DNA miniprep as above. For all seven plates, plasmid DNA from the breakdown plates was transfected into BHK cells and assayed by proliferation as above, except that transfections were not done in duplicate.

Two positive clones 62.6C7 and 62.6E9 were identified by activity from a total of 672 clones. Plasmid DNA miniprep from clone 62.6E9 was sequenced and a tentative identification was obtained, but a mixed sequence was obtained from this positive clones. To further isolate the zcytor17lig cDNA to a single clone, 1 μ l of DNA from pool 62.6E9 was used to electroporate DH10B cells and the transformants plated on LB + amp (100 μ g/ml) plates to give single colonies. Plasmid DNA miniprep from several colonies was sequenced to give the exact DNA sequence. The polynucleotide sequence of zcytor17lig was full-length (SEQ ID NO:1) and its corresponding amino acid sequence is shown (SEQ ID NO:2).

Example 8

Construction of Mammalian Expression Vectors That Express zcytor17 Soluble

Receptors: zcytor17CEE, zcytor17CFLG, zcytor17CHIS and zcytor17-Fc4

A. Construction of zcytor17 Mammalian Expression Vector containing zcytor17CEE, zcytor17CFLG and zcytor17CHIS

An expression vector was prepared for the expression of the soluble, extracellular domain of the zcytor17 polypeptide, pZp9zcytor17CEE, where the construct is designed to express a zcytor17 polypeptide comprised of the predicted initiating methionine and truncated adjacent to the predicted transmembrane domain, and with a C-terminal Glu-Glu tag (SEQ ID NO:32).

An approximately 1500 bp PCR product was generated using ZC29,451 (SEQ ID NO:33) and ZC29,124 (SEQ ID NO:34) as PCR primers to add EcoRI and

BamHI restriction sites. A human HPVS in-house cDNA library was used as a template and PCR amplification was performed as follows: 30 cycles at 94°C for 1 minute, 65°C for 1 minute, 72°C for 1.5 minutes, then 72°C for 7 minutes; 10°C soak. The PCR reaction was ethanol precipitated and digested with EcoRI and BamHI
5 restriction enzymes. The digested PCR product was gel purified on a 1.0% agarose gel and the approximately 1500 bp band excised. This band was then re-amplified using identical primers with the following cycling: 30 cycles at 94°C for 1 minute, 65°C for 1 minute, 72°C for 3 minutes, then 72°C for 7 minutes; 10°C soak. The PCR reaction was ethanol precipitated and digested with EcoRI and BamHI restriction enzymes. The
10 digested PCR product was gel purified on a 1.0% agarose gel and the approximately 1500 bp band excised. The excised DNA was subcloned into plasmid CEEpZp9 that had been cut with EcoRI and BamHI, to generate plasmid with a GLU-GLU C-terminally tagged soluble receptor for zcytor17, zcytor17CEEpZp9. The extracellular domain in the zcytor17CEE cDNA in zcytor17CEEpZp9 has a silent mutation that
15 changes the T to C at position 1705 of SEQ ID NO:4 (encoding a Pro residue at residue 403 of SEQ ID NO:5). As this mutation is silent, the zcytor17 cDNA in zcytor17CEEpZp9 encodes the polypeptide as shown in SEQ ID NO:5. Moreover, because of the construct used, a Gly-Ser residue pair is inserted C-terminal to the end of the soluble, extracellular domain of zcytor17 and prior to the C-terminal Glu-Glu Tag
20 (SEQ ID NO:32). As such, the tag at the C-terminus of the zcytor17 extracellular domain, was a Glu-Glu tag as shown in (SEQ ID NO:17). Plasmid CEEpZp9 is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 promoter, multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also has an *E. coli* origin of
25 replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene and the SV40 terminator. Using standard molecular biological techniques zcytor17CEEpZp9 was electroporated into DH10B competent cells (GIBCO BRL, Gaithersburg, MD) according to manufacturer's direction and plated onto LB plates containing 100 µg/ml ampicillin, and incubated
30 overnight. Colonies were screened by restriction analysis, or PCR from DNA prepared from individual colonies. The insert sequence of positive clones was verified by

sequence analysis. A large scale plasmid preparation was done using a QIAGEN® Maxi prep kit (Qiagen) according to manufacturer's instructions.

The same process is used to prepare the zcytor17 soluble receptors with a C-terminal his tag, composed of 6 His residues in a row; and a C-terminal FLAG® tag (SEQ ID NO:36), zcytor17CFLAG. To construct these constructs, the
5 aforementioned vector has either the HIS or the FLAG® tag in place of the glu-glu tag (e.g., SEQ ID NO:17; SEQ ID NO:32 or SEQ ID NO:35).

B. Mammalian Expression Construction of Soluble human zcytor17 receptor:

10 *zcytor17-Fc4*

An expression vector, pEZE-2 hzcytor17/Fc4, was prepared to express a C-terminally Fc4 tagged soluble version of hzcytor17 (human zcytor17-Fc4) in PF CHO cells. PF CHO cells are an in house CHO cell line adapted for growth in protein-free medium (ExCell 325 PF medium; JRH Biosciences). The in house CHO cell line
15 was originally derived from CHO DG44 cells (G. Urlaub, J. Mitchell, E. Kas, L.A. Chasin, V.L. Funanage, T.T. Myoda and J.L. Hamlin, "The Effect Of Gamma Rays at the Dihydrofolate Reductase Locus: Deletions and Inversions," *Somatic Cell and Molec. Genet.*, 12: 555-566 (1986). A fragment of zcytor17 cDNA that includes the polynucleotide sequence from extracellular domain of the zcytor17 receptor was fused
20 in frame to the Fc4 polynucleotide sequence (SEQ ID NO:37) to generate a zcytor17-Fc4 fusion (SEQ ID NO:38 and SEQ ID NO:39). The pEZE-2 vector is a mammalian expression vector that contains the Fc4 polynucleotide sequence and a cloning site that allows rapid construction of C-terminal Fc4 fusions using standard molecular biology techniques.

25 A 1566 base pair fragment was generated by PCR, containing the extracellular domain of human zcytor17 and the first two amino acids of Fc4 (Glu and Pro) with FseI and BglII sites coded on the 5' and 3' ends, respectively. This PCR fragment was generated using primers ZC29,157 (SEQ ID NO:40) and ZC29,150 (SEQ ID NO:41) by amplification from a plasmid containing the extracellular domain of
30 human zcytor17 (pZp9zcytor17CEE) (Example 8). The PCR reaction conditions were as follows: 25 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 2 minutes;

1 cycle at 72°C for 10 minutes; followed by a 4°C soak. The fragment was digested with FseI and BglII restriction endonucleases and subsequently purified by 1% gel electrophoresis and band purification using QiaQuick gel extraction kit (Qiagen). The resulting purified DNA was ligated for 5 hours at room temperature into a pEZE-2
5 vector previously digested with FseI and BglII containing Fc4 3' of the FseI and BglII sites.

Two µl of the ligation mix was electroporated in 37 µl DH10B electrocompetent *E. coli* (Gibco) according to the manufacturer's directions. The transformed cells were diluted in 400 µl of LB media and plated onto LB plates
10 containing 100 µg/ml ampicillin. Clones were analyzed by restriction digests and positive clones were sent for DNA sequencing to confirm the sequence of the fusion construct. 1 µl of a positive clone was transformed into 37 µl of DH10B electrocompetent *E. coli* and streaked on a LB/amp plate. A single colony was picked from this streaked plate to start a 250 ml LB/amp culture that was then grown overnight
15 at 37°C with shaking at 250 rpm. This culture was used to generate 750 µg of purified DNA using a Qiagen plasmid Maxi kit (Qiagen).

Example 9

Transfection And Expression Of Zcytor17 Soluble Receptor Polypeptides

20 BHK 570 cells (ATCC No. CRL-10314), DG-44 CHO, or other mammalian cells are plated at about 1.2×10^6 cells/well (6-well plate) in 800 µl of appropriate serum free (SF) media (e.g., DMEM, Gibco/BRL High Glucose) (Gibco BRL, Gaithersburg, MD). The cells are transfected with expression plasmids containing zcytor17CEE, zcytor17CFLG, zcytor17CHIS or zcytor17-Fc4 (Example 8),
25 using Lipofectin™ (Gibco BRL), in serum free (SF) media according to manufacturer's instruction. Single clones expressing the soluble receptors are isolated, screened and grown up in cell culture media, and purified using standard techniques.

A. Mammalian expression of soluble human zcytor17CEE receptor

30 BHK 570 cells (ATCC NO: CRL-10314) were plated in T-75 tissue culture flasks and allowed to grow to approximately 50 to 70% confluence at 37°C, 5%

CO₂, in DMEM/FBS media (DMEM, Gibco/BRL High Glucose, (Gibco BRL, Gaithersburg, MD), 5% fetal bovine serum, 1 mM L-glutamine (JRH Biosciences, Lenea, KS), 1 mM sodium pyruvate (Gibco BRL)). The cells were then transfected with the plasmid containing zcytor17CEE (Example 8) using LipofectamineTM (Gibco BRL), in serum free (SF) media formulation (DMEM, 10 mg/ml transferrin, 5 mg/ml insulin, 2 mg/ml fetuin, 1% L-glutamine and 1% sodium pyruvate). Ten µg of the plasmid DNA pZp9zcytor17CEE (Example 8) was diluted into a 15ml tube to a total final volume of 500 µl with SF media. 50 µl of Lipofectamine was mixed with 450 µl of SF medium. The Lipofectamine mix was added to the DNA mix and allowed to incubate approximately 30 minutes at room temperature. Four ml of SF media was added to the DNA:Lipofectamine mixture. The cells were rinsed once with 5 ml of SF media, aspirated, and the DNA:Lipofectamine mixture was added. The cells were incubated at 37°C for five hours, and then 5 ml of DMEM/10%FBS media was added. The flask was incubated at 37°C overnight after which time the cells were split into the selection media (DMEM/FBS media from above with the addition of 1 µM methotrexate or 10 µM Methotrexate (Sigma Chemical Co., St. Louis, Mo.) in 150 mm plates at 1:2, 1:10, and 1:50. Approximately 10 days post-transfection, one 150 mm plate of 1 µM methotrexate resistant colonies was trypsinized, the cells were pooled, and one-half of the cells were replated in 10 µM methotrexate; to further amplify expression of the zcytor17CEE protein. A conditioned-media sample from this pool of amplified cells was tested for expression levels using SDS-PAGE and Western analysis.

B. Mammalian expression of soluble human zcytor17-Fc4 receptor

Five replicates of 200 µg of pEZE-2hzcytor17Fc4 plasmid DNA (Example 8) were linearized by restriction digestion with FspI, a restriction enzyme that cuts once within the vector and does not disturb genes necessary for expression. 200 µg of CHO cell genomic DNA was added to each replicate as carrier DNA and then the DNA was precipitated by addition of 0.1 volumes of 3M Sodium Acetate pH 5.2 and 2.2 volumes ethanol followed by a 15 minute ice incubation and microcentrifugation at 4°C. The resulting DNA pellets were washed in 70% ethanol and air dried before being resuspended in 100 µl protein free (PF) CHO non-selection growth media (21 g/L PF

CHO Ex Cell 325 /200 mM L-glutamine (Gibco)/100 mM sodium pyruvate (Gibco)/1x HT Supplement (Gibco). Ten million PF CHO passage 61 cells were added to the DNA in 600 µl of PF CHO non-selection growth media and then electroporated in a Gene Pulser II Electroporation system (BioRad) using 950 µF capacitance and 300 Kv
5 using a 0.4 cm gap Gene Pulser (BioRad) electroporation cuvette. All 5 replicates of the electroporated cells were pooled and directly selected in –HT media (21 g/L PF CHO Ex Cell 325/ 200 mM L-glutamine (Gibco)/100 mM sodium pyruvate (Gibco). Cells were selected for 15 days in –HT media before being passaged at 4×10^5 ml into 50 nM MTX selection. Eight days later cells were seeded at 3.5×10^5 cells/ml into 200
10 mM MTX selection. After one week, cells were seeded at 4×10^5 cells/ml into 1 µM MTX selection. After two weeks at 1 µM MTX, cells were seeded at 1×10^6 cells/ml into 50 ml to generate conditioned medium. The resulting 72 hour conditioned media was analyzed by probing western blots with an antibody generated against human Ig. The cells produced hzcytor17/Fc4 protein at approximately 1 mg/L.

15

C. Larger-scale mammalian expression of soluble human zcytor17-Fc4 receptor

Two hundred micrograms of pEZE-2hzcytor17Fc4 plasmid DNA (Example 8) was linearized by restriction digestion with FspI, a restriction enzyme that cuts once within the pEZE-2 vector and does not disturb genes necessary for
20 expression. 200 µg of CHO genomic DNA (prepared in-house) was added as carrier DNA and then the DNA was precipitated by addition of 0.1 volumes of 3M Sodium Acetate pH 5.2 and 2.5 volumes ethanol followed by microcentrifugation at Room temperature. Five replicate DNA pellets were made and transformed. The resulting DNA pellet was washed in 70% ethanol and air dried before being resuspended in 100
25 µl PF CHO non-selection growth media (21 g/L PF CHO Ex Cell 325 /200 mM L-glutamine (Gibco)/100 mM sodium pyruvate (Gibco)/1x HT Supplement (Gibco). Ten million PF CHO cells were added to the DNA in 600 µl of PF CHO non-selection growth media and then electroporated in a Gene Pulser II Electroporation system (BioRad) using 950 µF capacitance and 300 volts using a 0.4 cm gap Gene Pulser
30 (BioRad) electroporation cuvette. The electroporated cells were pooled and put directly into selection in –HT media (21 g/L PF CHO Ex Cell 325/ 200 mM L-glutamine

(Gibco)/100 mM sodium pyruvate (Gibco). Cells were selected for 14 days in -HT media before being passaged at 4×10^5 /ml into 50 nM MTX selection. Cells were amplified to 200nM MTX and then to 1uM MTX. The -HT, 50nM, and 1uM pools were seeded at 1×10^6 c/ml for 48 hours, and the resulting conditioned media was
5 analyzed by probing western blots with an antibody generated against human Ig.

Example 10

Purification of zcytor17 soluble receptors from BHK 570 and CHO cells

A. Transient mammalian expression and purification of soluble human zcytor17-Fc4 10 receptor

pEZE-2hzcytor17Fc4 plasmid DNA (Example 11B) was introduced into 40 maxi plates of BHK cells using Lipofectamine (Gibco BRL) as described herein and in manufacturer's instructions. Cells were allowed to recover overnight, then were rinsed and refed with serum-free medium (SL7V4, made in-house). After 72 hours, the
15 media was collected and filtered, and cells were refed with serum-free medium. After 72 hours, the media was again collected and filtered.

The serum-free conditioned media (2 x 1.5 L batches) from transiently transfected BHK cells was pumped over a 1.5 ml Protein A-agarose column in 20 mM Tris, pH 7.5, 0.5 M NaCl. The column was washed extensively with this buffer and
20 then the bound protein was eluted with 1 ml of 0.2 M glycine, pH 2.5, 0.5 M NaCl. The eluted protein was collected into 0.1 ml of 2 M Tris, pH 8.5.

Aliquots were collected for SDS-polyacrylamide gel electrophoresis and the bulk zcytor17-Fc was dialyzed overnight against PBS. The soluble receptor was sterile filtered and placed in aliquots at -80°C.
25

B. Purification of zcytor17-Fc4

Recombinant carboxyl terminal Fc4 tagged zcytor17 (Example 8 and Example 9) was produced from transfected CHO cells. The CHO transfection was performed using methods known in the art. Approximately five-liters of conditioned
30 media were harvested and sterile filtered using Nalgene 0.2µm filters.

Protein was purified from the filtered media by a combination of Poros

50 protein A affinity chromatography (PerSeptive Biosystems, 1-5559-01, Framingham, MA) and Superdex 200 gel exclusion chromatography column (Amersham Pharmacia Biotech, Piscataway, NJ). Culture medium was directly loaded onto a 10x70mm (5.5-ml bed volume) protein A affinity column at a flow of about 3-10 ml/minute. Following column washing for ten column volumes of PBS, bound protein was eluted by five column volumes of 0.1 M glycine, pH 3.0 at 10 ml/minute). Fractions of 2 ml each were collected into tubes containing 100 μ l of 2.0 M Tris, pH 8.0, in order to neutralize the eluted proteins. Samples from the affinity column were analyzed by SDS-PAGE with coomassie staining and Western blotting for the presence of zcytor17-Fc4 using human Ig-HRP. Zcytor17-Fc4-containing fractions were pooled and concentrated to 1-2 ml using Biomax-30 concentrator (Millipore), and loaded onto a 20x580 mm Superdex 200 gel filtration column. The fractions containing purified zcytor17-Fc4 were pooled, filtered through 0.2 μ m filter, aliquoted into 100 μ l each, and frozen at -80°C. The concentration of the final purified protein was determined by BCA assay (Pierce, Rockford, IL).

C. SDS-PAGE and Western blotting analysis of zcytor17/Fc4

Recombinant zcytor17-Fc4 was analyzed by SDS-PAGE (Nupage 4-12%, Invitrogen, Carlsbad, CA) with coomassie staining method and Western blotting using human Ig-HRP. Either the conditioned media or purified protein was electrophoresed using an Invitrogen Novex's Xcell II mini-cell, and transferred to nitrocellulose (0.2 mm; Invitrogen, Carlsbad, CA) at room temperature using Novex's Xcell II blot module with stirring according to directions provided in the instrument manual. The transfer was run at 500 mA for one hour in a buffer containing 25 mM Tris base, 200 mM glycine, and 20% methanol. The filters were then blocked with 10% non-fat dry milk in PBS for 10 minutes at room temperature. The nitrocellulose was quickly rinsed, then the human Ig-HRP antibody (1:2000) was added in PBS containing 2.5% non-fat dry milk. The blots were incubated for two hours at room temperature, or overnight at 4°C, with gentle shaking. Following the incubation, the blots were washed three times for 10 minutes each in PBS, then quickly rinsed in H₂O. The blots were developed using commercially available chemiluminescent substrate

reagents (SuperSignal® ULTRA reagents 1 and 2 mixed 1:1; reagents obtained from Pierce, Rockford, IL), and the signal was captured using Lumi-Imager's Lumi Analyst 3.0 software (Boehringer Mannheim GmbH, Germany) for exposure times ranging from 10 second to 5 minutes or as necessary.

5 The purified zcytor17-Fc4 appeared as a single band with either the coomassie or silver staining at about 220 kDa under non-reducing conditions, and at about 120 kDa under reducing conditions, suggesting the dimeric form of zcytor17-Fc4 under non-reducing conditions as expected.

10 *Example 11*

Assay using zcytor17 soluble receptor zcytor17-Fc4 Soluble receptor in competitive inhibition assay

BaF3/zcytor17/WSX-1/OSMRbeta cells or BaF3/zcytor17/OSMRbeta cells were spun down and washed in mIL-3 free media. The cells were spun and washed 3 times to ensure the removal of the mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at 5000 cells per well in a volume of 100 μ l per well using the mIL-3 free media.

Both conditioned media from the CCRF-CEM and CCRF-HSB2 cell activation and the human CD3+ selected cells, described in Example 5, were added in separate experiments at 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.75%, 0.375%, and 0.187% concentrations, with or without zcytor17 soluble receptors (Zcytor17-Fc4; See, Example 9 and Example 10) at 1-10 $\mu\text{g/ml}$. The total assay volume was 200 μl .

The assay plates were incubated at 37°C, 5% CO₂ for 3-5 days at which time Alamar Blue (Accumed) was added at 20 µl/well. Plates were again incubated at 37°C, 5% CO₂ for 16-24 hours. Plates were read on the Fmax™ plate reader (Molecular Devices) as described in Example 2. Results demonstrated partial inhibition of cell growth with zcytor17-Fc4 soluble receptor at 10 µg/ml, confirming that the factor in each sample was specific for the zcytor17 receptor.

Titration curves, diluting out the soluble receptor, or soluble receptor
30 heterodimers and trimers comprising zcytor17 receptor (e.g., zcytor17/OSMR,
zcytor17/WSX-1, or zcytor17/OSMR/WSX-1, or other Class I cytokine receptor

subunits) are also run using the above stated assay to determine whether zcytor17 receptors are able to completely inhibit growth, for example, at low or physiologic concentrations.

5

Example 12

Secretion trap assay

A secretion trap assay is used to test the binding of the zcytor17lig to receptors comprising zcytor17 receptor, such as the zcytor17 receptor or receptor heterodimers and trimers comprising zcytor17 receptor (e.g., zcytor17/OSMR, zcytor17/WSX-1, or zcytor17/OSMR/WSX-1, or other Class I cytokine receptor subunits). Zcytor17lig plasmid DNA is transfected into COS cells, and used to assess binding of the zcytor17lig to receptors comprising zcytor17 receptor by secretion trap as described below.

15 *A. COS Cell Transfections*

The COS cell transfection is performed as follows: Mix about 800 ng of zcytor17lig cDNA and 5 μ l LipofectamineTM in 92 μ l serum free DMEM media (55mg sodium pyruvate, 146mg L-glutamine, 5mg transferrin, 2.5mg insulin, 1 μ g selenium and 5mg fetuin in 500ml DMEM), incubate at room temperature for 30 minutes and then add 400 μ l serum free DMEM media. Add this 500 μ l mixture onto 1.5x10⁵ COS cells/well plated on 12-well tissue culture plate and incubate for 5 hours at 37°C. Add 500 μ l 20% FBS DMEM media (100 ml FBS, 55 mg sodium pyruvate and 146mg L-glutamine in 500ml DMEM) and incubate overnight.

25 *B. Secretion Trap Assay*

The secretion trap is performed as follows: Media is rinsed off cells with PBS and then fixed for 15 minutes with 1.8% Formaldehyde in PBS. Cells are then washed with TNT (0.1M Tris-HCL, 0.15M NaCl, and 0.05% TweenTM-20 in H₂O), and permeated with 0.1% TritonTM-X in PBS for 15 minutes, and again washed with TNT. Cells are blocked for 1 hour with TNB (0.1M Tris-HCL, 0.15M NaCl and 0.5% Blocking Reagent (NEN Renaissance TSA-Direct Kit) in H₂O), and washed again with

TNT. If using the biotinylated receptor protein, the cells are blocked for 15 minute incubations with Avidin and then Biotin (Vector Labs) washing in-between with TNT. Depending on which soluble receptor is used, the cells are incubated for 1 hour in TNB with: (A) 1-3 $\mu\text{g/ml}$ zcytor17 soluble receptor zcytor17-Fc4 fusion protein (Example 5 10); (B) 1-3 $\mu\text{g/ml}$ zcytor17/OSMRbeta soluble receptor protein; (C) 1-3 $\mu\text{g/ml}$ zcytor17/WSX-1 soluble receptor protein; or (D) 1-3 $\mu\text{g/ml}$ zcytor17/OSMR/WSX-1 soluble receptor protein. Cells are then washed with TNT. Depending on which soluble receptor is used (e.g., if labeled with an Fc4 tag (SEQ ID NO:37), C-terminal FLAG tag (SEQ ID NO:36), or CEE tag (SEQ ID NO:32; SEQ ID NO:35)), cells are incubated for 10 another hour with: (A) 1:200 diluted goat-anti-human Ig-HRP (Fc specific); (B) 1:1000 diluted M2-HRP; (C) 1:1000 diluted anti-GluGlu antibody-HRP; or (D) 1:300 diluted streptavidin-HRP (NEN kit) in TNB, for example. Again cells are washed with TNT.

Positive binding is detected with fluorescein tyramide reagent diluted 1:50 in dilution buffer (NEN kit) and incubated for 4-6 minutes, and washed with TNT. 15 Cells are preserved with Vectashield Mounting Media (Vector Labs Burlingame, CA) diluted 1:5 in TNT. Cells are visualized using a FITC filter on fluorescent microscope.

Example 13

Chromosomal Assignment and Placement of the gene sequence for the zcytor17lig

20 The zcytor17lig gene sequence was mapped to human chromosome 12 using the commercially available version of the "Stanford G3 Radiation Hybrid Mapping Panel" (Research Genetics, Inc., Huntsville, AL). The "Stanford G3 RH Panel" contains DNA from each of 83 radiation hybrid clones of the whole human genome, plus two control DNAs (the RM donor and the A3 recipient). A publicly 25 available WWW server (e.g., Standford University) allows chromosomal localization of markers and genes.

For the mapping of the zcytor17lig gene sequence with the "Stanford G3 RH Panel", 20 μl reactions were set up in a 96-well microtiter plate compatible for PCR (Stratagene, La Jolla, CA) and used in a "RoboCycler Gradient 96" thermal cycler 30 (Stratagene). Each of the 95 PCR reactions consisted of 2 μl 10X PCR reaction buffer (Qiagen, Inc., Valencia, CA), 1.6 μl dNTPs mix (2.5 mM each, PERKIN-ELMER,

Foster City, CA), 1 μ l sense primer, ZC41,458 (SEQ ID NO:42), 1 μ l antisense primer, ZC41,457 (SEQ ID NO:43), 2 μ l "RediLoad" (Research Genetics, Inc., Huntsville, AL), 0.1 μ l Qiagen HotStarTaq DNA Polymerase (5 units/ μ l), 25 ng of DNA from an individual hybrid clone or control and distilled water for a total volume of 20 μ l. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cyclers conditions were as follows: an initial 1 cycle 15 minute denaturation at 95°C, 35 cycles of a 45 second denaturation at 95°C, 1 minute annealing at 53°C and 1 minute and 15 seconds extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 2% agarose gel (EM Science, Gibbstown, NJ) and visualized by staining with ethidium bromide.

The results showed linkage of the zcytor17lig gene sequence to the chromosome 12 marker SHGC-83339 with a LOD score of >11 and at a distance of 17 cR_10000 from the marker. This marker positions zcytor17lig gene in the 12q24.31 chromosomal region.

15

Example 14

Identification and cloning of murine zcytor17lig

A. Identification of full length murine zcytor17lig

Using the human zcytor17lig peptide sequence (SEQ ID NO:2) to query an in house DNA database, a murine cDNA, Genbank Accession No. AK005939, was identified as a potential partial sequence for the murine zcytor17lig. The AK005939 cDNA sequence was used to query a database containing murine genomic fragments. A genomic contig of the murine zcytor17lig was assembled (SEQ ID NO:76). Prediction of coding potential on this genomic fragment with the program Genscan revealed a likely cDNA sequence, with the same gene structure as the human zcytor17lig. A murine cDNA sequence is represented in SEQ ID NO:10, and corresponding polypeptide sequence is shown in SEQ ID NO:11.

B. Cloning of mouse zcytor17lig from a mouse testis cDNA library by PCR

Based on the genomic sequence (SEQ ID NO:76), two PCR primers were designed and used to identify a cDNA source of mouse zcytor17lig by PCR.

These Primers ZC41498 (SEQ ID NO:86) and ZC41496 (SEQ ID NO:87) were designed to the putative 5' and 3' untranslated regions of the mouse sequences (SEQ ID NO:76 and SEQ ID NO:10). Several cDNA sources were screened by PCR, including Marathon-ready cDNAs (Clontech) and aliquots of locally made cDNA libraries. Products were visualized on 1% agarose gels. Bands of the expected size were observed in reactions utilizing a mouse testis cDNA library template. These PCR reactions were successfully performed in approximately 50 μ l volumes with or without 10% DMSO, using pfu turbo polymerase (Stratagene) according to the manufacturer's recommendations; with an additional application of a wax hot-start employing hot start 50s (Molecular Bioproducts, Inc. San Diego, CA). PCR thermocycling was performed with a single cycle of 94°C for 4 min; followed by 40 cycles of 94°C: 30 seconds, 48°C: 30 seconds, 72°C: 50 seconds; with additional final 72°C extension for 7 minutes. The two PCR reactions were pooled and purified using low melt agarose and Gelase agarose digesting enzyme (Epicenter, Inc. Madison, WI) according to the manufacturer's recommendations.

DNA sequence determination of these PCR products revealed a murine zcytor17 cDNA sequence (SEQ ID NO:90) which comprised an ORF identical to SEQ ID NO:10, confirming that SEQ ID NO:10 encoded the mouse zcytor17lig polypeptide. PCR primers, ZC41583 (SEQ ID NO:88) and ZC41584 (SEQ ID NO:89), were then used to add FseI and AscI restriction sites and a partial Kozak sequence to the mcytor17lig open reading frame and termination codon (SEQ ID NO:92). A Robocycler 40 thermocycler (Stratagene) was used to run a temperature gradient of annealing temperatures and cycling as follows. Pfu turbo polymerase (Stratagene) was applied as described above, but only in 10% DMSO. Cycling was performed with a single cycle of 94°C for 4 min; followed by 20 cycles of 94°C: 30 seconds, 65°C to 51°C gradient: 30 seconds, 72°C: 1 minute; and a single 72°C extension for 7 minutes. The template for this second thermocycling reaction was 1 μ l of the initial gel-purified mcytor17lig PCR product, above. Resulting PCR product from the three lowest temperature reactions were pooled and gel purified using the Gelase (Epicenter) method described above. This purified mzcytor17lig was digested with FseI and AscI and ligated into a pZP7X vector modified to have FseI and AscI sites in its cloning site.

Plasmid pZP7X is a mammalian expression vector containing an expression cassette having the mouse metallothionein-1 (MT-1) promoter, multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selective marker expression unit
5 having an SV40 promoter, enhancer and origin of replication, a DHFR gene, and the SV40 terminator. The cloned murine cDNA sequence is represented in SEQ ID NO:90, and corresponding polypeptide sequence is shown in SEQ ID NO:91 (which is identical to SEQ ID NO:11).

10

Example 15

Isolation of mouse zcytor17lig cDNA clone from an activated mouse spleen library

A. Murine Primary Source used to isolate mouse zcytor17lig

Mouse spleens from Balb/C mice, are collected and mashed between frosted-end slides to create a cell suspension. The isolated primary mouse cell yield is
15 expected to be about 6.4×10^8 cells prior to selection described below.

The spleen cells are suspended in 9.6 ml MACS buffer (PBS, 0.5% EDTA, 2mM EDTA). 1.6 ml of cell suspension is removed and 0.4 ml CD90 (Thy1.2) microbeads (Miltenyi Biotec) added. The mixture is incubated for 15 min. at 4°C. These cells labeled with CD90 beads are washed with 30 ml MACS buffer, and then
20 resuspended in 2 ml MACS buffer.

A VS+ column (Miltenyi) is prepared according to the manufacturer's instructions. The VS+ column is then placed in a VarioMACS™ magnetic field (Miltenyi). The column is equilibrated with 5 ml MACS buffer. The isolated primary mouse cells are then applied to the column. The CD90 negative cells are allowed to
25 pass through. The column is rinsed with 9 ml (3 X 3 ml) MACS buffer. The column is then removed from the magnet and placed over a 15 ml falcon tube. CD90+ cells are eluted by adding 5 ml MACS buffer to the column and bound cells flushed out using the plunger provided by the manufacturer. The incubation of the cells with the CD90 magnetic beads, washes, and VS+ column steps (incubation through elution) above are
30 repeated once more. The resulting CD90+ fractions from the 2 column separations are pooled. The yield of CD90+ selected mouse spleen cells are expected to be about

1X10⁸ total cells.

A sample of the pooled CD90+ selected mouse cells is removed for staining and sorting on a fluorescent antibody cell sorter (FACS) to assess their purity. A PE-conjugated hamster anti-mouse CD3ε antibody (PharMingen) is used for staining
5 and sorting the CD90+ selected cells. The mouse CD90+ selected cells should be about 93% CD3+ cells, suggesting the cells are 93% T-cells.

The murine CD90+ selected cells are activated by incubating 3X10⁶ cells/ml in RPMI + 5% FBS + PMA 10 ng/ml and Ionomycin 0.5 μg/ml (Calbiochem) for overnight at 37°C. The supernatant from these activated CD90+ selected mouse
10 cells is tested for zcytor17lig activity as described below. Moreover, the activated CD90+ selected mouse cells are used to prepare a cDNA library, as described in Example 16, below.

Example 16

15 Cloning of mouse zcytor17lig from a mouse CD90+ selected cell library

Screening of a primary mouse activated CD90+ selected cell cDNA library can reveal isolated cDNA that is a novel member of the four-helix bundle cytokine family that would encode the mouse ortholog of the human zcytor17lig. The cDNA is identified by hybridization screening.

20

A. The vector for CD90+ selected library construction

The vector, pZP7N is used for CD3+ selected library construction (See Example 6A)

25 B. Preparation of primary mouse activated CD90+ selected cell cDNA library

Approximately 1.5X10⁸ primary mouse CD90+ selected cells stimulated in ionomycin/PMA (Example 15) are isolated by centrifugation. Total RNA is isolated from the cell pellet, and converted to double stranded cDNA as described in Example 6B. This DNA is subsequently transfected into BHK cells, as described in Example 6B,
30 and proliferation is assessed using an "Alamar blue" fluorescence assay (Example 2B).

For the purpose of screening the library by secretion trap cloning, a

complex, amplified form of the library is needed to transfect COS-7 cells. 4.8 million clones are plated on 110 15cm LB-agar plates supplemented with 100 µg/ml ampicillin, 10 µg/ml methicillin. After growing the plates overnight at 37°C the bacteria are harvested by scraping and pelleted. Plasmid DNA is extracted from the pelleted
5 bacteria using a Nucleobond-giga™ (Clontech) following the manufacturer's instructions. This plasmid is then used to transfect COS-7 cells on slides and screened using the secretion trap technique described below (Example 17).

C. Screening the activated mouse cDNA library

10 Approximately 5×10^5 clones are plated on 10 LB/Amp Maxi plates. The colonies are lifted, denatured, neutralized, and cross-linked using the standard procedure (Sambrook, J. et al. *supra.*). Fifty nanograms of the 300 bp 5' RACE PCR fragment (Example 14) is labeled with ^{32}P using Prime-It[®] RmT random primer labeling kit (Stratagene). The 10 filters are hybridized with this labeled probe at 65°C overnight
15 using ExpressHyb™ Hybridization Solution (Clontech). The filters are then washed sequentially at 60°C for 1 hour three times with 0.2xSSC (30 mM NaCl, 3 mM sodium citrate, pH 7.0), 0.1% SDS; and then at 65°C for 1 hour. The filters are exposed at -80°C overnight, and the X-ray film are developed. Agar plugs containing the positive colonies are pulled, and the clones plated on 10-cm LB/Amp plates. The colonies are
20 then filter-lifted and hybridized again following the same procedure described above. Single DNA clones are isolated and sequenced using standard methods, to identify the mouse cDNA.

Example 17

25 *Mouse zcytor17lig does not bind to human zcytor17 soluble receptor in secretion trap assay*

The DNA for mouse clone mzcyc17lig/pZP7 was transfected into COS cells, and the binding of zcytor17 comprising soluble receptors (human zcytor17 soluble receptor zcytor17-Fc4 (Example 10), or soluble receptor heterodimers
30 (zcytor17/WSX-1 or BaF3/zcytor17/OSMRbeta), to the transfected COS cells were tested by a secretion trap assay (Example 12). The assay confirmed that the mouse

zcytor17lig does not bind to human zcytor17 soluble receptor.

The COS cell transfection was performed as per Example 12 using about 0.7 µg mouse zcytor17lig cDNA (Example 16) in 3 µl.

The secretion trap was performed as per example 12 using, for example,
5 1 µg/ml zcytor17 soluble receptor Fc4 fusion protein (Example 10) (or zcytor17 comprising soluble receptor heterodimers as described herein) in TNB, and 1:200 diluted goat-anti-human Ig-HRP (Fc specific) in TNB for the detectable antibody. Positive binding of the soluble human zcytor17 receptor to the prepared fixed cells was not detected with fluorescein tyramide reagent as per Example 12. Cells were
10 preserved and visualized according to Example 12.

Results indicated that the mouse zcytor17lig does not bind to human zcytor17 soluble receptor (or zcytor17 comprising soluble receptor heterodimers as described herein).

15

Example 18

Expression of mouse zcytor17lig in mammalian cells

Mammalian expression of mouse zcytor17lig

BHK 570 cells (ATCC No: CRL-10314) were plated in 10 cm tissue culture dishes and allowed to grow to approximately 20% confluence overnight at 37°C,
20 5% CO₂, in DMEM/FBS media (DMEM, Gibco/BRL High Glucose media; Gibco BRL, Gaithersburg, MD), 5% fetal bovine serum (Hyclone, Logan, UT), 1 mM L-glutamine (JRH Biosciences, Lenexa, KS), 1 mM sodium pyruvate (Gibco BRL). The cells were then transfected with the plasmid mzytor17lig/pZP7X (Example 14) using a mammalian stable Lipofectamine (GibcoBRL) transfection kit according to the
25 manufacturer's instructions.

One day after transfection, the cells were split 1:10 and 1:20 into the selection media (DMEM/FBS media with the addition of 1 µM methotrexate (Sigma Chemical Co., St. Louis, MO)) in 150 mm plates. The media on the cells was replaced with fresh selection media at day 5 post-transfection. Approximately 10 days post-
30 transfection, methotrexate resistant colonies were trypsinized and the cells pooled and plated into large-scale culture flasks. Once the cells were grown to approximately 90%

confluence, they were rinsed with PBS three times, and cultured with serum-free ESTEP2 media (DMEM (Gibco BRL), 0.11 g/l Na Pyruvate, 3.7 g/l NaHCO₃, 2.5 mg/l insulin, 5 mg/l transferrin, pH7.0) conditioned media. The conditioned media was collected three days later, and put into a BaF3 proliferation assay using Alamar Blue,
5 described in Example 19 below.

Example 19

Mouse zcytor17lig does not activate human zcytor17 receptor in BaF3 assay using Alamar Blue

10 Proliferation of BaF3/zcytor17, BaF3/zcytor17/OSMRbeta and BaF3/zcytor17/WSX-1 cells (Example 4, and 5B) was assessed using serum-free conditioned media from BHK cells expressing mouse zcytor17lig (Example 18). BaF3/Zcytor17, BaF3/zcytor17/OSMRbeta and BaF3/zcytor17/WSX-1 cells were spun down, washed and plated in mIL-3 free media as described in Example 5B.
15 Conditioned media from BHK cells expressing mouse zcytor17lig (Example 18) was diluted with mIL-3 free media to 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.75% and 0.375% concentrations. The proliferation assay was performed as per Example 5B. The results of this assay were negative, indicating that mouse zcytor17lig does not activate human zcytor17, zcytor17/OSMRbeta, or zcytor17/WSX-1 receptor complexes.

20

Example 20

Human zcytor17lig activates human, zcytor17/OSMRbeta receptor, in luciferase assay

A. Construction of BaF3/KZ134/zcytor17 cell line

The KZ134 plasmid was constructed with complementary
25 oligonucleotides ZC12,749 (SEQ ID NO:44) and ZC12,748 (SEQ ID NO:45) that contain STAT transcription factor binding elements from 4 genes, which includes a modified c-fos Sis inducible element (m67SIE, or hSIE) (Sadowski, H. et al., *Science* 261:1739-1744, 1993), the p21 SIE1 from the p21 WAF1 gene (Chin, Y. et al., *Science* 272:719-722, 1996), the mammary gland response element of the β -casein gene
30 (Schmitt-Ney, M. et al., *Mol. Cell. Biol.* 11:3745-3755, 1991), and a STAT inducible element of the Fcg RI gene, (Seidel, H. et al., *Proc. Natl. Acad. Sci.* 92:3041-3045,

1995). These oligonucleotides contain Asp718-XhoI compatible ends and were ligated, using standard methods, into a recipient firefly luciferase reporter vector with a c-fos promoter (Poulsen, L.K. et al., *J. Biol. Chem.* 273:6229-6232, 1998) digested with the same enzymes and containing a neomycin selectable marker. The KZ134 plasmid was
5 used to stably transfect BaF3 cells, using standard transfection and selection methods, to make the BaF3/KZ134 cell line.

A stable BaF3/KZ134 indicator cell line, expressing the full-length zcytor17 receptor or zcytor17/OSMRbeta receptor was constructed as per Example 4. Clones were diluted, plated and selected using standard techniques. Clones were
10 screened by luciferase assay (see Example 20B, below) using the human zcytor17lig conditioned media or purified zcytor17lig protein (see Example 35, below) as an inducer. Clones with the highest luciferase response (via STAT luciferase) and the lowest background were selected. Stable transfectant cell lines were selected. The cell lines were called BaF3/KZ134/zcytor17 or BaF3/KZ134/zcytor17/OSMRbeta
15 depending on the receptors transfected into the cell line.

Similarly, BHK cell lines were also constructed using the method described herein, and were used in luciferase assays described herein. The cell lines were called BHK/KZ134/zcytor17 or BHK/KZ134/zcytor17/OSMRbeta depending on the receptors transfected into the cell line.

20

B. Human zcytor17lig activates human zcytor17 receptor in BaF3/KZ134/zcytor17/OSMRbeta or BHK/KZ134/zcytor17/OSMRbeta luciferase assay

BaF3/KZ134/zcytor17 and BaF3/KZ134/zcytor17/OSMRbeta cells were spun down and washed in mIL-3 free media. The cells were spun and washed 3 times
25 to ensure removal of mIL-3. Cells were then counted in a hemacytometer. Cells were plated in a 96-well format at about 30,000 cells per well in a volume of 100 µl per well using the mIL-3 free media. The same procedure was used for untransfected BaF3/KZ134 cells for use as a control in the subsequent assay. BHK/KZ134/zcytor17 or BHK/KZ134/zcytor17/OSMRbeta cells were plated in a 96-well format at 15,000
30 cells per well in 100 µl media. Parental BHK/KZ134 cells were used as a control.

STAT activation of the BaF3/KZ134/Zcytor17,

BaF3/KZ134/zcytor17/OSMRbeta, BHK/KZ134/zcytor17, or BHK/KZ134/zcytor17/OSMRbeta cells was assessed using (1) conditioned media from BHK570 cells transfected with the human zcytor17lig (Example 7), (2) conditioned media from BHK570 cells transfected with the mouse zcytor17lig (Example 18), (3) purified human zcytor17lig (Example 35), or (4) mIL-3 free media to measure media-only control response. Conditioned media was diluted with RPMI mIL-3 free media to 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5%, 0.75% and 0.375% concentrations. Purified human zcytor17lig was diluted to a concentration of 1200, 600, 300, 150, 75, 37.5, 18.75, or 9.4 pM. 100 µl of the diluted conditioned media or protein was added to the BaF3/KZ134/Zcytor17, BaF3/KZ134/zcytor17/OSMRbeta, BHK/KZ134/zcytor17, or BHK/KZ134/zcytor17/OSMRbeta cells. The assay using the conditioned media was done in parallel on untransfected BaF3/KZ134 or BHK/KZ134 cells as a control. The total assay volume was 200 µl. The assay plates were incubated at 37°C, 5% CO₂ for 24 hours at which time the BaF3 cells were pelleted by centrifugation at 2000 rpm for 10 min., and the media was aspirated and 25 µl of lysis buffer (Promega) was added. For the BHK cell lines, the centrifugation step was not necessary as the cells are adherant. After 10 minutes at room temperature, the plates were measured for activation of the STAT reporter construct by reading them on a luminometer (Labsystems Luminoskan, model RS) which added 40 µl of luciferase assay substrate (Promega) at a five second integration.

The results of this assay confirmed that the STAT reporter response of the BaF3/KZ134/zcytor17/OSMRbeta and BHK/KZ134/zcytor17/OSMRbeta cells to the human zcytor17lig when compared to either the BaF3/KZ134/zcytor17 cells, the BHK/KZ134/zcytor17 cells or the untransfected BaF3/KZ134 or BHK/KZ134 control cells, showed that the response was mediated through the zcytor17/OSMRbeta receptors. The results also showed that the mouse zcytor17lig does not activate the STAT reporter assay through the human receptor complex.

Example 21

Mouse zcytor17lig is active in mouse bone marrow assay

A. Isolation of Non-adherent Low Density Marrow Cells:

Fresh mouse femur aspirate (marrow) is obtained from 6-10 week old male Balb/C or C57BL/6 mice. The marrow is then washed with RPMI+10% FBS (JRH, Lenexa KS; Hyclone, Logan UT) and suspended in RPMI+10% FBS as a whole marrow cell suspension. The whole marrow cell suspension is then subjected to a density
5 gradient (Nycoprep, 1.077, Animal; Gibco BRL) to enrich for low density, mostly mononuclear, cells as follows: The whole marrow cell suspension (About 8 ml) is carefully pipetted on top of about 5 ml Nycoprep gradient solution in a 15 ml conical tube, and then centrifuged at 600X g for 20 minutes. The interface layer, containing the low density mononuclear cells, is then removed, washed with excess RPMI+10% FBS, and
10 pelleted by centrifugation at 400X g for 5-10 minutes. This pellet is resuspended in RPMI +10% FBS and plated in a T-75 flask at approximately 10^6 cells/ml, and incubated at 37°C 5% CO₂ for approximately 2 hours. The resulting cells in suspension are Non-Adherent Low Density (NA LD) Marrow Cells.

15 *B. 96-Well Assay*

NA LD Mouse Marrow Cells are plated at 25,000 to 45,000 cells/well in 96 well tissue culture plates in RPMI +10% FBS + 1ng/mL mouse Stem Cell Factor (mSCF) (R&D Systems, Minneapolis, MN), plus 5% conditioned medium from one of the following: (1) BHK 570 cells expressing mouse zcytor17lig (Example 18), (2)
20 BHK 570 cells expressing human zcytor17lig (Example 7), or (3) control BHK 570 cells containing vector and not expressing either Ligand. These cells are then subjected to a variety of cytokine treatments to test for expansion or differentiation of hematopoietic cells from the marrow. For testing, the plated NA LD mouse marrow cells are subjected to human Interleukin-15 (hIL-15) (R&D Systems), or one of a panel
25 of other cytokines (R&D Systems). Serial dilution of hIL-15, or the other cytokines, are tested, with 2-fold serial dilution from about 50 ng/ml down to about 0.5 ng/ml concentration. After 8 to 12 days the 96-well assays are scored for cell proliferation by Alamar blue assay as described in Example 5B.

30 *C. Results from the 96-well NA LD Mouse Marrow assay*

Conditioned media from the BHK cells expressing both mouse and

human zcytor17lig can promote the expansion of a population of hematopoietic cells either alone or in synergy with other cytokines in the NA LD mouse marrow in comparison to control BHK conditioned medium. The population hematopoietic cells expanded by the mouse zcytor17lig with or without other cytokines, and those
5 hematopoietic cells expanded by the human zcytor17lig with or without other cytokines, are further propagated in cell culture. These hematopoietic cells are stained with a Phycoerythrin labeled anti-Pan NK cell antibody (PharMingen) and subjected to flow cytometry analysis, which demonstrated that the expanded cells stained positively for this natural killer (NK) cell marker. Similarly, other specific hematopoietic cell
10 markers can be used to determine expansion of, for example, CD4+ or CD8+ T-cells, other T-cell populations, B-cells, and other immune cell markers.

The same 96-well assay is run, using fresh human marrow cells bought from Poietic Technologies, Gaithersburg, MD. Again, a positive result shows that zcytor17lig alone or in synergy with other cytokines, the mouse and human zcytor17lig
15 can expand a hematopoietic cell population that is stained positively for specific cell markers as disclosed above.

Example 22

Constructs for generating zcytor17lig Transgenic Mice

20 A. *Construct for expressing human zcytor17lig from the MT-1 promoter*
Oligonucleotides are designed to generate a PCR fragment containing a consensus Kozak sequence and the human zcytor17lig coding region. These oligonucleotides are designed with an FseI site at the 5' end and an AscI site at the 3' end to facilitate cloning into (a) pMT12-8, our standard transgenic vector, or (b) pKFO51, a lymphoid-
25 specific transgenic vector (Example 22B).

PCR reactions are carried out with about 200 ng human zcytor17lig template (SEQ ID NO:1) and oligonucleotides designed to amplify the full-length or active portion of the zcytor17lig. PCR reaction conditions are determined using methods known in the art. PCR products are separated by agarose gel electrophoresis
30 and purified using a QiaQuick™ (Qiagen) gel extraction kit. The isolated, correct sized DNA fragment is digested with FseI and AscI (Boehringer-Mannheim), ethanol

precipitated and ligated into pMT12-8 previously digested with FseI and AscI. The pMT12-8 plasmid, designed for expressing a gene of interest in liver and other tissues in transgenic mice, contains an expression cassette flanked by 10 kb of MT-1 5' DNA and 7 kb of MT-1 3' DNA. The expression cassette comprises the MT-1 promoter, the
5 rat insulin II intron, a polylinker for the insertion of the desired clone, and the human growth hormone (hGH) poly A sequence.

About one microliter of each ligation reaction is electroporated into DH10B ElectroMax™ competent cells (GIBCO BRL, Gaithersburg, MD) according to manufacturer's direction and plated onto LB plates containing 100 µg/ml ampicillin,
10 and incubated overnight. Colonies are picked and grown in LB media containing 100 µg/ml ampicillin. Miniprep DNA is prepared from the picked clones and screened for the human zcytor17lig insert by restriction digestion with EcoRI alone, or FseI and AscI combined, and subsequent agarose gel electrophoresis. Maxipreps of the correct pMT-human zcytor17lig are performed. A SalI fragment containing with 5' and 3' flanking
15 sequences, the MT-1 promoter, the rat insulin II intron, human zcytor17lig cDNA and the hGH poly A sequence is prepared to be used for microinjection into fertilized murine oocytes. Microinjection and production of transgenic mice are produced as described in Hogan, B. et al. *Manipulating the Mouse Embryo*, 2nd ed., Cold Spring Harbor Laboratory Press, NY, 1994.

20

B. Construct for expressing human zcytor17lig from the lymphoid-specific EµLCK promoter

Oligonucleotides are designed to generate a PCR fragment containing a consensus Kozak sequence and the human zcytor17lig coding region. These
25 oligonucleotides are designed with an FseI site at the 5' end and an AscI site at the 3' end to facilitate cloning into pKFO51, a lymphoid-specific transgenic vector.

PCR reactions are carried out with about 200 ng human zcytor17lig template (SEQ ID NO:1) and oligonucleotides designed to amplify the full-length or active portion of the zcytor17lig. A PCR reaction is performed using methods known
30 in the art. The isolated, correct sized DNA fragment is digested with FseI and AscI (Boehringer-Mannheim), ethanol precipitated and ligated into pKFO51 previously

digested with FseI and AscI. The pKFO51 transgenic vector is derived from p1026X (Iritani, B.M., et al., *EMBO J.* 16:7019-31, 1997) and contains the T cell-specific lck proximal promoter, the B/T cell-specific immunoglobulin μ heavy chain enhancer, a polylinker for the insertion of the desired clone, and a mutated hGH gene that encodes
5 an inactive growth hormone protein (providing 3' introns and a polyadenylation signal).

About one microliter of each ligation reaction is electroporated, plated, clones picked and screened for the human zcytor17lig insert by restriction digestion as described above. A correct clone of pKFO51-zcytor17lig is verified by sequencing, and a maxiprep of this clone is performed. A NotI fragment, containing the lck proximal
10 promoter and immunoglobulin μ enhancer (E μ LCK), zcytor17lig cDNA, and the mutated hGH gene is prepared to be used for microinjection into fertilized murine oocytes.

C. Construct for expressing mouse zcytor17lig from the EF1alpha promoter

15 Primers ZC41,498 (SEQ ID NO:86) and ZC41,496 (SEQ ID NO:87) were used to PCR a mouse testis cDNA library template. These PCR reactions were successfully performed in approximately 50 μ l volumes with or without 10% DMSO, using pfu turbo polymerase (Stratagene) according to the manufacturer's recommendations; with an additional application of a wax hot-start employing hot start
20 50s (Molecular Bioproducts, Inc. San Diego, CA). PCR thermocycling was performed with a single cycle of 94°C for 4 min; followed by 40 cycles of 94°C: 30 seconds, 48°C: 30 seconds, 72°C: 50 seconds; with additional final 72°C extension for 7 minutes. The two PCR reactions were pooled and purified using low melt agarose and Gelase agarose digesting enzyme (Epicenter, Inc. Madison, WI) according to the
25 manufacturer's recommendations.

DNA sequenced PCR products revealed a murine zcytor17 cDNA sequence (SEQ ID NO:90) which comprised an ORF identical to SEQ ID NO:10, confirming that SEQ ID NO:10 encoded the mouse zcytor17lig polypeptide. PCR primers, ZC41583 (SEQ ID NO:88) and ZC41584 (SEQ ID NO:89), were then used to
30 add FseI and AscI restriction sites and a partial Kozak sequence to the mcytor17lig open reading frame and termination codon (SEQ ID NO:92). A Robocycler 40

thermocycler (Stratagene) was used to run a temperature gradient of annealing temperatures and cycling as follows. Pfu turbo polymerase (Stratagene) was applied as described above, but only in 10% DMSO. Cycling was performed with a single cycle of 94°C for 4 min; followed by 20 cycles of 94°C: 30 seconds, 65°C to 51°C gradient: 30
5 seconds, 72°C: 1 minute; and a single 72°C extension for 7 minutes. The template for this second thermocycling reaction was 1 µl of the initial gel-purified mcytor17lig PCR product, above. Resulting PCR product from the three lowest temperature reactions were pooled and gel purified using the Gelase (Epicenter) method described above. This purified fragment was then digested with FseI and AscI and ligated into a pZP7X
10 vector modified to have FseI and AscI sites in its cloning site. This was sent to sequencing to confirm the correct sequence. The cloned murine cDNA sequence is represented in SEQ ID NO:90, and corresponding polypeptide sequence is shown in SEQ ID NO:91 (which is identical to SEQ ID NO:11).

The isolated, correct sized DNA fragment digested with FseI and AscI
15 (Boehringer-Mannheim) was subcloned into a plasmid containing EF1alpha promoter previously digested with FseI and AscI. Maxipreps of the correct EF1alpha mouse zcytor17lig were performed. The expression cassette contains the EF1alpha promoter (with a deleted FseI site), the EF1alpha intron, SUR IRES like site to facilitate expression, a polylinker flanked with rat insulin II sites on the 5' end which adds FseI
20 PmeI AscI sites for insertion of the desired clone, and the human growth hormone (hGH) poly A sequence. A 7.5kb NotI fragment containing the EF1alpha promoter expression cassette and mouse zcytor17lig was prepared to be used for microinjection into fertilized murine oocytes. The EF1alpha plsdmid was obtained from Louis-Marie of the Laboratoire de Differentiation Cellulaire, as described in Taboit-Dameron et al.,
25 1999, *Transgenic Research* 8:223-235.

D. Construct for expressing mouse zcytor17lig from the lymphoid-specific EµLCK promoter

Oligonucleotides were designed to generate a PCR fragment containing
30 a consensus Kozak sequence and the mouse zcytor17lig coding region. These oligonucleotides were designed with an FseI site at the 5' end and an AscI site at the 3'

end to facilitate cloning into pKFO51 (see Example 22B, above).

The isolated, correct sized zcytor17lig DNA fragment used in EF1alpha constructs, digested with FseI and AscI (Boehringer-Mannheim), was subcloned into a plasmid containing pKFO51, a lymphoid-specific transgenic vector. The pKFO51
5 transgenic vector is derived from p1026X (Iritani, B.M., et al., *EMBO J.* 16:7019-31, 1997) and contains the T cell-specific lck proximal promoter, the B/T cell-specific immunoglobulin μ heavy chain enhancer, a polylinker for the insertion of the desired clone, and a mutated hGH gene that encodes an inactive growth hormone protein (providing 3' introns and a polyadenylation signal). A 6.5kb NotI fragment, containing
10 the lck proximal promoter and immunoglobulin μ enhancer (E μ LCK), mouse zcytor17lig cDNA, and the mutated hGH gene was prepared to be used for microinjection into fertilized murine oocytes (Example 41).

Example 23

15 Construction of mammalian expression vectors that express zcytor17lig-CEE

A. Construction of zCyt17Lig-CEE/pZMP21

An expression plasmid containing all or part of a polynucleotide encoding human zCyt17Lig was constructed via homologous recombination. The plasmid was called zCyt17Lig-CEE/pZMP21.

20 The construction of zCyt17Lig-CEE/pZMP21 was accomplished by generating a zCyt17Lig-CEE fragment (SEQ ID NO:95) using PCR amplification. The DNA template used for the production of the zCyt17Lig-CEE fragment was zCyt17Lig/pZP7nx. The primers used for the production of the zCyt17Lig-CEE fragment were: (1) ZC41607 (SEQ ID NO:97) (sense sequence), which includes from
25 the 5' to the 3' end: 28bp of the vector flanking sequence (5' of the insert) and 21 bp corresponding to the 5' sequence of zCyt17Lig; and (2) ZC41605 (SEQ ID NO:98) (anti-sense sequence), which includes from the 5' to the 3' end: 37 bp of the vector flanking sequence (3' of the insert), 3 bp of the stop codon, 21 bp encoding a C-terminal EE tag, and 21 bp corresponding to the 3' end of zCyt17Lig sequence. The
30 fragment resulting from the above PCR amplification is a copy of the template zCyt17Lig with the addition of a C-terminal EE tag, yielding a final product

zCytol17Lig-CEE.

PCR reactions were run as follows: To a 100 μ l final volume was added: 10 μ l of 10x Taq Polymerase Reaction Buffer with 15mM MgCl (Gibco), 1 μ l of Taq DNA Polymerase (5 units/ μ l, Gibco), 3 μ l of 10mM dNTPs, 78 μ l dH₂O, 3 μ l of a 20 pmol/ μ l stock of primer ZC41607 (SEQ ID NO:97) 3 μ l of a 20 pmol/ μ l stock of primer ZC41605 (SEQ ID NO:98), and 2 μ l of a 0.13 μ g/ μ l stock of zCytol17lig template DNA. A volume equal to 50 μ l of mineral oil was added to the mixture. The reaction was heated to 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute; 55°C for 2 minutes; 72°C for 3 minutes; followed by a 10 minute extension at 72°C and held at 4°C until the reaction was collected.

The plasmid pZMP21 was restriction digested with BglII enzyme, cleaned with a QiaQuick PCR Purification Kit (Qiagen) using a microcentrifuge protocol, and used for recombination with the PCR fragment. Plasmid pZMP21 was constructed from pZMP20 which was constructed from pZP9 (deposited at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, and is designated No. 98668) with the yeast genetic elements taken from pRS316 (deposited at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, and designated No. 77145), an IRES element from poliovirus, and the extracellular domain of CD8, truncated at the carboxyl terminal end of the transmembrane domain. PZMP21 is a mammalian expression vector containing an expression cassette having the MPSV promoter, immunoglobulin signal peptide intron, multiple restriction sites for insertion of coding sequences, a stop codon and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene, the SV40 terminator, as well as the URA3 and CEN-ARS sequences required for selection and replication in *S. cerevisiae*.

Fifty microliters of competent yeast cells (*S. cerevisiae*) were independently combined with 100 ng of cut plasmid, 5 μ l of previously described PCR mixture, and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV (5 kV/cm), ∞ ohms, 25 μ F. Each cuvette had 600 μ l of 1.2 M sorbitol added, and the yeast was plated in one 100 μ l aliquot and one 300 μ l

aliquot onto two URA-D plates and incubated at 30°C. After about 72 hours, the Ura+ yeast transformants from a single plate were resuspended in 1 ml H₂O and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 500 µl of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). The 500 µl of the lysis mixture was added to an Eppendorf tube containing 300 µl acid washed 600µm glass beads and 300 µl phenol-chloroform, vortexed for 1 minute intervals two or three times, followed by a 5 minute spin in a EppendorfTM centrifuge at maximum speed. Three hundred microliters of the aqueous phase was transferred to a fresh tube, and the DNA precipitated with 600 µl 100% ethanol (EtOH), followed by centrifugation for 10 minutes at 4°C. The DNA pellet was then washed with 500µl 70% EtOH, followed by centrifugation for 1 minute at 4°C. The DNA pellet was resuspended in 30 µl H₂O.

Transformation of electrocompetent *E. coli* cells (MC1061) was done with 5 µl of the yeast DNA prep and 50 µl of MC1061 cells. The cells were electropulsed at 2.0 kV, 25 µF and 400 ohms(Ω). Following electroporation, 600µl SOC (2% Bacto[®] Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose) was added. The electroporated *E.coli* cells were plated in a 200µl and a 50µl aliquot on two LB AMP plates (LB broth (Lennox), 1.8% Bacto Agar (Difco), 100 mg/L Ampicillin). The plates were incubated upside down for about 24 hours at 37°C. Three Ampicillin-resistant colonies were selected at random and submitted for sequence analysis of the insert. Large scale plasmid DNA was isolated from a sequence-confirmed clone using the Qiagen Maxi kit (Qiagen) according to manufacturer's instructions.

25 B. Mammalian Expression of human zcytor17lig

Full-length zCytot17Lig protein was produced in BHK cells transfected with zCytot17Lig-CEE/pZMP21 (Example 23A). BHK 570 cells (ATCC CRL-10314) were plated in T75 tissue culture flasks and allowed to grow to approximately 50 to 70% confluence at 37°C, 5% CO₂, in growth media (SL7V4, 5%FBS, 1% pen/strep). The cells were then transfected with zCytot17Lig-CEE/pZMP21 by liposome-mediated transfection (using LipofectamineTM; Life Technologies), in serum free (SF) media

(SL7V4). The plasmid (16 μ g) was diluted into 1.5 ml tubes to a total final volume of 640 μ l with SF media. Thirty-five microliters of the lipid mixture was mixed with 605 μ l of SF medium, and the resulting mixture was allowed to incubate approximately 15 minutes at room temperature. Five milliliters of SF media was then added to the DNA:lipid mixture. The cells were rinsed once with 10 ml of PBS, the PBS was decanted, and the DNA:lipid mixture was added. The cells are incubated at 37°C for five hours, then 15 ml of media (SL7V4, 5% FBS, 1% pen/strep) was added to each plate. The plates were incubated at 37°C overnight, and the DNA:lipid media mixture was replaced with selection media (SL7V4, 5% FBS, 1% pen/strep, 1 μ M methotrexate) the next day. Approximately 10 days post-transfection, methotrexate-resistant colonies from the T75 transfection flask were trypsinized, and the cells were pooled and plated into a T-162 flask and transferred to large-scale culture.

Example 24

Expression of zcytor17 Soluble Receptor in E. coli

A. Construction of expression vector pCMH01 that expresses huzcytor17/MBP-6H fusion polypeptide

An expression plasmid containing a polynucleotide encoding a zcytor17 soluble receptor fused C-terminally to maltose binding protein (MBP) was constructed via homologous recombination. The fusion polypeptide contains an N-terminal approximately 388 amino acid MBP portion fused to any of the zcytor17 soluble receptors described herein. A fragment of zcytor17 cDNA (SEQ ID NO:4) was isolated using PCR as described herein. Two primers were used in the production of the zcytor17 fragment in a standard PCR reaction: (1) one containing about 40 bp of the vector flanking sequence and about 25 bp corresponding to the amino terminus of the zcytor17, and (2) another containing about 40 bp of the 3' end corresponding to the flanking vector sequence and about 25 bp corresponding to the carboxyl terminus of the zcytor17. Two μ l of the 100 μ l PCR reaction was run on a 1.0% agarose gel with 1 x TBE buffer for analysis, and the expected approximately fragment was seen. The remaining PCR reaction was combined with the second PCR tube and precipitated with 400 μ l of absolute ethanol. The precipitated DNA used for recombining into the SmaI

cut recipient vector pTAP170 to produce the construct encoding the MBP-zcytor17 fusion, as described below.

Plasmid pTAP170 was derived from the plasmids pRS316 and pMAL-c2. The plasmid pRS316 is a *Saccharomyces cerevisiae* shuttle vector (Hieter P. and
5 Sikorski, R., *Genetics* 122:19-27, 1989). pMAL-C2 (NEB) is an *E. coli* expression plasmid. It carries the *tac* promoter driving *MalE* (gene encoding MBP) followed by a His tag, a thrombin cleavage site, a cloning site, and the *rrnB* terminator. The vector pTAP170 was constructed using yeast homologous recombination. 100ng of EcoR1 cut pMAL-c2 was recombined with 1µg Pvu1 cut pRS316, 1µg linker, and 1µg
10 Sca1/EcoR1 cut pRS316. The linker consisted of oligos zc19,372 (SEQ ID NO:172) (100pmole): zc19,351 (SEQ ID NO:173) (1pmole): zc19,352 (SEQ ID NO:174) (1pmole), and zc19,371 (SEQ ID NO:175) (100pmole) combined in a PCR reaction. Conditions were as follows: 10 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 30 seconds; followed by 4°C soak. PCR products were concentrated via
15 100% ethanol precipitation.

One hundred microliters of competent yeast cells (*S. cerevisiae*) were combined with 10 µl of a mixture containing approximately 1 µg of the human zcytor17 insert, and 100 ng of SmaI digested pTAP170 vector, and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV (5
20 kV/cm), infinite ohms, 25 µF. To each cuvette was added 600 µl of 1.2 M sorbitol. The yeast was then plated in two 300 µl aliquots onto two -URA D plates and incubated at 30°C.

After about 48 hours, the Ura+ yeast transformants from a single plate were picked, DNA was isolated, and transformed into electrocompetent *E. coli* cells
25 (e.g., MC1061, Casadaban et. al. *J. Mol. Biol.* 138, 179-207), and plated on MM/CA +KAN 25 µg/L plates (Pryor and Leiting, *Protein Expression and Purification* 10:309-319, 1997) using standard procedures. Cells were grown in MM/CA with 25 µg/ml Kanomycin for two hours, shaking, at 37°C. One ml of the culture was induced with 1mM IPTG. Two to four hours later the 250 µl of each culture was mixed with 250 µl
30 acid washed glass beads and 250 µl Thorner buffer with 5% βME and dye (8M urea,

100 mM Tris pH7.0, 10% glycerol, 2mM EDTA, 5% SDS). Samples were vortexed for one minute and heated to 65°C for 10 minutes. 20 µl were loaded per lane on a 4%-12% PAGE gel (NOVEX). Gels were run in 1XMES buffer. The positive clones were designated pCMH01 and subjected to sequence analysis.

5 One microliter of sequencing DNA was used to transform strain BL21. The cells were electropulsed at 2.0 kV, 25 µF and 400 ohms. Following electroporation, 0.6 ml MM/CA with 25 µg/L Kanomycin. Cells were grown in MM/CA and induced with ITPG as described above. The positive clones were used to grow up for protein purification of the huzcytor17/MBP-6H fusion protein using
10 standard techniques.

B. Purification of huzcytor17/MBP-6H soluble receptor from E.coli fermentation

Unless otherwise noted, all operations were carried out at 4°C. The following procedure was used for the purification of recombinant huzcytor17/MBP-6H
15 soluble receptor polypeptide. *E. coli* cells containing the pCMH01 construct and expressing huzcytor17/MBP-6H soluble receptor polypeptide were constructed using standard molecular biology methods and cultured in SuperBroth II (12 g/L Casien, 24 g/L Yeast Extract, 11.4 g/L di-potassium phosphate, 1.7 g/L Mono-potassium phosphate; Becton Dickenson, Cockeysville, MD). The resulting cells were harvested
20 and frozen in 0.5% glycerol. Twenty grams of the frozen cells were used for protein purification.

Thawed cells were resuspended in 500mL Amylose Equilibration buffer (20mM Tris, 100mM NaCl, pH 8.0). A French Press cell breaking system (Constant Systems Ltd., Warwick, UK) with a temperature setting of -7°C to -10°C and 30K PSI
25 was used to lyse the cells. The resuspended cells were checked for breakage by A₆₀₀ readings before and after cycling through the French Press. The lysed cell suspension was pelleted at 10,000G for 30 minutes. Supernatant was harvested from the debris pellet for protein purification.

Twenty-five milliliters of Amylose resin (New England Biolabs,
30 Beverly, MA) was poured into a Bio-Rad, 2.5 cm D x 10 cm H glass column. The column was packed and equilibrated by gravity with 10 column volumes (CVs) of

Amylose Equilibration buffer. The harvested cell supernatant was batch loaded to the Amylose resin, overnight with rocking. The loaded resin was returned to the glass column, washed with 10 CVs Amylose Equilibration buffer, and eluted by gravity with ~2 CVs Amylose Elution buffer (Amylose Equilibration buffer, 10 mM Maltose, Fluka Biochemical, Switzerland). Ten 5 ml fractions were collected over the elution profile and assayed for absorbance at 280 and 320 nM. The Amylose resin was regenerated with 1 CV of distilled H₂O, 5 CVs of 0.1% (w/v) SDS (Sigma), 5 CVs of distilled H₂O, 5 CVs of Amylose Equilibration buffer, and finally 1 CV of Amylose Storage buffer (Amylose Equilibration buffer, 0.02% (w/v) Sodium Azide). The regenerated resin was stored at 4°C.

Elution profile fractions of interest were pooled and dialyzed in a 10K dialysis chamber (Slide-A-Lyzer, Pierce Immunochemical) against 4 changes of 4L PBS pH 7.4 (Sigma) over an 8 hour time period. Following dialysis, the material harvested represented the purified huzcytor17/MBP-6H polypeptide. The purified huzcytor17/MBP-6H polypeptide was filter sterilized and analyzed via SDS-PAGE Coomassie staining for an appropriate molecular weight product. The concentration of the huzcytor17/MBP-6H polypeptide was determined by BCA analysis to be 0.76 mg/ml.

Purified huzcytor17/MBP-6H polypeptide was appropriately formulated for the immunization of rabbits and sent to R & R Research and Development (Stanwood, WA) for polyclonal antibody production (Example 25, below).

Example 25

Human zcytor17 Receptor Polyclonal Antibody

A. Preparation and Purification

Polyclonal antibodies were prepared by immunizing 2 female New Zealand white rabbits with the purified recombinant protein huzcytor17/MBP-6H (Example 24). The rabbits were each given an initial intraperitoneal (IP) injection of 200 µg of purified protein in Complete Freund's Adjuvant followed by booster IP injections of 100 µg protein in Incomplete Freund's Adjuvant every three weeks. Seven to ten days after the administration of the second booster injection (3 total

injections), the animals were bled and the serum was collected. The animals were then boosted and bled every three weeks.

The huzcytor17/MBP-6H specific rabbit serum was pre-adsorbed of anti-MBP antibodies using a CNBr-SEPHAROSE 4B protein column (Pharmacia LKB) that was prepared using 10 mg of non-specific purified recombinant MBP-fusion protein per gram of CNBr-SEPHAROSE. The huzcytor17/MBP-6H-specific polyclonal antibodies were affinity purified from the pre-adsorbed rabbit serum using a CNBr-SEPHAROSE 4B protein column (Pharmacia LKB) that was prepared using 10 mg of the specific antigen purified recombinant protein huzcytor17/MBP-6H. Following purification, the polyclonal antibodies were dialyzed with 4 changes of 20 times the antibody volume of PBS over a time period of at least 8 hours. Human zcytor17-specific antibodies were characterized by ELISA using 500 ng/ml of the purified recombinant protein huzcytor17/MBP-6H as antibody target. The lower limit of detection (LLD) of the rabbit anti-huzcytor17/MBP-6H affinity purified antibody is 500 pg/ml on its specific purified recombinant antigen huzcytor17/MBP-6H.

B. SDS-PAGE and Western blotting analysis of Rabbit Anti-human ZcytoR17 MBP-6H antibody

Rabbit anti-human ZcytoR17 MBP-6H antibody was tested by SDS-PAGE (NuPage 4-12%, Invitrogen, Carlsbad, CA) with coomassie staining method and Western blotting using goat anti-rabbit IgG-HRP. Either purified protein (200-25 ng) or conditioned media containing zcytor17 was electrophoresed using an Invitrogen Novex's Xcell II mini-cell, and transferred to nitrocellulose (0.2 mm; Invitrogen, Carlsbad, CA) at room temperature using Novex's Xcell blot module with stirring according to directions provided in the instrument manual. The transfer was run at 300 mA for one hour in a buffer containing 25 mM Tris base, 200 mM glycine, and 20% methanol. The filter was then blocked with Western A buffer (in house, 50 mM Tris, pH 7.4, 5 mM EDTA, pH 8.0, 0.05% Igepal CA-630, 150 mM NaCl, and 0.25% gelatin) overnight with gentle rocking at 4°C. The nitrocellulose was quickly rinsed, then the rabbit anti-human zcytoR17 MBP-6H (1:1000) was added in Western A buffer. The blot was incubated for 1.5 hours at room temperature with gentle rocking. The blot

was rinsed 3 times for 5 minutes each in Western A, then goat anti-rabbit IgG HRP antibody (1:1000) was added in Western A buffer. The blot was incubated for 1.25 hours at room temperature with gentle rocking. The blot was rinsed 3 times for 5 minutes each in Western A, then quickly rinsed in H₂O. The blot was developed using
5 commercially available chemiluminescent substrate reagents (ECL Western blotting detection reagents 1 and 2 mixed 1:1; reagents obtained from Amersham Pharmacia Biotech, Buckinghamshire, England) and the blot was exposed to X-ray film for up to 15 minutes.

The rabbit anti-human zcytoR17 MBP-6H was able to detect human
10 zcytoR17 present in conditioned media as well as zcytoR17 purified protein as a band at 120 kDa under reducing conditions.

Example 26

Tissue Distribution of Mouse zcytoR17 in Tissue Panels Using PCR

15 A panel of cDNAs from murine tissues was screened for mouse zcytoR17 expression using PCR. The panel was made in-house and contained 94 marathon cDNA and cDNA samples from various normal and cancerous murine tissues and cell lines are shown in Table 6, below. The cDNAs came from in-house libraries or marathon cDNAs from in-house RNA preps, Clontech RNA, or Invitrogen RNA. The
20 mouse marathon cDNAs were made using the marathon-Ready™ kit (Clontech, Palo Alto, CA) and QC tested with mouse transferrin receptor primers ZC10,651 (SEQ ID NO:46) and ZC10,565 (SEQ ID NO:47) and then diluted based on the intensity of the transferrin band. To assure quality of the amplified library samples in the panel, three tests for quality control (QC) were run: (1) To assess the RNA quality used for the
25 libraries, the in-house cDNAs were tested for average insert size by PCR with vector oligos that were specific for the vector sequences for an individual cDNA library; (2) Standardization of the concentration of the cDNA in panel samples was achieved using standard PCR methods to amplify full length alpha tubulin or G3PDH cDNA using a 5' vector oligo: ZC14,063 (SEQ ID NO:48) and 3' alpha tubulin specific oligo primer
30 ZC17,574 (SEQ ID NO:49) or 3' G3PDH specific oligo primer ZC17,600 (SEQ ID NO:50); and (3) a sample was sent to sequencing to check for possible ribosomal or

mitochondrial DNA contamination. The panel was set up in a 96-well format that included a mouse genomic DNA (Clontech, Palo Alto, CA) positive control sample. Each well contained approximately 0.2-100 pg/ μ l of cDNA. The PCR was set up using oligos ZC38,065 (SEQ ID NO:51) and ZC38,068 (SEQ ID NO:52), TaKaRa Ex TaqTM (TAKARA Shuzo Co LTD, Biomedicals Group, Japan), and Rediload dye (Research Genetics, Inc., Huntsville, AL). The amplification was carried out as follows: 1 cycle at 94°C for 5 minutes; 5 cycles of 94°C for 30 seconds, 68°C for 30 seconds; 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds and 72°C for 30 seconds, followed by 1 cycle at 72°C for 5 minutes. About 10 μ l of the PCR reaction product was subjected to standard Agarose gel electrophoresis using a 4% agarose gel. The correct predicted DNA fragment size was observed in brain, CD90+ cells, dendritic, embryo, MEWt#2, Tuvak-prostate cell line, salivary gland, skin and testis.

The DNA fragment for skin and testis were excised and purified using a Gel Extraction Kit (Qiagen, Chatsworth, CA) according to manufacturer's instructions. Fragments were confirmed by sequencing to show that they were indeed mouse zcytor17.

Table 6

Tissue/Cell line	#samples		Tissue/Cell line	#samples
229	1			
7F2	1			
Adipocytes-Amplified	1			
aTC1.9	1			
Brain	4			
CCC4	1			
CD90+ Amplified	1			
OC10B	1			
Dentritic	1			
Embyro	1			
Heart	2			
Kidney	3			
Liver	2			
Lung	2			
MEWt#2	1			
P388D1	1			
Pancreas	1			
Placenta	2			
Jakotay-Prostate Cell Line	1			
Nelix-Prostate Cell Line	1			
Paris-Prostate Cell Line	1			
Torres-Prostate Cell Line	1			
Tuvak-Prostate Cell Line	1			
Salivary Gland	2			
Skeletal Muscle	1			
Skin	2			
Small Intestine	1			
Smooth Muscle	2			
Spleen	2			
Stomach	1			
Testis	3			
Thymus	1			

Example 27: Human Zcytor17 Expression in Various Tissues Using Real-Time

5

Quantitative RT/PCR

A. Primers and Probes for Human Zcytor17, OSMRbeta and Zcytor17lig for Conventional and Quantitative RT-PCR

Real-time quantitative RT-PCR using the ABI PRISM 7900 Sequence Detection System (PE Applied Biosystems, Inc., Foster City, CA) has been previously

described (See, Heid, C.A. et al., *Genome Research* 6:986-994, 1996; Gibson, U.E.M. et al., *Genome Research* 6:995-1001, 1996; Sundaresan, S. et al., *Endocrinology* 139:4756-4764, 1998). This method incorporates use of a gene specific probe containing both reporter and quencher fluorescent dyes. When the probe is intact the
5 reporter dye emission is negated due to the close proximity of the quencher dye. During PCR extension using additional gene-specific forward and reverse primers, the probe is cleaved by 5' nuclease activity of Taq polymerase which releases the reporter dye from the probe resulting in an increase in fluorescent emission.

The primers and probes used for real-time quantitative RT-PCR analyses
10 of human Zcytor17, OSMRbeta and Zcytor17ligand expression were designed using the primer design software Primer Express™ (PE Applied Biosystems, Foster City, CA). Primers for human Zcytor17 were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC37,877 (SEQ ID NO:53) and the reverse primer, ZC37,876 (SEQ ID NO:54) were used in a
15 PCR reaction at a 200 nM concentration to synthesize a 73 bp product. The corresponding Zcytor17 TaqMan® probe, designated ZC37,776 (SEQ ID NO:55) was synthesized and labeled by PE Applied Biosystems and used in each PCR reaction at a concentration of 200nM. The ZC37,776 (SEQ ID NO:55) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied
20 Biosystems) and at the 3' end with a fluorescent quencher dye (6-carboxy-tetramethyl-rhodamine) (TAMRA) (Epoch Biosciences, Bothell, WA).

Primers for human OSMRbeta were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC43,891 (SEQ ID NO:137) and the reverse primer, ZC43,900 (SEQ ID NO:138) were
25 used in a PCR reaction (below) at a 200 nM concentration. The corresponding OSMRbeta TaqMan® probe, designated ZC43,896 (SEQ ID NO:139) was synthesized and labeled by PE Applied Biosystems and used in each PCR reaction at a concentration of 200nM. The ZC43,896 (SEQ ID NO:139) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied
30 Biosystems) and at the 3' end with a non-fluorescent quencher dye (ECLIPSE) (Epoch Biosciences).

Primers for human Zcytor17ligand were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC43,280 (SEQ ID NO:140) and the reverse primer, ZC43,281 (SEQ ID NO:141) were used in a PCR reaction (below) at about 200 nM concentration. The
5 corresponding Zcytor17ligand TaqMan® probe, designated ZC43,275 (SEQ ID NO:142) was synthesized and labeled by PE Applied Biosystems and used in each PCR reaction at a concentration of 200nM. The ZC43,275 (SEQ ID NO:142) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied Biosystems) and at the 3' end with a non-fluorescent quencher dye (ECLIPSE)
10 (Epoch Biosciences).

As a control to test the integrity and quality of RNA samples tested, all RNA samples were screened for either rRNA or GUS using primer and probe sets either ordered from PE Applied Biosystems (rRNA kit) or designed in-house (GUS). The rRNA kit contained the forward primer (SEQ ID NO:56), the rRNA reverse primer
15 (SEQ ID NO:57), and the rRNA TaqMan® probe (SEQ ID NO:58). The rRNA probe was labeled at the 5'end with a reporter fluorescent dye VIC (PE Applied Biosystems) and at the 3' end with the quencher fluorescent dye TAMRA (PE Applied Biosystems). The GUS primers and probe were generated in-house and used in each PCR reaction at 200nM and 100nM, respectively. The forward primer was ZC40,574 (SEQ ID
20 NO:143) and the reverse primer was ZC40,575 (SEQ ID NO:144). The GUS probe ZC43,017 (SEQ ID NO:145) was labeled at the 5'end with a reporter fluorescent dye (Yakima-Yellow) (Epoch Biosciences) and at the 3'end with a non-fluorescent quencher dye (ECLIPSE) (Epoch Biosciences). The rRNA and GUS results also serve as an endogenous control and allow for the normalization of the Zcytor17 mRNA
25 expression results seen in the test samples.

For conventional non-quantitative RT-PCR, primers were designed using the primer design software Primer Express™ (PE Applied Biosystems, Foster City, CA). The human zcytor17 primers generate an approximately 1000 base pair product and are as follows: forward primer ZC28,917 (SEQ ID NO:83), and reverse
30 primer ZC28,480 (SEQ ID NO:146). The human OSMRbeta primers generate a 202 base pair product and are as follows: forward primer ZC41,653(SEQ ID NO:147) and

reverse primer ZC41,655 (SEQ ID NO:148). The human Zcytor17ligand primers generate a 305 base pair product and are as follows: forward primer ZC41,703 (SEQ ID NO:149) and reverse primer ZC41,704 (SEQ ID NO:150).

5 *B. Primers and Probes for Murine Zcytor17, OSMRbeta and Zcytor17ligand for Conventional and Quantitative RT-PCR*

The primers and probes used for real-time quantitative RT-PCR analyses of murine Zcytor17, OSMRbeta and Zcytor17lig expression were designed using the primer design software Primer Express™ (PE Applied Biosystems, Foster City, CA).
10 Primers for murine Zcytor17 were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC43,272 (SEQ ID NO:151) and the reverse primer, ZC43,273 (SEQ ID NO:152) were used in the PCR reactions (below) at 300 nM concentration. The corresponding Zcytor17 TaqMan® probe, designated ZC43,478 (SEQ ID NO:153) was synthesized and labeled
15 by PE Applied Biosystems. The ZC43,478 (SEQ ID NO:153) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied Biosystems) and at the 3' end with a quencher fluorescent dye (6-carboxy-tetramethyl-rhodamine) (TAMRA) (PE Applied Biosystems). The ZC43,478 (SEQ ID NO:153) probe was used in the PCR reactions at a concentration of 100nM.

20 Primers for murine Zcytor17ligand were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer, ZC43,278 (SEQ ID NO:154) and the reverse primer, ZC43,279 (SEQ ID NO:155) were used in the PCR reactions at 500 nM concentration. The corresponding Zcytor17ligand TaqMan® probe, designated ZC43,276 (SEQ ID NO:156) was
25 synthesized and labeled by PE Applied Biosystems. The ZC43,478 (SEQ ID NO:153) probe was labeled at the 5'end with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied Biosystems) and at the 3' end with a non-fluorescent quencher dye (ECLIPSE) (Epoch Biosciences). The ZC43,276 (SEQ ID NO:156) probe was used in the PCR reactions (below) at a concentration of 200nM.

30 Primers for murine OSMRbeta were designed spanning an intron-exon junction to eliminate possible amplification of genomic DNA. The forward primer,

ZC43,045 (SEQ ID NO:157) and the reverse primer, ZC43,046 (SEQ ID NO:158) were used in the PCR reactions at a 300 nM concentration. The corresponding OSMRbeta TaqMan® probe, designated ZC43,141(SEQ ID NO:159) was synthesized and labeled by Epoch Biosciences. The ZC43,141 (SEQ ID NO:159) probe was labeled at the 5'end
5 with a reporter fluorescent dye (6-carboxy-fluorescein) (FAM) (PE Applied Biosystems) and at the 3' end with a non-fluorescent quencher dye (ECLIPSE) (Epoch Biosciences). The ZC43,141 (SEQ ID NO:159) probe was used in the PCR reactions (below) at a concentration of 100nM.

As a control to test the integrity and quality of RNA samples tested, all
10 RNA samples were screened for either murine GUS or transferrin receptor using primers and probes designed using the primer design program Primer Express™ (PE Applied Biosystems Inc., Foster City, CA). The murine GUS primers are as follows: forward primer, ZC43,004 (SEQ ID NO:160), reverse primer, ZC43,005 (SEQ ID NO:161), and TaqMan® probe ZC43,018 (SEQ ID NO:162). The murine GUS probe
15 ZC43,018 (SEQ ID NO:162) was labeled at the 5'end with a reporter fluorescent dye Yakima-Yellow (Epoch Biosciences) and at the 3' end with the non-fluorescent quencher dye ECLIPSE (Epoch Biosciences). The murine GUS primers were used in the PCR reactions at 300 nM and the probe, ZC43,018 (SEQ ID NO:162), was used at 100nM. In some cases murine Transferrin Receptor was used instead of GUS as the
20 endogenous control. The transferrin receptor forward primer, ZC40,269 (SEQ ID NO:163) and the reverse primer, ZC40,268 (SEQ ID NO:164) were used at 300nM. The transferrin receptor probe, ZC40,298 (SEQ ID NO:165) was used in PCR at 100nM and was labeled at the 5'end with a reporter fluorescent dye VIC (PE Applied Biosystems) and at the 3'end with a fluorescent quencher dye (TAMRA) (PE Applied
25 Biosystems). The murine GUS and transferrin receptor results also serve as an endogenous control and allow for the normalization of the Zcytor17, OSMRbeta and Zcytor17ligand mRNA expression results seen in the test samples.

For conventional semi-quantitative RT-PCR, primers were designed using the primer design software Primer Express™ (PE Applied Biosystems). The
30 murine Zcytor17 primers generate a 276 base pair product and are as follows: forward primer ZC43,140 (SEQ ID NO:166), and reverse primer ZC43,139 (SEQ ID NO:167).

The murine OSMRbeta primers generate a 575 base pair product and are as follows: forward primer ZC41,608 (SEQ ID NO:168) and reverse primer ZC41,609 (SEQ ID NO:169). The murine Zcytor17ligand primers generate a 657bp product and are as follows: forward primer ZC41,502 (SEQ ID NO:170) and reverse primer ZC41,500 (SEQ ID NO:171).

C. Protocols for Realtime Quantitative RT-PCR and Conventional Semi-quantitative RT-PCR

Relative levels of Zcytor17, OSMRbeta and Zcytor17ligand mRNA were determined by analyzing total RNA samples using the one-step RT-PCR method (PE Applied Biosystems). Total RNA from Zcytor17- and OSMRbeta-transfected BAF cells (human) or BHK cells (murine) was isolated by standard methods and used to generate a standard curve used for quantitation of Zcytor17 and OSMRbeta. The curve consisted of 10-fold serial dilutions ranging from 100-0.01ng/ μ l with each standard curve point analyzed in triplicate. Similarly, for Zcytor17ligand, activated CD4+ T cell RNA (previously shown to make Zcytor17ligand) was used to generate a standard curve in the same 100-0.01ng/ μ l range. Total RNA from human or murine cells was analyzed in triplicate for either human or murine Zcytor17, OSMRbeta and Zcytor17ligand transcript levels and for one of the following endogenous control genes: rRNA, GUS or transferrin receptor. In a total volume of 10 μ l, each RNA sample was subjected to a One-Step RT-PCR reaction containing: approximately 50-100ng of total RNA in a preformulated 2X master mix containing an internal control dye (ROX)(carboxy-x-rhodamine) and Thermo-Start® DNA Polymerase (Abgene, Surrey, UK); appropriate primers for the gene of interest (see parts A and B of current example); the appropriate probe (see parts A and B for concentration); Superscript® reverse transcriptase (50 U/ μ l) (PE Applied Biosystems), and an appropriate volume of RNase-free water. PCR thermal cycling conditions were as follows: an initial reverse transcription (RT) step of one cycle at 48°C for 30 minutes; followed by a Thermo-Start® enzyme activation step of one cycle at 95°C for 10 minutes; followed by 40 cycles of amplification at 95°C for 15 seconds and 60°C for 1 minute. Relative Zcytor17, OSMRbeta and Zcytor17ligand RNA levels were determined by using the Standard Curve Method as described by the

manufacturer, PE Biosystems (User Bulletin #2: ABI Prism 7700 Sequence Detection System, Relative Quantitation of Gene Expression, December 11, 1997). The rRNA, GUS or Transferrin Receptor measurements were used to normalize the levels of the gene of interest.

5 The semi-quantitative RT-PCR reactions used the 'Superscript One-StepTM RT-PCR System with Platinum Taq' (Invitrogen, Carlsbad, CA). Each 25 μ l reaction consisted of the following: 12.5 μ l of 2X Reaction Buffer, 0.5 μ l (20pmol/ μ l) of forward primer, 0.5 μ l (20pmol/ μ l) of reverse primer, 0.4 μ l RT/Taq polymerase mix, 5.0 μ l of Rediload Gel Loading Buffer (Invitrogen), 5.1 μ l RNase-free water, and 1.0 μ l total
10 RNA (100ng/ μ l). The amplification was carried out as follows: one cycle at 45°C for 30 minutes followed by 35-38 cycles of 94°C, 20 seconds; Variable annealing temp (See Table 7 below), 20 seconds; 72°C, 45 seconds; then ended with a final extension at 72°C for 5 minutes. Eight to ten microliters of the PCR reaction product was subjected to standard agarose gel electrophoresis using a 2% agarose gel.

15

Table 7

Murine Zcytor17	58°C anneal temp
Murine OSMRbeta	60°C anneal temp
Murine Zcytor17ligand	52°C anneal temp
Human Zcytor17	55°C anneal temp
Human OSMRbeta	59°C anneal temp
Human Zcytor17ligand	59°C anneal temp

D. Isolation of RNA from Human and Murine PBMC Subsets and Cell Lines

20 Blood was drawn from several anonymous donors and peripheral blood mononuclear cells (PBMC) isolated using standard Ficoll gradient methodology. Monocytes were then isolated using the Monocyte Isolation Kit and the Magnetic Cell Separation System (Miltenyi Biotec, Auburn, CA). The monocytes were then plated onto ultra-low adherence 24-well plates in endotoxin-free media. They were either
25 unstimulated or treated with recombinant human IFN γ (R&D Systems Inc.) at 10ng/ml. Cells were collected at 24 and 48 hours. In similar manner, CD4+ and CD8+ T cells were isolated from PBMC's using the anti-CD4 or anti-CD8 magnetic beads from

Miltenyi Biotec. Cells were then activated for 4 or 16 hours in tissue culture plates coated with 0.5 μ g/ml anti-CD3 antibodies in media containing 5 μ g/ml anti-CD28 antibodies. NK cells were also isolated from PBMC's using Miltenyi's anti-CD56 coated magnetic beads. Some of the NK cells were collected at time zero for RNA and
5 the others were plated in media containing Phorbol Myristate Acetate (PMA) (5ng/ml) and ionomycin (0.5 μ g/ml) for 24 hours. Additionally, several human monocyte-like cell lines, U937, THP-1 and HL-60, were collected in either their resting or activated states. U937 cells were activated overnight with PMA (10ng/ml). HL-60's were activated overnight with PMA (10ng/ml) or for 72 and 96 hours with IFNg (10ng/ml) to
10 drive them down a monocytic pathway. THP-1 cells were activated overnight with a combination of LPS (10ng/ml) and IFNg (10ng/ml). RNA was prepared from all primary cells using the RNeasy Midiprep™ Kit (Qiagen, Valencia, CA) as per manufacturer's instructions. Carryover DNA was removed using the DNA-Free™ kit (Ambion, Inc., Austin, TX). RNA concentration was determined using standard
15 spectrophotometry and RNA quality determined using the Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).

Murine T Cell RNA was collected using a variety of methods well-known in the art. Primary splenic CD4+ and CD8+ T cells were isolated from the spleens of C57Bl/6 mice using antibody-coated magnetic beads and the Magnetic Cell
20 Separation System from Miltenyi Biotec. The CD4+ and CD8+ T cells were then activated by culturing the cells in 24-well plates coated with anti-CD3 antibodies (500ng/ml) in media containing anti-CD28 antibodies at 5 μ g/ml. Cells were harvested for RNA at 0, 4 and 16 hours. Similarly, CD4+ T cells were isolated and then skewed towards a Th1 or Th2 phenotype using the following protocol. Since C57Bl/6 T cells
25 are already skewed in the Th1 direction, all that was required was to activate for 6 hours with 0.5 μ g/ml PMA and 10ng/ml ionomycin. 'Th2' skewing was obtained by plating naïve CD4+ T cells with 2.5 μ g/ml anti-CD28, 10ng/ml mIL-2 (R&D Systems Inc.) and 25ng/ml mIL-4 (R&D Systems) into plates coated with 0.5 μ g/ml anti-CD3. After 2 days in culture, cells were resuspended in media containing 10ng/ml mIL-2 (R&D
30 Systems) and 25ng/ml mIL-4. Cells were cultured for an additional three days then activated with PMA and ionomycin for 6 hours.

One additional set of Th1 and Th2 skewed T cells was derived using the T Cell Receptor Transgenic DO11.10 T cell line. All cells were plated into anti-CD3 and anti-CD28 coated plates. The 'Th1' cells were plated in media containing mIL-12 (1ng/ml) and anti-IL-4 (10 μ g/ml). The 'Th2' cells were plated in media containing
5 mIL-4 (10ng/ml) and anti-IFN γ (10 μ g/ml). After 24 hours, all cultures were given mIL-2 (10ng/ml). After two more days, the media on the cells was changed and new media containing the aforementioned cytokines was added and cells were cultured an additional 4 days before being harvested.

All of the murine T cell RNA was prepared using the RNeasy
10 Midiprep™ Kit (Qiagen) and contaminating DNA was removed using the DNA-free™ kit from Ambion.

E. Isolation of RNA from the Murine Models of Pancreatitis and Irritable Bowel Disease

15 To induce a condition similar to human Irritable Bowel Disease (IBD), the hybrid mouse strain C57Bl6/129S6F1 was used. Mice were divided into 4 groups with an average size of six mice per group. Group 1 was given no Dextran Sulfate Sodium (DSS) and was sacrificed on day 14. Group 2 received 2% DSS for two days prior to being sacrificed. Group 3 received 2% DSS for seven days prior to sacrifice.
20 Group 4 received 2% DSS for seven days then allowed to recover for seven days and was sacrificed on day 14. On the day of sacrifice, the distal colon sections were removed and placed in RNAlater™ (Ambion). The colon sections were homogenized using standard techniques and RNA was isolated using the RNeasy Midiprep™ Kit (Qiagen). Contaminating DNA was removed by DNA-free™ (Ambion) treatment as
25 per manufacturer's instructions.

In a different study, acute pancreatitis was induced in male CD-1 mice by caerulein injection. Mice were divided into three groups (n= 8 mice/group). Group 1 animals were given seven i.p. injections (1 injection per hour) with Vehicle (saline), and then sacrificed at 12 and 24 hours following the first injection. Groups 2 and 3
30 were given seven i.p. injections of caerulein (Sigma) (Catalog#C-9026) at a dose of 50 μ g/kg/hr for six hours (1 injection per hour). Group 2 was sacrificed at 12 hrs after

the first injection and Group 3 was sacrificed at 24 hrs following the first injection. Pancreases were removed at the time of sacrifice and snap frozen for RNA isolation. Tissues were homogenized and RNA was isolated using the Qiagen RNeasy Midiprep™ Kit.

5 In yet another study, murine Zcytor17ligand transgenic mice were generated and observed for phenotypic changes (see Example 41). Piloerection and hair loss was observed in many of the transgenic mice. Four transgenic mice were sacrificed and skin samples from both normal and hairless areas were removed and snap frozen for later RNA isolation. Skin sections from two non-transgenic control mice
10 were collected as well. Skin samples were homogenized and then digested with Proteinase K (Qiagen) (Catalog# 19133) for 20 minutes at 60°C. RNA was then isolated using the Qiagen RNeasy Midiprep™ Kit following manufacturer's instructions. Carryover DNA was removed using DNA-free™ kit from Ambion.

15 *F. Results of Quantitative and Semi-Quantitative RT-PCR for Human Zcytor17, OSMRbeta and Zcytor17ligand*

 Zcytor17 and OSMRbeta expression was examined by quantitative RT-PCR in four sets of primary human monocytes that were either in their resting state or activated with IFNγ for 24 or 48 hours. Zcytor17 expression was below detection in the
20 unstimulated cells but increased dramatically after the 24-hour activation with IFNγ, and was the highest after 48 hours of activation. In all cases OSMRbeta was below detection. Zcytor17ligand was not tested in these samples.

 In the primary T cells, Zcytor17 was below detection in both the resting CD4+ and CD8+ subsets. After a four-hour activation, however, expression of
25 Zcytor17 went up in both subsets and then decreased to a slightly lower level at the 16 hour time point. OSMRbeta was below detection in these samples. Zcytor17ligand expression was examined using semi-quantitative RT-PCR. No expression was detected in the unstimulated CD4+ and CD8+ T cells. However, after the four hour activation, high levels of Zcytor17ligand were detected. This level dropped somewhat
30 at the 16 hour time point.

 Expression of Zcytor17 was not examined in NK cells. OSMRb was

below detection in these samples. Zcytor17ligand expression was below detection in the resting NK cells, however there was a faint signal generated by the activated NK cells suggesting that these cells may make Zcytor17ligand under certain conditions.

In the human monocyte-like cell lines, U937, THP-1 and HL-60, OSMRbeta expression was below detection in all of the resting and activated samples except for activated THP-1 samples where a faint signal was detected. Zcytor17 expression was high in both the U937 and THP-1 resting cell lines and showed a strong upregulation following activation. Expression in U937's was the highest of any cell type. In the HL-60's, Zcytor17 was expressed at moderate levels in the unstimulated cells and decreased upon stimulation with PMA. However, the expression of Zcytor17 was dramatically upregulated in the HL-60's when stimulated with IFNg for 72 and 96 hours. All of the human expression data is summarized in Table 8 below.

Table 8

Primary Human Monocytes	Activation Status	Zcytor17	OSMRbeta	Zcytor17Ligand
Human Monocytes	Unstim	-	-	
Human Monocytes	Act. 24hr IFNg	+	-	
Human Monocytes	Act. 48hr IFNg	++	-	
Human CD4+	Unstim	-	-	-
Human CD4+	Act 4hr	++	-	++
Human CD4+	Act. 16hr	+	-	+
Human CD8+	Unstim	-	-	-
Human CD8+	Act 4hr	++	-	++
Human CD8+	Act. 16hr	+	-	+
Human NK Cells	Unstim		-	-
Human NK Cells	Act 24hr		-	+
U937	Unstim	++	-	-
U937	Act. 16hr	+++	-	-
THP-1	Unstim	++	-	-
THP-1	Act. 16hr	+++	+	-
HL-60	Unstim	++	-	-
HL-60	Act. 16hr PMA	+	-	-
HL-60	Act. 72hr IFNg	+++	-	-
HL-60	Act. 96hr IFNg	+++	-	-

15

G. Results of Quantitative and Semi-Quantitative RT-PCR for Murine Zcytor17, OSMRbeta and Zcytor17ligand

Murine Zcytor17, OSMRbeta and Zcytor17ligand expression levels were examined in several murine T cells populations and the results are summarized in Table 9 below. Murine Zcytor17 expression was tested by semi-quantitative RT-PCR and shown to be at low levels on both resting and activated primary CD4+ T cells.

5 Expression of Zcytor17 was detected on resting CD8+ T cells and then seemed to drop upon activation with anti-CD3 and anti-CD28 antibodies at both the 4- and 16-hour time points. OSMRbeta expression was measured by quantitative RT-PCR and shown to be expressed in resting and activated CD4+ and CD8+ T cells. The expression of OSMRbeta went up after a 4-hour activation and then returned to the unstimulated
10 levels by 16 hours in both the CD4+ and CD8+ T cells. Zcytor17ligand was detected by quantitative RT-PCR and shown to be expressed at very low levels in unstimulated CD4+ T cells. However, following a 4-hour activation, Zcytor17ligand expression was dramatically upregulated and then dropped slightly by the 16-hour time point. In CD8+ T cells, no Zcytor17ligand was detected in the unstimulated cells. There was some
15 Zcytor17ligand expression at the 4-hour time point, but by 16 hours expression levels had dropped back below detection.

In the DO11.10 T cells, Zcytor17 expression was detected in the naïve and Th2 skewed cells, but not in the Th1 skewed cells. OSMRbeta expression was at low levels in the naïve DO11.10 cells. There was a dramatic increase in OSMRbeta
20 expression levels in the Th1 skewed cells and a moderate increase of expression in the Th2-skewed cells. The Zcytor17ligand expression in these cells was shown to be predominantly by the Th2 skewed subset. Low levels were detected in the Th1 subset and no expression was detected in the naïve cells. These results are summarized in the Table 9 below.

25 In the primary CD4+ T cells that were skewed in either the Th1 or Th2 direction, Zcyto17 wasn't examined. OSMRbeta expression was detected in all three samples with the highest levels found in the Th2 sample. Similar to the DO11.10 results, Zcytor17ligand expression was detected at high levels in the Th2 skewed subset, with a small amount detected in the Th1 subset and levels were below detection
30 in the unstimulated cells. These results are summarized in the Table 9 below.

Table 9

Murine T Cells	Zcytor17	OSMRbeta	Zcytor17ligand
CD4+ T Cells Unstimulated	+	+	+/-
CD4+ T Cells 4hr Activation	+	++	++
CD4+ T Cells 16hr Activation	+	+	+
CD8+ T Cells Unstimulated	+	+	-
CD8+ T Cells 4hr Activation	+/-	++	+
CD8+ T Cells 16hr Activation	-	+	-
DO11.10 Naïve	+	+	-
DO11.10 Th1	-	+++	+
DO11.10 Th2	+	++	++
CD4+ T Cells Unstimulated		++	-
CD4+ T Cells - Th1 Skewed		+++	+
CD4+ T Cells - Th2 Skewed		++	+++

In the Zcytor17ligand transgenic skin samples, Zcytor17, OSMRbeta and Zcytor17ligand expression levels were determined using quantitative RT-PCR.

- 5 Zcytor17 was shown to be present in all samples at roughly equivalent levels. There were dramatically higher levels of OSMRbeta expression in the non-transgenic control animals than the transgenic samples. Zcytor17ligand expression was below detection in the non-transgenic control animals with moderate to high levels of expression in the transgenic animals. The results are summarized in Table 10 below.

10

Table 10

Murine Zcytor17ligand	Skin			
Transgenic Skin	Phenotype	Zcytor17	OSMRbeta	Zcytor17ligand
Wild Type Mouse	Normal	+	+++	-
Wild Type Mouse	Normal	+	+++	-
Transgenic #1	Normal	+	+	+
Transgenic #1	Hair Loss	+	+	+
Transgenic #2	Normal	+	+	+
Transgenic #2	Hair Loss	+	+	+
Transgenic #3	Normal	+	+	+
Transgenic #3	Hair Loss	+	+	+
Transgenic #4	Normal	+	+	+++
Transgenic #4	Hair Loss	+	+	+++

In a different experiment, Zcytor17, OSMRbeta and Zcytor17ligand expression levels were measured by quantitative RT-PCR in the pancreases of mice

subjected to acute pancreatitis. Zcytor17 expression was below detection in all of the samples. OSMRbeta expression was seen at low levels in the normal control samples (Group 1), but showed a strong upregulation at the 12-hour time point (Group 2) and slightly lower levels at the 24-hour time point (Group 3). Zcytor17ligand expression was below detection in the control animals, but showed high levels in both of the caerulein injected groups. The data is summarized in Table 11 below.

Table 11

Pancreatitis Model	Description	Zcytor17	OSMRbeta	Zcytor17ligand
Group 1	Normal Control	-	+	-
Group 2	12hr Post Injection	-	+++	++
Group 3	24hr Post Injection	-	++	++

In another experiment, Zcytor17 and OSMRbeta expression levels were examined in the distal colons of mice subjected to DSS treatment. In this murine model of Inflammatory Bowel Disease, expression levels of both genes were determined by quantitative RT-PCR and are summarized in Table 12 below. Zcytor17 expression levels increased with the severity of the disease, with low levels of expression in the Group 1 normal animals and increasing amounts seen Groups 2 and 3. In the Group 4 animals, the Zcytor17 levels had returned to more normal levels. Unlike Zcytor17 expression, OSMRbeta levels were the highest in the control animals and levels actually decreased in all three DSS treated groups.

Table 12

IBD Model	Description	SAC Day		Zcytor17	OSMRbeta
Group 1	Normal Control	14		+	++
Group 2	DSS-Treated 2 days	2		++	+
Group 3	DSS-Treated 7 days	7		+++	+
Group 4	DSS-Treated 7 days	14		+	+

Example 28

Human Zcytor17lig Tissue Distribution Expression based on RT-PCR Analysis of Multiple Tissue First-Strand cDNAs

Gene expression of the zcytor17lig was examined using commercially

available normalized multiple tissue first-strand cDNA panels (OriGene Technologies, Inc. Rockville, MD; BD Biosciences Clontech, Palo Alto, CA). These included the OriGene "Human Tissue Rapid-Scan™ Panel" (Cat. #CHSCA-101, containing 22 different tissues, bone marrow, and plasma blood leucocytes) and the BD Biosciences
5 Clontech "Human Blood Fractions MTC™ Panel" (Cat. #K1428-1, containing 9 different blood fractions).

PCR reactions were set up using the zcytor17lig specific oligo primers ZC41,458 (SEQ ID NO:60), and ZC41,457 (SEQ ID NO:61), which yield a 139 bp product, and ZC41,459 (SEQ ID NO:62), and ZC41,460 (SEQ ID NO:63), which yield
10 a 92 bp product, Qiagen HotStarTaq DNA polymerase and buffer (Qiagen, Inc., Valencia, CA), dH₂O, and RediLoad™ dye (Research Genetics, Inc., Huntsville, AL). The PCR cyclor conditions were as follows: an initial 1 cycle 15 minute denaturation at 95°C, 35 cycles of a 45 second denaturation at 95°C, 1 minute annealing at 53°C or 56°C and 1 minute and 15 seconds extension at 72°C, followed by a final 1 cycle
15 extension of 7 minutes at 72°C. The reactions were separated by electrophoresis on a 2% agarose gel (EM Science, Gibbstown, NJ) and visualized by staining with ethidium bromide.

A DNA fragment of the correct size was observed in the following human adult tissues using the OriGene "Human Tissue Rapid-Scan™ Panel": testis,
20 plasma blood leucocytes (PBL), and bone marrow.

A DNA fragment of the correct size was observed in the following human blood fractions using the BD Biosciences Clontech "Human Blood Fractions MTC™ Panel": activated mononuclear cells (B- & T-cells and monocytes), activated CD8+ cells (T-suppressor/cytotoxic), activated CD4+ cells (T-helper/inducer) and
25 faintly in resting CD8+ cells.

Example 29

Cloning the human Oncostatin M receptor

The OncostatinM beta receptor (OSMRbeta) is a type I cytokine receptor
30 with structural similarity to IL12R-B2. ZcytoR17 has structural similarity to IL12R - B1. The OSMRbeta and zcytor17 were tested to see whether they could interact as

subunits in a cytokine signaling complex, and whether together they could act as a signaling receptor, or soluble receptor antagonist, for zcytor17lig.

To isolate OSMRbeta, oligonucleotide PCR primers ZC39982 (SEQ ID NO:64) and ZC39983 (SEQ ID NO:65) were designed to amplify the full length coding
5 region of the human OncostatinM beta cDNA sequence (SEQ ID NO:6) (Genbank Accession No. U60805; Mosley B, *JBC Volume 271*, Number 50, Issue of December 20, 1996 pp. 32635-32643).

PCR reactions were run on an array of cDNA library templates using a robust polymerase, Advantage II (Clontech, PaloAlto, CA), in order to identify a
10 source of the cDNA. The template DNA used was from amplified cDNA plasmid libraries each containing 5 million independent cDNA clones. Reactions were assembled as per manufacturer's instructions using 400 fmol/μl of each oligonucleotide and 2-20 ng/μl purified plasmid library DNA as template. The cDNA libraries were derived from the following human tissues and cell lines: fetal brain, prostate smooth
15 muscle, bone marrow, RPMI1588, thyroid, WI-38, testis, stimulated peripheral blood mononuclear cells, stimulated CD3+ cells, THP-1, activated tonsil, HACAT and fetal liver. Reactions were performed on a thermocycler machine using the following conditions: 30 cycles of 95°C for 20 seconds, 68°C for 3 minutes. At the conclusion of 30 cycles an additional single extension cycle of 8 minutes at 68°C was run. PCR
20 products were visualized by TAE agarose, gel electrophoresis in the presence of ethidium bromide followed by UV illumination. The most abundant product was found to be from a prostate smooth muscle cDNA library. The PCR reaction using prostate smooth muscle template and oligonucleotides ZC39982 (SEQ ID NO:64) and ZC39983 (SEQ ID NO:65) was repeated using a less robust but higher fidelity thermostable DNA
25 polymerase "turboPFu", (Stratagene, La Jolla, CA). Thirty amplification cycles were run with the following conditions: denaturing at 94°C, 30 seconds, annealing at 63°C 45 seconds, extension at 72°C 3.5 minutes. A single band product was gel purified on a 0.8% TAE, agarose gel.

This DNA was then amplified again using primers ZC39980 (SEQ ID
30 NO:66) and ZC39981 (SEQ ID NO:67) designed to include restriction enzyme recognition sequences to allow the cloning of this cDNA into a mammalian expression

vector.

The PCR reaction was performed using "TurboPfu" and the purified PCR product for 15 cycles of: 95°C 1 minute, 64°C 1 minute 20 seconds, 72°C 4.5 minutes. The PCR reaction was then digested with EcoR1 and Xho1 (Invitrogen, Carlsbad,CA) and gel purified as described above. A mammalian expression vector, pZ7NX, was prepared by digesting with EcoR1 and Xho1 and the PCR product was ligated to this vector and electroporated into *E. coli* DH10b cells. Several bacterial colonies were isolated and sequenced. One clone was correct with the exception of a single non-conservative mutation. In order to change this base to match the expected sequence, an oligonucleotide spanning mutation and a neighboring Pst1 restriction site was used in a PCR reaction with "TurboPfu" using the pZP7Nx-h. OncostatinM R plasmid previously sequenced as a template. The PCR amplified DNA was digested with Pst1 and Xho1 and cloned back into the pZP7Nx-h OncostatinM R plasmid in place of the Pst1/Xho1 fragment containing the offending mutation. This new plasmid was sequenced over the recently amplified Pst1 to Xho1 region to confirm the correction and make sure no other errors were created in the amplification process. This analysis confirmed sequence that matched the expected sequence over the coding region. The sequence is shown in SEQ ID NO:6, and corresponding amino acid sequence shown in SEQ ID NO:7.

20

Example 30

Constructs for Generating a Human Zcytor17 / OncostatinM receptor (OSMRbeta)

Heterodimer

A system for construction, expression and purification of such soluble heterodimeric receptors is known in the art, and has been adapted to the receptor pair, human oncostatin M receptor (OSMRbeta) and human zcytor17. For this construct, the polynucleotide for the soluble receptor for OSMRbeta is shown in SEQ ID NO:68 and corresponding polypeptide is shown in SEQ ID NO:69; and the polynucleotide for the soluble receptor for human zcytor17 is shown in SEQ ID NO:70 and corresponding polypeptide is shown in SEQ ID NO:71.

30

To construct a cell line expressing a secreted soluble hzcytor17/human

OSMRbeta heterodimer, a construct was made so that the resulting heterodimeric soluble receptor comprises the extracellular domain of human OSMRbeta fused to the heavy chain of IgG gamma1 (Fc4) (SEQ ID NO:37) with a Glu-Glu tag (SEQ ID NO:35) at the C-terminus; while the extracellular domain of zcytoR17 is fused to Fc4 (SEQ ID NO:37) with a His tag (SEQ ID NO:72) at the C-terminus. For both of the hzcytor17 and human OSMRbeta arms of the heterodimer a Gly-Ser spacer of 12 amino acids (SEQ ID NO:73) was engineered between the extracellular portion of the receptor and the N-terminus of Fc4.

10 *A. Construction of human soluble OSMRbeta/Fc4-CEE*

For construction of the human soluble OSMRbeta/Fc4-CEE portion of the heterodimer the extracellular portion of human OSMRbeta was isolated using PCR with oligos ZC14063 (SEQ ID NO:48) and ZC41557 (SEQ ID NO:74) under PCR reaction conditions as follows: 30 cycles of 95°C for 60 sec., 57°C for 30 sec., and 72°C for 100 sec.; and 72°C for 7 min. PCR products were purified using QIAquick PCR Purification Kit (Qiagen), digested with EcoR1 and BglII (Boehringer-Mannheim), separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen).

The expression cassette, plasmid backbone and Fc4-GluGlu tag portion of the chimera were contained within a previously made in house plasmid vector. The plasmid vector was digested with EcoR1 and BamH1 (Boehringer-Mannheim), separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen). The digested and purified fragments of human OSMRbeta and Fc4-CEE containing plasmid were ligated together using T4 DNA Ligase (Life Technologies, Bethesda, MD) using standard ligation methods. Minipreps of the resulting ligation were screened for an EcoRI/SmaI insert of the correct size (772bp) for the soluble OSMRbeta and positive minipreps were sequenced to confirm accuracy of the PCR reaction. This new plasmid construction is termed pZP9-ONCOMR-Fc4CEE.

30 *B. Construction of human soluble Zcytor17 / Fc4-CHIS*

For construction of the hzcytor17/Fc4-CHIS portion of the heterodimer,

the extracellular portion of human zcytor17 was isolated by digestion of a plasmid previously containing Zcytor17-Fc4 soluble receptor. The plasmid was first digested with Sal1 (New England Biolabs, Beverly, MA) after which the reaction was serially phenol chloroform extracted and ethanol precipitated. The digested DNA was then
5 treated with T4 DNA Polymerase (Boehringer-Mannheim), to fill in the 5' overhangs created by the Sal1 digestion, leaving the DNA ends blunt, after which the reaction was serially phenol chloroform extracted and ethanol precipitated. The blunted DNA was then further digested with BglII to cut at the 3' end.), separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen) as per manufacturer's
10 instruction. The resulting DNA fragment containing the sequence coding for the extracellular domain of zcytoR17 was ligated into an Fc4-CHIS tag containing mammalian expression vector prepared as follows.

The expression cassette, plasmid backbone and Fc4-CHIS tag portion of the chimera were contained within a previously made in house plasmid vector. This
15 plasmid vector was digested with EcoR1(Boehringer-Mannheim) after which the reaction was serially phenol chloroform extracted and ethanol precipitated. The digested DNA was then treated with T4 DNA Polymerase (Boehringer-Mannheim), to fill in the 5' overhangs created by the EcoR1 digestion, leaving the DNA ends blunt, after which the reaction was serially phenol chloroform extracted and ethanol
20 precipitated. The blunted DNA was then further digested with BamH1 (Boehringer-Mannheim) to cut at the 3' end, separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen). The digested and purified fragments of human zcytor17 and Fc4-CHIS containing plasmid were ligated together using T4 DNA Ligase (Life Technologies, Bethesda, MD) using standard ligation methods.

25 Minipreps of the resulting ligation were screened by PCR using the zcytor17 specific sense primer ZC29180 (SEQ ID NO:22) and the Fc4 specific antisense primer ZC29232 (SEQ ID NO:75) with the following PCR reaction conditions: 30 cycles of 94°C for 60 sec., 68°C for 150 sec; and 72°C for 7 min. An expected product size of 848 bp confirmed the correct assembly of the plasmid termed
30 pZEM228 hzcytor17/Fc4HIS.

A second zcytor17-Fc4 construction was created for use in generating

homodimer protein from COS cells. Briefly the coding region for the full fusion protein was isolated by digestion of a plasmid previously containing Zcytor17-Fc4 soluble receptor with SalI (Boehringer-Mannheim). The reaction was serially phenol chloroform extracted and ethanol precipitated. The digested DNA was then treated
5 with T4 DNA Polymerase (Boehringer-Mannheim), to fill in the 5' overhangs created by the EcoRI digestion, leaving the DNA ends blunt, after which the reaction was serially phenol chloroform extracted and ethanol precipitated. The blunted DNA was then further digested with NotI (Boehringer-Mannheim) to cut at the 3' end, separated by gel electrophoresis and purified using a QIAquick gel extraction kit (Qiagen). A
10 mammalian expression vector containing a CMV driven expression cassette was digested to generate compatible ends and the 2 fragments were ligated together. Minipreps of the resulting ligation were screened by PCR using the vector specific sense primer ZC14063 (SEQ ID NO:48) and the zcytor17 specific antisense primer ZC27899 (SEQ ID NO:19) with the following PCR reaction conditions: 30 cycles of
15 94°C for 30 sec., 64°C for 30 sec; 70°C for 90 sec; and 72°C for 7 min. An expected product size of approximately 1000 bp confirmed the correct assembly of the plasmid termed pZP7NX-hzcytor17-Fc4. This plasmid was subsequently transfected into COS cells using Lipofectamine (Gibco/BRL), as per manufacturer's instructions. The cells were conditioned for 60 hours in DMEM + 5%FBS (Gibco/BRL) after which the
20 protein was purified over a protein G-sepharose 4B chromatography column and made available for *in vitro* bioassays, for example, such as those described herein.

C. Generating a Human Zcytor17 / OncostatinM receptor (OSMRbeta)

About 16 µg each of the pZP9-ONCOMR-Fc4CEE and pZEM228
25 hzcytor17/Fc4HIS were co-transfected into BHK-570 (ATCC No. CRL-10314) cells using lipofectamine (Gibco/BRL), as per manufacturer's instructions. The transfected cells were selected for 10 days in DMEM + 5%FBS (Gibco/BRL) containing 0.5 mg/ml G418 (Gibco/BRL) and 250 nM methyltrexate (MTX)(Sigma, St. Louis, MO) for 10 days.

30 The resulting pool of doubly-selected cells was used to generate the heterodimeric protein. Three cell Factories (Nunc, Denmark) of this pool were used to

generate 10 L of serum free conditioned medium. This conditioned media was passed over a 1 ml protein-A column and eluted in (10) 750 microliter fractions. Four of these fractions found to have the highest concentration were pooled and dialyzed (10 kD MW cutoff) against PBS. The desired heterodimeric soluble zcytor17/OSMRbeta protein
5 complex was isolated from other media components by passing the pool over a Nickel column and washing the column with various concentrations of Imidazole. The soluble zcytor17/OSMRbeta protein eluted at intermediate concentrations of Imidazole, while hzcytor17/Fc4HIS homodimer eluted at higher concentrations of Imidazole.

10 *Example 31*

Tissue Distribution of Human zcytor17 in Tissue Panels Using Northern Blot and PCR

A. Human zcytor17 Tissue Distribution using Northern Blot

Human Multiple Tissue Northern Blots (Human 12-lane MTN Blot I and II, and Human Immune System MTN Blot II; Human Endocrine MTN, Human Fetal
15 MTN Blot II, Human Multiple Tissue Array) (Clontech) as well as in house blots containing various tissues were probed to determine the tissue distribution of human zcytor17 expression. The in-house prepared blots included the following tissue and cell line mRNA: SK-Hep-1 cells, THP1 cells, Adrenal gland (Clontech); Kidney (Clontech), Liver (Clontech and Invitrogen); Spinal cord (Clontech), Testis (Clontech),
20 Human CD4+ T-cells, Human CD8+ T-cells, Human CD19+ T-cells, human mixed lymphocyte reaction (MLR), THP1 cell line (ATCC No. TIB-202), U937 cell line, p388D1 mouse lymphoblast cell line (ATCC No. CCL-46) with or without stimulation by Ionomycin; and WI-38 human embryonic lung cell line (ATCC No. CRL-2221) with or without stimulation by Ionomycin.

25 An approximately 500 bp PCR derived probe for zcytor17 (SE ID NO:4) was amplified using oligonucleotides ZC28,575 (SEQ ID NO:77) and ZC27,899 (SEQ ID NO:19) as primers. The PCR amplification was carried out as follows: 30 cycles of 94°C for 1 minute, 65°C for 1 minute, and 72°C for 1 minute; followed by 1 cycle at 72°C for 7 minutes. The PCR product was visualized by agarose gel electrophoresis
30 and the approximately 500 bp PCR product was gel purified as described herein. The probe was radioactively labeled using the PRIME IT II™ Random Primer Labeling Kit

(Stratagene) according to the manufacturer's instructions. The probe was purified using a NUCTRAP™ push column (Stratagene). EXPRESSHYB™ (Clontech) solution was used for the prehybridization and as a hybridizing solution for the Northern blots. Prehybridization was carried out at 68°C for 2 hours. Hybridization took place
5 overnight at 68°C with about 1.5×10^6 cpm/ml of labeled probe. The blots were washed three times at room temperature in 2X SSC, 0.05% SDS, followed by 1 wash for 10 minutes in 2X SSC, 0.1% SDS at 50°C. Several faint bands were seen after several days exposure. An approximately 9 kb transcript was seen in trachea, skeletal muscle and thymus; an approximately 2 kb transcript was seen in PBL, HPV, U937 and THP-1
10 cells; and about a 1.2 kb transcript was seen in placenta, bone marrow and thyroid, and HPV and U937 cells. In all the tissues listed above, the signal intensity was faint. There appeared to be little expression in most normal tissues, suggesting that zcytor17 expression may be dependent on activation of the cell or tissues in which it is expressed.

15

B. Tissue Distribution in tissue panels using PCR

A panel of cDNAs from human tissues was screened for zcytor17 expression using PCR. The panel was made in-house and contained 94 marathon cDNA and cDNA samples from various normal and cancerous human tissues and cell
20 lines as shown below in Table 13. The cDNAs came from in-house libraries or marathon cDNAs from in-house RNA preps, Clontech RNA, or Invitrogen RNA. The marathon cDNAs were made using the marathon-Ready™ kit (Clontech, Palo Alto, CA) and QC tested with clathrin primers ZC21195 (SEQ ID NO:78) and ZC21196 (SEQ ID NO:79) and then diluted based on the intensity of the clathrin band. To assure
25 quality of the panel samples, three tests for quality control (QC) were run: (1) To assess the RNA quality used for the libraries, the in-house cDNAs were tested for average insert size by PCR with vector oligos that were specific for the vector sequences for an individual cDNA library; (2) Standardization of the concentration of the cDNA in panel samples was achieved using standard PCR methods to amplify full
30 length alpha tubulin or G3PDH cDNA using a 5' vector oligo ZC14,063 (SEQ ID NO:48) and 3' alpha tubulin specific oligo primer ZC17,574 (SEQ ID NO:49) or 3'

G3PDH specific oligo primer ZC17,600 (SEQ ID NO:50); and (3) a sample was sent to sequencing to check for possible ribosomal or mitochondrial DNA contamination. The panel was set up in a 96-well format that included a human genomic DNA (Clontech, Palo Alto, CA) positive control sample. Each well contained approximately 0.2-100
5 pg/ μ l of cDNA. The PCR reactions were set up using oligos ZC26,358 (SEQ ID NO:80) and ZC26,359 (SEQ ID NO:81), TaKaRa Ex TaqTM (TAKARA Shuzo Co LTD, Biomedicals Group, Japan), and Rediload dye (Research Genetics, Inc., Huntsville, AL). The amplification was carried out as follows: 1 cycle at 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 66.3°C for 30 seconds and 72°C for 30
10 seconds, followed by 1 cycle at 72°C for 5 minutes. About 10 μ l of the PCR reaction product was subjected to standard agarose gel electrophoresis using a 4% agarose gel. The correct predicted DNA fragment size was observed in lymph node, prostate, thyroid, HPV (prostate epithelia), HPVS (prostate epithelia, selected), lung tumor, uterus tumor reactions, along with the genomic DNA reaction.

15 The DNA fragment for prostate tissue (2 samples), HPV (prostate epithelia), HPVS (prostate epithelia, selected), and genomic were excised and purified using a Gel Extraction Kit (Qiagen, Chatsworth, CA) according to manufacturer's instructions. Fragments were confirmed by sequencing to show that they were indeed zcytor17.

Table 13

Tissue/Cell line	#samples		Tissue/Cell line	#samples
Adrenal gland	1		Bone marrow	3
Bladder	1		Fetal brain	3
Bone Marrow	1		Islet	2
Brain	1		Prostate	3
Cervix	1		RPMI #1788 (ATCC # CCL-156)	2
Colon	1		Testis	4
Fetal brain	1		Thyroid	2
Fetal heart	1		WI38 (ATCC # CCL-75	2
Fetal kidney	1		ARIP (ATCC # CRL-1674 - rat)	1
Fetal liver	1		HaCat - human keratinocytes	1
Fetal lung	1		HPV (ATCC # CRL-2221)	1
Fetal muscle	1		Adrenal gland	1
Fetal skin	1		Prostate SM	2
Heart	2		CD3+ selected PBMC's Ionomycin + PMA stimulated	1
K562 (ATCC # CCL-243)	1		HPVS (ATCC # CRL-2221) - selected	1
Kidney	1		Heart	1
Liver	1		Pituitary	1
Lung	1		Placenta	2
Lymph node	1		Salivary gland	1
Melanoma	1		HL60 (ATCC # CCL-240)	3
Pancreas	1		Platelet	1
Pituitary	1		HL-100	1
Placenta	1		Renal mesangial	1
Prostate	1		T-cell	1
Rectum	1		Neutrophil	1
Salivary Gland	1		MPC	1

Skeletal muscle	1		Hut-102 (ATCC # TIB-162)	1
Small intestine	1		Endothelial	1
Spinal cord	1		HepG2 (ATCC # HB-8065)	1
Spleen	1		Fibroblast	1
Stomach	1		E. Histo	1
Testis	2			
Thymus	1			
Thyroid	1			
Trachea	1			
Uterus	1			
Esophagus tumor	1			
Gastric tumor	1			
Kidney tumor	1			
Liver tumor	1			
Lung tumor	1			
Ovarian tumor	1			
Rectal tumor	1			
Uterus tumor	1			

C. Expression analysis of zcytoR17 by PCR and Northern

Annotation of the cell types and growth conditions that affect expression of the receptor is a useful means of elucidating its function and predicting a source of ligand. To that end a wide variety of tissue and cell types were surveyed by PCR. The thermostable polymerase Advantage II™ (Clontech, La Jolla, CA) was used with the oligonucleotide primers ZC29,180 (SEQ ID NO:22) and ZC29,179 (SEQ ID NO:82) and 1-10ng of the various cDNA templates listed below for 30 amplification cycles of (94°C, 30 sec.; 66°C, 20 sec.; 68°C, 1 min. 30 sec.). Following this, 20% of each reaction was run out on 0.8% agarose, TAE /ethidium bromide gels and visualized with UV light. Samples were then scored on the basis of band intensity. See Table 14 below.

Table 14

Cells and Conditions	Score 0-5
Hel stimulated with PMA	0
U937	3
MCF-7	0
HuH7	1
Human follicle	0
HT-29	0
HEPG2	0
HepG2 stimulated with IL6	0
Human dermal endothelial	0
Human venous endothelial	0
Human CD4+	0
BEWO	0
Human CD19+	1
Human PBMC stimulated with PHA, PMA, Ionomycin, IL2, IL4, TNF α 24 hours	0
Human PBMC stimulated with LPS, PWM, IFN γ , TNF α , 24 hours	0
Human PBMC all of the above conditions for 48 hours	4
HUVEC p.2	4
RPMI1788	0
TF1	0
Monkey spleen T cells stimulated with PMA, Ionomycin	0
Human prostate epithelia HPV transformed	5
Human tonsils, inflamed	0
HACAT	0
Human chondrocyte	1
Human synoviacyte	1
THP1	5
REH	0

Of the strong positive PCR signals, two were from the human monocyte cell lines U937 and THP1.

These two cell lines along with a prostate epithelia line were selected for further analysis by Northern blot. Previous attempts at visualizing a transcript by
5 northern analysis using mRNA from various tissues yielded weak and diffuse signals in the surprisingly large size range of 7-10kb making this data difficult to interpret. A denaturing formaldehyde/MOPS/0.8%agarose gel was prepared (RNA Methodologies, Farrell, RE Academic Press) and 2 µg of polyA+ mRNA was run for each sample along
10 side an RNA ladder (Life Technologies, Bethesda, MD). The gel was then transferred to Hybond nylon (Amersham, Buckinghamshire, UK), UV crosslinked, and hybridized in ExpressHyb solution (Clontech, LaJolla, CA) at 68°C overnight using a probe to human zcytoR17 generated by PCR with the oligos ZC28,575 (SEQ ID NO:77), and ZC27,899 (SEQ ID NO:19) and labeled with a Megaprime ³²P kit (Amersham). The
15 northern blot was subsequently washed with 0.2xSSC+0.1%SDS at 65C for 15 minutes and exposed to film for 7 days with intensifying screens. A prominent 8kb band was seen in both the prostate epithelia and U937 lanes while a fainter band was present in the THP1 lane.

To optimize the cDNA used as a hybridization probe, four different regions of the full-length human zcytoR17 sequence were amplified by PCR, labeled
20 and hybridized as described above to southern blots containing genomic and amplified cDNA library DNA. The four probes, herein designated probes A-D, were amplified using the following primer pairs: (A) ZC28,575 (SEQ ID NO:77), ZC27,899 (SEQ ID NO:19); (B) ZC27,895 (SEQ ID NO:20), ZC28,917 (SEQ ID NO:83); (C) ZC28,916 (SEQ ID NO:84), ZC28,918 (SEQ ID NO:85); and (D) ZC28,916 (SEQ ID NO:84),
25 ZC29,122 (SEQ ID NO:21). Human genomic DNA along with amplified cDNA libraries demonstrated to contain zcytor17 by PCR were digested with EcoR1 and Xho1 to liberate inserts and run out on duplicate TAE/0.8%agarose gels, denatured with 0.5M NaOH, 1.5 M NaCl, blotted to Hybond, UV crosslinked and each hybridized with a distinct probe. Probe B was found to have the least nonspecific binding and strongest
30 signal. Thus, Probe B was used for all subsequent hybridizations.

Given that the THP1 cells are an excellent model of circulating monocytes and expressed zcytor17 at low levels we treated them with a variety of compounds in an effort to increase expression of zcytoR17. The cells were grown to a density of 2e5/ml, washed and resuspended in various stimulating media, grown for 5 four or thirty hours, and harvested for RNA preparations. Each media was supplemented with one of the following drugs or pairs of cytokines: LPS 2ug/ml (Sigma Chemicals, St. Louis, MO), hTNF α 2 ng/ml (R&D Systems, Minneapolis, MN), hGM-CSF 2ng/ml (R&D Systems, Minneapolis, MN), hIFN γ 50 ng/ml (R&D Systems, Minneapolis, MN), hMCSF 1ng/ml (R&D Systems, Minneapolis, MN), hIL6 1ng/ml 10 (R&D Systems, Minneapolis, MN), hIL1 β 2ng/ml (R&D Systems, Minneapolis, MN), hIFN γ 50ng/ml+hIL4 0.5ng/ml (R&D Systems, Minneapolis, MN), hIFN γ 50ng/ml+hIL10 1ng/ml (R&D Systems, Minneapolis, MN), PMA 10ng/ml (Calbiochem, San Diego, CA) and an untreated control. At the end of the culture period Total RNA was prepared using an RNAeasy Midi-kit (Qiagen, Valencia, CA). 15 Poly A+ RNA was selected from the total RNA using an MPG kit (CPG, Lincoln Park, NJ). Two micrograms of polyA+ RNA from each condition was run on formaldehyde/MOPS/agarose gels, transferred to nylon and UV crosslinked as described above. These northern blots were then hybridized, as above, to probe B at 68°C overnight, washed at high stringency with 0.2XSSC, 0.1%SDS at 65C, exposed to 20 film overnight then exposed to phosphor screens for signal quantitation. A dominant 8 kb mRNA as well a relatively weaker 2.8 kb band were seen in all lanes. A 20-fold increase in zcytor17 mRNA was seen in RNA from cells treated with hIFN γ for 30 hours, this effect was slightly muted with simultaneous treatment with IL4. Minor 3 fold increases in mRNA were seen in RNA from cells treated with LPS, TNF α and 25 GM-CSF while MCSF, IL6, and IL1 β had no effect on zcytor17 mRNA levels. Taken together this data suggests a role for the zcytor17 receptor and its ligand in monocyte macrophage biology and by extension any number of disease processes in which these cells participate.

A human zcytor17lig cDNA fragment was obtained using PCR with gene specific primers: Sense primer ZC41438 (SEQ ID NO:93) and antisense primer ZC41437 (SEQ ID NO:94) and template human zcytor17lig cDNA (SEQ ID NO:90). This fragment was purified using standard methods and about 25ng labeled with ³²P alpha dCTP using the Prime-It RmT random primer labeling kit (Stratagene) and hybridized in Ultrahyb, (Ambion) and used to expose Biomax film/intensifying screens per the manufacturer's recommendations in each case. New, previously unused blots including the Clontech Human 12 lane MTN, the human brain MTN II, and the human brain MTN blot IV, the human immune system MTN II, and the human MTE array II, from Clontech were hybridized overnight at 42°C per the Ambion ultrahyb method. Non-specific radioactive counts were washed off using .1SSC/.5% SDS at 55°C. The positive blots included the human 12 lane MTN (Clontech). Of the 12 tissues examined, only placenta was positive for an approximately 1.2 KB transcript.

15

Example 33

Construction of mammalian expression vectors that express human zcytor17lig-CEE

A. Construction of zCyt17Lig-CEE/pZMP21

An expression plasmid containing all or part of a polynucleotide encoding zCyt17Lig-CEE (SEQ ID NO:95) was constructed via homologous recombination. The plasmid was called zCyt17Lig-CEE/pZMP21.

The construction of zCyt17Lig-CEE/pZMP21 was accomplished by generating a zCyt17Lig-CEE fragment using PCR amplification. The DNA template used for the production of the zCyt17Lig-CEE fragment was zCyt17Lig/pZP7nx. The primers used for the production of the zCyt17Lig-CEE fragment were: (1) ZC41,607 (SEQ ID NO:97) (sense sequence), which includes from the 5' to the 3' end: 28bp of the vector flanking sequence (5' of the insert) and 21 bp corresponding to the 5' sequence of zCyt17Lig; and (2) ZC41,605 (SEQ ID NO:98) (anti-sense sequence), which includes from the 5' to the 3' end: 37 bp of the vector flanking sequence (3' of the insert), 3 bp of the stop codon, 21 bp encoding a C-terminal EE tag, and 21 bp corresponding to the 3' end of zCyt17Lig sequence. The fragment resulting from the above PCR amplification is a copy of the template zCyt17Lig with the addition of a

C-terminal EE tag, yielding a final product zCyt17Lig-CEE.

PCR reactions were run as follows: To a 100 μ l final volume was added: 10 μ l of 10x Taq Polymerase Reaction Buffer with 15mM MgCl (Gibco), 1 μ l of Taq DNA Polymerase (5 units/ μ l, Gibco), 3 μ l of 10mM dNTPs, 78 μ l dH₂O, 3 μ l of a 20 pmol/ μ l stock of primer ZC41,607 (SEQ ID NO:97) 3 μ l of a 20 pmol/ μ l stock of primer ZC41,605 (SEQ ID NO:98), and 2 μ l of a 0.13 μ g/ μ l stock of zCyt17lig template DNA. A volume equal to 50 μ l of mineral oil was added to the mixture. The reaction was heated to 94°C for 5 minutes, followed by 35 cycles at 94°C for 1 minute; 55°C for 2 minutes; 72°C for 3 minutes; followed by a 10 minute extension at 72°C and held at 4°C until the reaction was collected.

The plasmid pZMP21 was restriction digested with BglII enzyme, cleaned with a QiaQuick PCR Purification Kit (Qiagen) using a microcentrifuge protocol, and used for recombination with the PCR fragment. Plasmid pZMP21 was constructed from pZMP20 which was constructed from pZP9 (deposited at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, and is designated No. 98668) with the yeast genetic elements from pRS316 (deposited at the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, and designated No. 77145), an IRES element from poliovirus, and the extracellular domain of CD8, truncated at the carboxyl terminal end of the transmembrane domain. PZMP21 is a mammalian expression vector containing an expression cassette having the MPSV promoter, immunoglobulin signal peptide intron, multiple restriction sites for insertion of coding sequences, a stop codon and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a DHFR gene, the SV40 terminator, as well as the URA3 and CEN-ARS sequences required for selection and replication in *S. cerevisiae*.

Fifty microliters of competent yeast cells (*S. cerevisiae*) were independently combined with 100 ng of cut plasmid, 5 μ l of previously described PCR mixture, and transferred to a 0.2 cm electroporation cuvette. The yeast/DNA mixture was electropulsed at 0.75 kV (5 kV/cm), ∞ ohms, 25 μ F. Each cuvette had 600 μ l of 1.2 M sorbitol added, and the yeast was plated in one 100 μ l aliquot and one 300 μ l

aliquot onto two URA-D plates and incubated at 30°C. After about 72 hours, the Ura+ yeast transformants from a single plate were resuspended in 1 ml H₂O and spun briefly to pellet the yeast cells. The cell pellet was resuspended in 500 µl of lysis buffer (2% Triton X-100, 1% SDS, 100 mM NaCl, 10 mM Tris, pH 8.0, 1 mM EDTA). The 500
5 µl of the lysis mixture was added to an Eppendorf tube containing 300 µl acid washed 600µm glass beads and 300 µl phenol-chloroform, vortexed for 1 minute intervals two or three times, followed by a 5 minute spin in a Eppendorf centrifuge at maximum speed. Three hundred microliters of the aqueous phase was transferred to a fresh tube, and the DNA precipitated with 600 µl 100% ethanol (EtOH), followed by
10 centrifugation for 10 minutes at 4°C. The DNA pellet was then washed with 500µl 70% EtOH, followed by centrifugation for 1 minute at 4°C. The DNA pellet was resuspended in 30 µl H₂O.

Transformation of electrocompetent *E. coli* cells (MC1061) was done with 5 µl of the yeast DNA prep and 50 µl of MC1061 cells. The cells were
15 electropulsed at 2.0 kV, 25 µF and 400 ohms(Ω). Following electroporation, 600µl SOC (2% Bacto[®] Tryptone (Difco, Detroit, MI), 0.5% yeast extract (Difco), 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl₂, 10 mM MgSO₄, 20 mM glucose) was added. The electroporated *E. coli* cells were plated in a 200µl and a 50µl aliquot on two LB AMP plates (LB broth (Lennox), 1.8% Bacto Agar (Difco), 100 mg/L Ampicillin). The plates
20 were incubated upside down for about 24 hours at 37°C. Three Ampicillin-resistant colonies were selected at random and submitted for sequence analysis of the insert. Large-scale plasmid DNA was isolated from a sequence-confirmed clone using the Qiagen Maxi kit (Qiagen) according to manufacturer's instructions.

25 B. Construction of mouse *zCyt17Lig(m)*-CEE/pZMP21

An expression plasmid containing the entire polynucleotide encoding murine *zCyt17Lig*-CEE (SEQ ID NO:104 and SEQ ID NO:105) was also constructed via homologous recombination using the method described in Example 33A above. The primers used were: (1) ZC41643 (SEQ ID NO:106) (forward, 5' to 3' sense) having
30 a 28bp vector overlap 5' of the insertion point; 21 bp of the 5' end of *zcytor17lig(m)* and (2) ZC41641 (SEQ ID NO:107) (reverse, 5' to 3' anti-sense) having a 37bp vector

overlap 3' of the insertion point; 3 bp stop codon; 21 bp C-terminal EE tag; 24 bp of the 3' end of zCytol17Lig(m)-CEE. The plasmid was called zcytol17lig(m)-CEE/pZMP21. The polynucleotide sequence of zcytol17lig(m)-CEE is shown in SEQ ID NO:104, and corresponding polypeptide sequence is shown in SEQ ID NO:105.

5

Example 34

Transfection And Expression Of zcytol17lig-CEE Polypeptides

A. Expression of human zCytol17Lig-CEE/pZMP21 in 293T cells

ZCytol17Lig-CEE was expressed transiently in 293T cells (Stanford
10 University School of Medicine, Stanford, CA; ATCC No. SD-3515) to generate initial purified protein. The day before the transfection, 293T cells were seeded at 6.5×10^4 cells/cm² in 30 T162 culture flasks with a total volume of 30ml of culture media (SL7V4 +5%FBS +1% Pen/Strep) per flask. The cells were allowed to incubate for 24 hours at 37°C.

15 A DNA/Liposome mixture was prepared as follows: Two 50 ml conical tubes were filled with 25 mLs of transfection media (SL7V4 +1%Pen/Strep) and 1.13 mg of zCytol17Lig-CEE/pZMP21 (Example 33) was added to each. A separate set of two 50 ml conical tubes were filled with 22 ml of transfection media (above) and 3 ml of liposomes (Lipofectamine, Gibco) was added to each. For each set of tubes, one
20 tube of DNA was added to one tube of liposomes and the DNA/liposome mix was incubated for 30 minutes. The two 50 ml conical tubes containing the DNA/liposome mixtures were pooled (about 100 ml) and 300 ml of transfection media was added. The 30 flasks of the 293T cells were decanted, washed 1x with about 15 ml of PBS, and 12.5 ml of the diluted DNA/liposome mixture was added to each flask. The flasks were
25 incubated for 3 hours at 37°C. After the incubation period, 25 ml of culture media (above) were added to each T162 flask. The transfection media was harvested after approximately 96 hours and was used for protein purification (Example 35).

B. Expression of mouse zCytol17Lig-CEE(m)/pZMP21 in 293T cells

30 Mouse zCytol17Lig(m)-CEE was expressed transiently in 293T cells as described in Example 34A and cultured media was used for protein purification (Example 35).

*Example 35**Purification of Zcytor17lig-CEE from 293T cells*

Unless otherwise noted, all operations were carried out at 4°C. The following procedure was used for purifying both mouse and human Zcytor17lig containing C-terminal Glu-Glu (EE) tags (SEQ ID NO:103). Conditioned media from 293T cells expressing Zcytor17lig-CEE (Example 34) was purified. Total target protein concentrations of the conditioned media were determined via SDS-PAGE and Western blot analysis with the anti-EE antibody.

10 A 5.5 ml column of anti-EE Poros 50 A (PE BioSystems, Framingham, MA) (prepared as described below) was poured in a Waters AP-1, 1 cm x 7cm glass column (Waters, Milford, MA). The column was flow packed and equilibrated on a BioCad Sprint (PE BioSystems, Framingham, MA) with phosphate buffered saline (PBS) pH 7.4. The conditioned media was adjusted with NaCl to 0.3 M and the pH
15 adjusted to 7.2. The conditioned media was then loaded on the column overnight with about 3 ml/minute flow rate. The column was washed with 10 column volumes (CVs) of PBS pH 7.4, and again washed with 3CVs 5X Sigma PBS pH 7.4. It was step eluted with 0.5 M Acetate, 0.5 M NaCl, pH 2.5 at 3 ml/minute. The fraction tubes contained 1ml Tris base (no pH adjustment) to neutralize the elution immediately. The column
20 was again washed for 2CVs with 5X Sigma PBS, pH 7.4 to neutralize the column and then equilibrated in PBS (pH 7.4). Two ml fractions were collected over the entire elution chromatography and absorbance at 280 and 215 nM were monitored; the pass through and wash pools were also saved and analyzed. The 5X PBS and the acid elution peak fractions were analyzed for the target protein via SDS-PAGE Silver
25 staining and Western Blotting with the primary antibody anti-EE and secondary antibody, anti mouse-HRP conjugated. The acid elution fractions of interest were pooled and concentrated from 38 ml to 0.8ml using a 5000 Dalton molecular weight cutoff membrane spin concentrator (Millipore, Bedford, MA) according to the manufacturer's instructions.

30 To separate Zcytor17lig-CEE from aggregated material and any other contaminating co-purifying proteins, the pooled concentrated fractions were subjected

to size exclusion chromatography on a 1.6 x 60 cm (120 ml) Superdex 75 (Pharmacia, Piscataway, NJ) column equilibrated and loaded in PBS at a flow rate of 1.0 ml/min using a BioCad Sprint. Three milliliter fractions were collected across the entire chromatography and the absorbance at 280 and 215 nm were monitored. The peak
5 fractions were characterized via SDS-PAGE Silver staining, and only the most pure fractions were pooled. This material represented purified Zcytor17lig -CEE protein.

On Western blotted, Coomassie Blue and Silver stained SDS-PAGE gels, the Zcytor17lig-CEE was one major band. The protein concentration of the purified material was performed by BCA analysis (Pierce, Rockford, IL) and the protein
10 was aliquoted, and stored at -80°C according to standard procedures.

To prepare PorosA50 anti-EE, a 65 ml bed volume of Poros A50 (PE Biosystems) was washed with 100 ml of water and then 0.1 M triethanolamine, pH 8.2 (TEA, ICN, Aurora, Ohio), 1 M Na₂SO₄, pH 8.8 containing 0.02% sodium azide using a vacuum flask filter unit. The EE monoclonal antibody solution, at a concentration of 2
15 mg/ml in a volume of 300 ml, was mixed with the washed resin in a volume of 250 ml. After an overnight incubation at room temperature, the unbound antibody was removed by washing the resin with 5 volumes of 200 mM TEA, 1 M Na₂SO₄, pH 8.8 containing 0.02% sodium azide as described above. The resin was resuspended in 2 volumes of TEA, 1 M Na₂SO₄, pH 8.8 containing 0.02% sodium azide and transferred to a suitable
20 container. Three ml of 25 mg/ml (68mM) Disuccinimidyl suberate (in DMSO supplied by Pierce, Rockford, IL) is added and the solution is incubated for three hours at room temperature. Nonspecific sites on the resin were then blocked by incubating for 10 min at room temperature with 5 volumes of 20 mM ethanolamine (Sigma, St. Louis, MO) in 200 mM TEA, pH 8.8 using the vacuum flask filter unit. The resin is washed with PBS,
25 pH 7.4, followed by 0.1 M Glycine, pH 3 and then neutralized with 10X PBS. After washing with distilled water, the final coupled anti-EE Poros-A 50 resin was stored at 4°C in 20% Ethanol.

30

Example 36

N-terminal sequencing of human and mouse Zcytor17lig

A. N-terminal sequencing of human Zcytor17lig

Standard automated N-terminal polypeptide sequencing (Edman degradation) was performed using reagents from Applied Biosystems. N-terminal sequence analysis was performed on a Model 494 Protein Sequencer System (Applied Biosystems, Inc., Foster City, CA). Data analysis was performed with Model 610A
5 Data Analysis System for Protein Sequencing, version 2.1a (Applied Biosystems).

A purified human zcytor17lig-CEE sample (Example 35) was supplied. The sample was loaded onto a prepared glass fiber filter for n-terminal sequencing. The glass fiber filter was prepared by precycling it with Biobrene™.

N-terminal sequence analysis of the secreted human zcytor17lig
10 polypeptide did not verify the predicted cleavage site of the signal sequence but resulted in a mature start at residue 27(Leu) in SEQ ID NO:2 of the human zcytor17lig precursor sequence.

B. N-terminal sequencing of human Zcytor17lig

15 Standard automated N-terminal polypeptide sequencing (Edman degradation) was performed using reagents from Applied Biosystems. N-terminal sequence analysis was performed on a Model 494 Protein Sequencer System (Applied Biosystems, Inc., Foster City, CA). Data analysis was performed with Model 610A Data Analysis System for Protein Sequencing, version 2.1a (Applied Biosystems).

20 A purified mouse zcytor17lig-CEE sample was supplied as captured on Protein G Sepharose/anti-EE beads (Example 35). The beads were placed in reducing SDS PAGE sample buffer and on a boiling water bath before running on SDS PAGE, using a Novex SDS PAGE system (4-12% Bis-Tris MES NuPAGE; Invitrogen) as per manufacturer's instructions. The gel was electrotransferred to a Novex PVDF
25 membrane (Invitrogen), and Coomassie blue stained (Sigma, St. Louis, MO) using standard methods. Corresponding anti-EE Western blots were performed to identify the zcytor17lig band for N-terminal protein sequencing. The mouse anti-EE IgG HRP conjugated antibody used was produced in house.

N-terminal sequence analysis of the secreted mouse zcytor17lig
30 polypeptide verified the predicted cleavage site of the signal sequence resulting in a mature start at 31 (Ala) in reference to SEQ ID NO:11 and SEQ ID NO:91 of the mouse

zcytor17lig precursor sequence.

Example 37

Cos cell binding assay

5 A binding assay was used to test the binding of the zcytor17lig to receptors comprising zcytor17 receptor, such as the zcytor17 receptor or receptor heterodimers and trimers comprising zcytor17 receptor (e.g., zcytor17/OSMR, zcytor17/WSX-1, or zcytor17/OSMR/WSX-1, or other Class I cytokine receptor subunits). Zcytor17 receptor plasmid DNA was transfected into COS cells and
10 transfected COS cells were used to assess binding of the zcytor17lig to receptors comprising zcytor17 receptor as described below.

A. COS Cell Transfections

The COS cell transfection was performed as follows: Mix 800 ng
15 receptor plasmid DNA in the following combinations: pZp7pX/zcytor17 alone; pZp7Z/WSX-1 alone; pZp7NX/OSMR alone; pZp7pX/zcytor17 + pZp7NX/OSMR; pZp7pX/zcytor17 + pZp7Z/WSX-1; pZp7NX/OSMR + pZp7Z/WSX-1; pZp7pX/zcytor17 + pZp7NX/OSMR + pZp7Z/WSX-1) and 4ul Lipofectamine™ in 80 µl serum free DMEM media (55mg sodium pyruvate, 146mg L-glutamine, 5mg
20 transferrin, 2.5mg insulin, 1µg selenium and 5mg fetuin in 500ml DMEM), incubate at room temperature for 30 minutes and then add 320 µl serum free DMEM media. Add this 400ul mixture onto 2×10^5 COS cells/well plated on 12-well tissue culture plate (fibronectin-coated) and incubate for 5 hours at 37°C. Add 500ul 20% FBS DMEM
25 media (100 ml FBS, 55 mg sodium pyruvate and 146mg L-glutamine in 500ml DMEM) and incubate overnight.

B. Binding Assay

The binding assay was performed as follows: media was rinsed off cells with PBS + 0.1% BSA, and then cells were blocked for 60 minutes with the same
30 solution. The cells were then incubated for 1 hour in PBS + 0.1% BSA with 1.0 µg/ml zcytor17ligCEE purified protein. Cells were then washed with PBS + 0.1% BSA and

incubated for another hour with 1:1000 diluted mouse anti-GluGlu antibody. Again cells were washed with PBS + 0.1% BSA, then incubated for 1 hour with 1:200 diluted goat anti-mouse-HRP conjugated antibody.

Positive binding was detected with fluorescein tyramide reagent diluted
5 1:50 in dilution buffer (NEN kit) and incubated for 4-6 minutes, and washed with PBS + 0.1% BSA. Cells were fixed for 15 minutes with 1.8% Formaldehyde in PBS, then washed with PBS + 0.1% BSA. Cells were preserved with Vectashield Mounting Media (Vector Labs Burlingame, CA) diluted 1:5 in PBS. Cells were visualized using a FITC filter on fluorescent microscope.

10 Positive binding was detected for cells transfected with zcytor17 only, zcytor17+OSMRbeta, zcytor17+WSX-1, and zcytor17+OSMRbeta+WSX-1. No binding was detected for cells transfected with WSX-1 + OSMRbeta, with OSMRbeta only, or with WSX-1 only.

15 *Example 38*

Mouse zcytor17lig activates mouse zcytor17/OSMRbeta receptor-in luciferase assay

A. Cloning of full-length mouse zcytor17 and mouse OSMRbeta for expression

A mouse testes cDNA library was screened for a full-length clone of mouse zcytoR17. The library was plated at 65,500 cfu/plate on 24 LB + Amp plates.
20 Filter lifts were prepared using Hybond N (Amersham-Pharmacia Biotech, Inc., Piscataway, NJ) on a total of approximately 1.6 million colonies. The filters were marked with a hot needle for orientation and then denatured for 6 minutes in 0.5 M NaOH and 1.5 M Tris-HCl, pH 7.2. The filters were then neutralized in 1.5 M NaCl and 0.5 M Tris-HCl, pH 7.2 for 6 minutes. The DNA was affixed to the filters using a
25 UV crosslinker (Stratalinker®, Stratagene, La Jolla, CA) at 1200 joules. The filters were then left to dry overnight at room temperature.

The next day, the filters were pre-washed at 65°C in pre-wash buffer consisting of 0.25X SSC, 0.25% SDS and 1mM EDTA. Cell debris was manually removed using Kimwipes® (Kimberly-Clark) and the solution was changed 3 times
30 over a period of 1 hour. Filters were air dried and stored at room temperature until needed. The filters were then prehybridized for approximately 3 hours at 63°C in 20 ml

of ExpressHyb™Hybridization Solution (Clontech, Palo Alto, CA).

Probe B (Example 31) was generated by PCR from human zcytoR17 template using oligonucleotide primers ZC27,895 (SEQ ID NO:20) and ZC28,917 (SEQ ID NO:83) and was radioactively labeled with ³²P using a commercially available
5 kit (Megaprime DNA Labeling System; Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions. The probe was purified using a Stratagene™ push column (NucTrap® column; Stratagene, La Jolla, CA). The probe was denatured at 100°C for 15 min and added to ExpressHyb™. Filters were hybridized in 15 ml hybridizing solution containing 1.6×10^6 cpm/ml of probe at 63°C overnight.
10 Filters were washed at 55°C in 2X SSC, 0.1 % SDS and 1 mM EDTA and exposed to X-ray film at -80°C for 4 1/2 days. Thirteen positives were picked from the plates as plugs and placed in 1 ml LB +amp in 1.7 ml tubes. Tubes were placed at 4°C overnight. These 13 positives were subjected to two further rounds of purification. The tertiary plates were outgrown at 37°C after filter lifts were taken and single colonies
15 were picked and sent to sequencing. Three of these were determined to contain sequence of the mouse ortholog of zcytoR17.

In addition, a PCR product was generated using CTLL-2 cDNA as a template and oligonucleotides ZC38,239 (SEQ ID NO:123) and ZC38,245 (SEQ ID NO:124) as primers. CTLL-2 is a mouse cytotoxic T lymphocyte cell line (ATCC No.
20 TIB-214). This PCR reaction was run as follows: 1 cycle at 95°C for 1 minute, 30 cycles at 95°C for 15 seconds, 68°C for 3 minutes, then 68°C for 10 minutes; 4°C soak. The PCR reaction used approximately 0.5 ng of cDNA, 20 pmoles of each oligonucleotide, and 1 µl of Advantage II polymerase mix (ClonTech). About 6% of the PCR product was used as a template in a new PCR reaction, as above, except with
25 oligonucleotides ZC38,239 (SEQ ID NO:123) and ZC38,238 (SEQ ID NO:125). This PCR reaction was run as follows: 30 cycles at 94°C for 45 seconds, 65°C for 45 seconds, 72°C for 1 minute, then 72°C for 7 minutes; 10°C soak. Most of the PCR reaction was loaded on a 1.0% agarose gel and the predominant band at approximately 360 bp was excised, the DNA fragment was eluted, and DNA sequencing was
30 performed.

The sequence of the mouse zcytor17 polynucleotide is shown in SEQ ID

NO:126 and the corresponding amino acid sequence shown in SEQ ID NO:127. In addition, a truncated soluble form of the mouse zcytor17 polynucleotide is shown in SEQ ID NO:128 and the corresponding amino acid sequence shown in SEQ ID NO:129.

5 To obtain a full-length mouse OSMRbeta cDNA, 5' and 3' PCR products were isolated and joined using an internal BamHI site. The PCR primers were designed using the nucleotide sequence SEQ ID NO:134 and include EcoRI and XbaI restriction sites for cloning purposes. The genomic mouse OSMRbeta nucleic acid sequence is shown in SEQ ID NO:134, wherein the coding sequence encompasses residues 780 to
10 3692 encoding a mouse OSMRbeta 970 amino acid polypeptide, which is shown in SEQ ID NO:135. A degenerate nucleic acid sequence which encodes the polypeptide of SEQ ID NO:135 is shown in SEQ ID NO:136.

A 5' PCR product was generated using an in-house 3T3-L1 (differentiated mouse adipocyte) cDNA library as a template and oligonucleotides
15 ZC41,764 (SEQ ID NO:130) and ZC41,598 (SEQ ID NO:131) as primers. This 5' PCR reaction was run as follows: 30 cycles at 95°C for 45 seconds, 55°C for 45 seconds, 72°C for 1 minute 30 seconds, then 72°C for 7 minutes; 4°C soak. The PCR reaction used approximately 3 µg of plasmid prepared from the cDNA library, 20 pmoles of each oligonucleotide, and five units of *Pwo* DNA polymerase (Roche). About 90% of
20 the 5' PCR product was digested with EcoRI and BamHI and gel purified on a 1.0% agarose gel. The approximately 1446 bp band was excised and used for ligation (see below).

A 3' PCR product was generated using a mouse placenta in-house cDNA library as a template and oligonucleotides ZC41,948 (SEQ ID NO:132) and ZC41,766
25 (SEQ ID NO:133) as primers. This 3' PCR reaction was run as follows: 30 cycles at 95°C for 45 seconds, 55°C for 45 seconds, 72°C for 1 minute 30 seconds, then 72°C for 7 minutes; 4°C soak. The PCR reaction used approximately 3 µg of plasmid prepared from the cDNA library, 20 pmoles of each oligonucleotide, and five units of *Pwo* DNA polymerase (Roche). About 90% of the 3' PCR product was digested with BamHI and
30 XbaI and gel purified on a 1.0% agarose gel. The approximately 2200 bp band was excised and used for ligation along with the 5' PCR product (described above) to the

expression vector pZP-5Z digested with EcoRI and XbaI. The three-part ligation was performed with the 5' EcoRI to BamHI fragment above, the 3' BamHI to XbaI fragment, and the expression vector pZP-5Z digested with EcoRI and XbaI. This generated a pZP-5Z plasmid containing a full-length cDNA for mouse OSMRbeta (nucleotides 780
5 to 3692 of SEQ ID NO:134), designated pZP-5Z/OSMRbeta. The full length mouse OSMRbeta cDNA in pZP5Z/OSMRbeta has two amino acid insertions from SEQ ID NO:135. There is a duplication of amino acid Glycine at position 370 and a duplication of amino acid Glutamic Acid at position 526. Plasmid pZP-5Z is a mammalian expression vector containing an expression cassette having the CMV promoter,
10 multiple restriction sites for insertion of coding sequences, and a human growth hormone terminator. The plasmid also has an *E. coli* origin of replication, a mammalian selectable marker expression unit having an SV40 promoter, enhancer and origin of replication, a zeocin resistance gene and the SV40 terminator.

The resulting transformants were sequenced to confirm the mouse
15 OSMRbeta cDNA sequence.

B. Construction of BaF3/KZ134/zcytor17m, BaF3/KZ134/zcytor17m/OSMRbetam, BHK/KZ134/zcytor17m, and BHK/KZ134/zcytor17m/OSMRbetam cell lines

Stable BaF3/KZ134 and BHK/KZ134 cell lines (Example 20) were
20 transfected with an expression plasmid encoding full-length mouse zcytor17, pZP-7P/zcytor17m (Example 38A), to create BaF3/KZ134/zcytor17m and BHK/KZ134/zcytor17m cells, respectively. The mouse OSMRbeta expression plasmid, pZP-5Z/OSMRbetam (Example 38A), was then transfected into these cells to create BaF3/KZ134/zcytor17m/OSMRbetam and BHK/KZ134/zcytor17m/OSMRbetam cell
25 lines, respectively. Methods were as described in Example 4 with the exception that Baf3/KZ134/zcytor17m and BHK/KZ134/zcytor17m were selected with, in addition to Geneticin, 2ug/ml puromycin while Baf3/KZ134/zcytor17m/OSMRbetam and BHK/KZ134/zcytor17m/OSMRbetam were selected with, in addition to Geneticin, 2ug/ml puromycin and 200 ug/ml zeocin.

30 Clones were diluted, plated and selected using standard techniques. Clones were screened by luciferase assay (see Example 20, above) using the mouse

zcytor17lig conditioned media or purified mouse zcytor17lig protein (Example 35) as an inducer. Clones with the highest luciferase response (via STAT luciferase) and the lowest background were selected. Stable transfectant cell lines were selected.

5 *C. Mouse Zcytor17lig activates mouse zcytor17 receptor in BaF3/KZ134/zcytor17m/OSMRbetam or BHK/KZ134/zcytor17m/OSMRbetam luciferase assay*

Cell lines were plated for luciferase assays as described in Example 20 above. STAT activation of the BaF3/KZ134/Zcytor17m,
10 BaF3/KZ134/zcytor17m/OSMRbetam, BHK/KZ134/zcytor17m, or BHK/KZ134/zcytor17m/OSMRbetam cells was assessed using (1) conditioned media from BHK570 cells transfected with the human zcytor17lig (Example 7), (2) conditioned media from BHK570 cells transfected with the mouse zcytor17lig (Example 18), (3) purified mouse and human zcytor17lig (Example 35), and (4) mIL-3
15 free media to measure media-only control response. Luciferase assays were performed as described in Example 20.

The results of this assay confirm the STAT reporter response of the BaF3/KZ134/zcytor17m/OSMRbetam and BHK/KZ134/zcytor17m/OSMRbetam cells to the mouse zcytor17lig when compared to either the BaF3/KZ134/zcytor17m cells,
20 the BHK/KZ134/zcytor17m cells or the untransfected BaF3/KZ134 or BHK/KZ134 control cells, and show that the response is mediated through the mouse zcytor17/OSMRbeta receptors. The results also show that the human zcytor17lig does not activate the STAT reporter assay through the mouse receptor complex.

25 *Example 39*

Human zcytor17 ligand binding to zcytor17 and zcytor17/OSMRbeta by Flow Cytometry

The biotinylation of human zcytor17L was done as follows: 100μL of zcytor17 at 5.26mg/mL was combined with 30μL of 10mg/mL EZ-link Sulfo-NHS-LC-
30 biotin (Pierce, Rockford, IL) dissolved in ddH₂O. This solution was incubated on a rocker for 30 minutes at room temperature. After biotinylation the solution was

dialyzed in PBS using a Slide-A-Lyzer dialysis cassette.

To test the binding properties of human zcytor17 ligand to different receptor combinations both BHK and BAF3 cells were transfected with expression plasmids using standard techniques well-known in the art. These plasmids were transfected into both cell lines in the following combinations: zcytor17 alone, OSMRbeta alone, and both zcytor17 and OSMRbeta. Transfection was performed as detailed above. Untransfected BHK and BAF3 cells were used as controls. Cells were stained by FACS as follows: 2E5 cells were stained with either: 2.0µg/mL, 100ng/mL, 10ng/mL, 1.0ng/mL, 100pg/mL, 10pg/mL, 1.0pg/mL of biotinylated zcytor17L or left unstained for 30 minutes on ice in FACS buffer (PBS + 2%BSA + 2%NHS (Gemini) + 2%NGS). Cells were washed 1.5 times and then stained with SA-PE (Jackson Immuno Laboratories) at 1:250 for 30 minutes on ice. Cells were then washed 1.5 times with FACS buffer and resuspended in FACS buffer and analyzed by FACS on a BD FACSCaliber using CellQuest software (Becton Dickinson, Mountain View, CA).

Both BHK and BAF3 cells showed that zcytor17 ligand bound to both zcytor17 alone and in combination with OSMRbeta with the binding to the zcytor17/OSMRbeta heterodimer being slightly stronger. No binding was seen in either cell lines expressing OSMRbeta alone. The zcytor17 ligand bound in a concentration dependent manner. The mean fluorescent intensity (MFI) values for the BHK binding are shown below in Table 15.

Table 15

zcytor17 µg/mL	2.0	0.100	0.010	0.001	0.0001	0.00001	0.000001	0.0
BHK C17+OSMRbeta	3780	2126	328	53	17	15	14	13
BHK-C17	3032	1600	244	39	16	15	14	15
BHK-OSMRbeta	13	X	X	X	X	X	X	0
BHK-WT	15	14	13	X	X	X	X	13

zcytor17 µg/mL	10.0	3.33	1.11	0.37	0.12	0.04	0.00
BAF3-C17+OSMRbeta	531	508	489	441	364	247	7
BAF3-OSMRbeta	6	5	5	5	5	5	11
BAF3-WT	13	13	12	12	12	12	13

zcytor17 ng/mL	100.0	10.0	1.0	0.0
BAF3-C17	347	72	17	7

*Example 40**Gene Expression Array Analysis of Human Zcytor17lig Treated Cells*

RNA was isolated from human zcytor17lig treated A549 cells,
5 zcytor17lig treated SK-LU-1 cells, and untreated control cells using a RNeasy Midi Kit (Qiagen, Valencia, CA) according to the manufactures instructions.

Gene expression profiling of the cells treated with zcytor17lig and the respective control cells was carried out using GEMatrix Q series cDNA expression arrays (SuperArray Inc., Bethesda, MD). The Q Series cDNA expression arrays contain
10 up to 96 cDNA fragments associated with a specific biological pathway, or genes with similar functions or structural features. Comparison of arrays from treated and control cells allows for a determination of the up and down regulation of specific genes. Probe labeling, hybridization and detection were carried out according to the manufactures instructions. Chemiluminescent signal detection and data acquisition was carried out on
15 a Lumi-Imager workstation (Roche, Indianapolis, IN). The resulting image data was analyzed using ImageQuant 5.2 (Amersham Biosciences, Inc., Piscataway, NJ) and GEMatrix Analyzer 1.2 (SuperArray Inc., Bethesda, MD) software.

Analysis of the results from the Human Interleukin and Receptor Q series HS-014N arrays, showed, after normalization, an approximate 4.7 fold increase
20 of IL13RA2 signal in the zcytor17lig treated human SK-LU-1 cells and an approximate 2.2 fold increase of the IL13RA2 signal in the zcytor17lig treated human A549 cells.

These results indicate that zcytor17lig significantly up regulated IL13RA2 in the SK-LU-1 and A549 cells. Both of these are established cell lines derived from human lung carcinomas (Blobel et al., *Virchows Arch B Cell Pathol Incl*
25 *Mol Pathol.*, 1984;45(4):407-29). More specifically, A549 is characterized as a human pulmonary epithelial cell line (Lin, et al., *J Pharm Pharmacol.*, 2002 Sep;54(9):1271-8; Martinez et al., *Toxicol Sci.*, 2002 Oct;69(2):409-23).

Interleukin-13 (IL13), a cytokine secreted by activated T lymphocytes, has been demonstrated to be both necessary and sufficient for the expression of allergic
30 asthma and for use in experimental models of asthma, which include airway hyperresponsiveness, eosinophil recruitment, and mucus overproduction (Wills-Karp et

al., *Science*, 1998;282:2258-2261). It has been shown, that selective neutralization of IL13 will ameliorate the asthma phenotype (Grunig et al., *Science*, 1998; 282:2261-2263). It has also been reported that IL13 is involved in the up regulation of mucin gene MUC8 expression in human nasal polyp epithelium and cultured nasal epithelium
5 (Kimm et al., *Acta Otolaryngol.*, 2002; Sep;122(6):638-643; Seong et al., *Acta Otolaryngol.*, 2002; Jun;122(4):401-407). MUC8, a major airway mucin glycoprotein, is implicated as playing a role in the pathogenesis of mucus hypersecretion in chronic sinusitis with polyps (Seong et al., *Acta Otolaryngol.*, 2002; Jun;122(4):401-407).

Functionally, IL13 signals through a receptor complex consisting of the
10 interleukin-13 receptor alpha-1 chain (IL13RA1) and IL-4 receptor alpha (IL4RA) (Daines and Hershey, *J Biol Chem.*, 2002; 22(12):10387-10393). It has also been shown, that the interleukin-13 receptor alpha-2 (IL13RA2) binds IL13 with high affinity, but by itself (Daines and Hershey, *J Biol Chem.*, 2002; 22(12):10387-10393). This receptor lacks, however, the cytoplasmic domain necessary for signaling and,
15 therefore, is considered to be a decoy receptor. It has been shown that IL13RA2 is predominately an intracellular molecule that can be quickly mobilized from intracellular stores and surface expressed following cellular treatment with interferon (IFN)-gamma. The surface expression of IL13RA2 after IFN-gamma treatment does not involve protein synthesis and results in diminished IL13 signaling (Daines and Hershey, *J Biol*
20 *Chem.*, 2002; 22(12):10387-10393).

The results of the gene expression array analysis for zcytor17lig indicate the action of zcytor17lig to be novel to that of IFN-gamma in that the zcytor17lig treatment of lung epithelial derived cell lines resulted in a significant increase of IL13RA2 gene expression. It is conceivable, therefore, that zcytor17lig treatment
25 would be beneficial in cases where long-term up regulation of IL13RA2 expression and down regulation of IL13 is desired such as in asthma, airway hyperactivity (AHR), and mucin regulation, including chronic sinusitis with polyps.

Example 41

30 *Murine zcytor17lig Transgenic Mice*

To evaluate the *in vivo* effects of zcytor17lig overexpression, multiple founders of transgenic mice expressing the murine form of the gene were generated, driven by two different promoters: the lymphocyte-specific promoter $E\mu/lck$, and the ubiquitous promoter, $EF1\alpha$ (Example 22). Serum protein levels range from approximately 20-300 ng/ml. The $E\mu/lck$ promoter generated mice with higher levels of serum protein than those in the $EF1\alpha$ -zcytor17lig transgenic mice.

The zcytor17lig transgenic mice developed a skin phenotype around 4-8 weeks of age. The fur of the transgenic mice became "ruffled," with obvious piloerection and mild to severe hair loss, usually on their backs, sides of the torso, and around their eyes. This phenotype was consistently found in mice with detectable levels of zcytor17lig protein in their serum. Among the founders, 100% incidence rate among the mice expressing the $E\mu/lck$ -driven gene, and a 50% incidence in the $EF1\alpha$ -zcytor17lig transgenic mice was noted, correlating well with the relative levels of zcytor17lig that was detected in their serum. The transgenic skin appeared to be pruritic, as evidenced by the scratching behavior of the mice, sometimes excessive enough to induce excoriation and lesions of the skin, which usually became infected (with at least *Staphylococcus aureus*). The mice were originally identified with metal ear tags, but in most cases, the ear tags were forcibly removed by the mice themselves. This often resulted in severe damage to the external ear. These wounded ears often did not heal properly, as reflected in the presence of long-lasting pustules and crusting, and a seeping, expanding wound would that developed in many of the animals, behind and between their ears. Some of the transgenic mice also developed scabby wounds on their shoulders and neck. Skin lesions were observed in a subset of the animals, generally evolving on areas of skin where hair loss had already been apparent, and were often exacerbated by the scratching behavior of the mice.

RealTime quantitative RT-PCR was used to detect zcytor17lig RNA transcripts in transgenic (but not non-transgenic) skin samples, with the $E\mu/lck$ transgenic skin expressing more zcytor17lig RNA than skin from $EF1\alpha$ -zcytor17lig transgenic mice. The genes encoding the zcytor17 receptor subunits, zcytor17 and OSM-Rbeta, were expressed in the skin of both non-transgenic and zcytor17lig-transgenic mice.

An examination of the lymphoid tissues from a subset of the E μ /lck-transgenic founders by flow cytometry revealed a significant increase in the proportion of activated T cells in the spleen and lymph nodes of these mice. Two of the four mice analyzed had severely enlarged cervical lymph nodes, possibly due to the presence of lesions on their necks. A subtle increase in spleen weight and a slight increase in monocytes and neutrophils circulating in the blood of the transgenic mice was observed. There was no increase in a variety of cytokines tested, nor were there changes in the circulating serum amyloid A levels in these mice. The effects on the immune cells in the transgenic mice may be a direct or an indirect result of zcytor17lig, or are secondary effects of the skin lesions.

Histopathology was performed on many tissues other than skin, including liver, thymus, spleen, kidney, and testes, and no significant abnormalities in these organs were noted. Analysis of the transgenic skin, however, did reveal a number of alterations, which varied greatly depending upon the source and location of skin (e.g., normal, hairless, or lesional). In many cases, the ears of the transgenic mice had a thickened epidermis as compared to the non-transgenic controls (e.g., approximately 4 layers versus 2 layers), and the underlying tissues contained low to moderate numbers of inflammatory cells, which were primarily mononuclear with occasional neutrophils. The epidermis over the abdomen appeared multifocally slightly thicker in the transgenic, but there was no apparent increase in inflammatory cells in the underlying dermis or subcutis. In the hairless portions of skin from these mice, there were dilated hair follicles that contained some debris but no hair shafts (e.g., the hairs fell out by the roots). In the lesioned areas, there was severe thickening of the epidermis (acanthosis), increased keratin on the surface of the skin (hyperkeratosis), scattered ulcers of varying size and significant numbers of inflammatory cells in the dermis (mainly neutrophils, with varying numbers of macrophages and lymphocytes). The dermis also contained numerous mast cells bordering the lesions. Some of the hair shafts in the lesioned areas of the transgenic skin were in the active stage (anagen), in contrast to many of the hair shafts in "normal" areas which were in the involuting (catagen) to inactive (telogen) stage.

The phenotype of the zcytor17lig transgenic mice strongly resembles that of atopic dermatitis (AD) patients, and mouse models of AD. AD is a common chronic inflammatory disease that is characterized by hyperactivated cytokines of the helper T cell subset 2 (Th2). Zcytor17lig is preferentially expressed by Th2 vs. Th1
5 cells, which lends further credence to this comparison. Although the exact etiology of AD is unknown, multiple factors have been implicated, including hyperactive Th2 immune responses, autoimmunity, infection, allergens, and genetic predisposition. Key features of the disease include xerosis (dryness of the skin), pruritus (itchiness of the skin), conjunctivitis, inflammatory skin lesions, *Staphylococcus aureus* infection,
10 elevated blood eosinophilia, elevation of serum IgE and IgG1, and chronic dermatitis with T cell, mast cell, macrophage and eosinophil infiltration. Colonization or infection with *S. aureus* has been recognized to exacerbate AD and perpetuate chronicity of this skin disease.

AD is often found in patients with asthma and allergic rhinitis, and is
15 frequently the initial manifestation of allergic disease. About 20% of the population in Western countries suffer from these allergic diseases, and the incidence of AD in developed countries is rising for unknown reasons. AD typically begins in childhood and can often persist through adolescence into adulthood. Current treatments for AD include topical corticosteroids, oral cyclosporin A, non-corticosteroid
20 immunosuppressants such as tacrolimus (FK506 in ointment form), and interferon-gamma. Despite the variety of treatments for AD, many patients' symptoms do not improve, or they have adverse reactions to medications, requiring the search for other, more effective therapeutic agents.

Epithelial cells, which express the heterodimeric receptor for zcytor17lig
25 (zcytoR17 and OSM-Rbeta), are located at the sites (e.g., skin, gut, lung, etc.) of allergen entry into the body and interact closely with dendritic cells (professional antigen presenting cells) *in situ*. Dendritic cells play an important role in the pathogenesis of allergic diseases, and it is possible that zcytor17lig can interact with its receptor on epithelial cells in the skin and lung and influence immune responses in
30 these organs. Zcytor17lig and its receptor(s) may therefore contribute to the pathogenesis of allergic diseases such as AD and asthma. Furthermore, the phenotype

of the zcytor17lig transgenic mice suggests that this ligand may play a role in wound healing, since the mice seem unable to repair damage to their ears, and often bear long-lasting lesions on their backs and sides. An antagonist of zcytor17lig might therefore represent a viable therapeutic for these and other indications.

5

Example 42

Luciferase Assay on Human Transformed Epithelial Cell Lines via Transient Infection with an Adenoviral STAT/SRE Reporter Gene

A wide variety of human transformed epithelial cell lines (see Table 16
10 below) were seeded in 96-well flat-bottom plates at 10,000 cell/well in regular growth media as specified for each cell type. The following day, the cells were infected with an adenovirus reporter construct, KZ136, at a multiplicity of infection of 5000. The KZ136 reporter contains the STAT elements in addition to a serum response element. The total volume was 100 μ l/well using DMEM supplemented with 2 mM L-glutamine
15 (GibcoBRL), 1 mM Sodium Pyruvate (GibcoBRL) and 1x Insulin-Transferrin-Selenium supplement (GibcoBRL) (hereinafter referred to as serum-free media). Cells were cultured overnight.

The following day, the media was removed and replaced with 100 μ l of induction media. The induction media was human zcytor17 ligand diluted in serum-
20 free media at 100ng/ml, 50 ng/ml, 25 ng/ml, 12.5 ng/ml, 6.25 ng/ml, 3.125 ng/ml and 1.56 ng/ml. A positive control of 20% FBS was used to validate the assay and to ensure the infection by adenovirus was successful. The cells were induced for 5 hours at which time the media was aspirated. The cells were then washed in 50 μ l/well of PBS, and subsequently lysed in 30 μ l/well of 1X cell lysis buffer (Promega). After a 10-
25 minute incubation at room temperature, 25 μ l/well of lysate was transferred to opaque white 96-well plates. The plates were then read on the Luminometer using 5-second integration with 40 μ l/well injection of luciferase substrate (Promega).

The results revealed the ability of multiple epithelial cell lines to respond to zcytor17 ligand as shown in Table 16 below.

Table 16

Cell Line	Species	Tissue	Morphology	Disease	Fold Induction
A549	Human	Lung	Epithelial	Carcinoma	2x
Sk-Lu-1	Human	Lung	Epithelial	Adenocarcinoma	6x
WI-38	Human	Embryonic Lung	Fibroblast		Negative
MRC-5	Human	Lung	Fibroblast		Negative
DU 145	Human	Prostate	Epithelial	Carcinoma	10x
PZ-HPV-7	Human	Prostate	Epithelial	Transformed with HPV	5x
PC-3	Human	Prostate	Epithelial	Adenocarcinoma	Negative
U2OS	Human	Bone	Epithelial	Osteosarcoma	15.5x
SaOS2	Human	Bone	Epithelial	Osteosarcoma	22x
MG-63	Human	Bone	Fibroblast	Osteosarcoma	Negative
143B	Human	Bone	Fibroblast	Osteosarcoma	3.5x
HOS	Human	Bone	Fibroblast and Epithelial		8x
TRBMeC	Human	Vascular Bone Marrow	Epithelial		2x
HT144	Human	Skin	Fibroblast	Melanoma	5x
C32	Human	Skin		Melanoma	Negative
Sk-Mel-2	Human	Skin	Polygonal	Melanoma	2.7x
WM-115	Human	Skin	Epithelial	Melanoma	2x
HCT-116	Human	Colon	Epithelial	Carcinoma	Negative
HT-29	Human	Colon	Epithelial	Carcinoma	Negative

CaCo2	Human	Colon	Epithelial	Adenocarcinoma	3x
HBL-100	Human	Breast	Epithelial		1.5x
ME-180	Human	Cervix	Epithelial	Carcinoma	Negative
HeLa 299	Human	Cervix	Epithelial	Adenocarcinoma	Negative
SK-N-SH	Human	Brain	Epithelial	Neuroblastoma	Negative
U138 MG	Human	Brain	Polygonal	Glioblastoma	Negative
HepG2	Human	Liver	Epithelial	Carcinoma	Negative
Chang liver	Human	Liver	Epithelial		Negative
Sk-Hep-1	Human	Liver	Epithelial	Adenocarcinoma	4x
Int 407	Human	Intestine	Epithelial		Negative
3a-Sub E	Human	Placenta	Epithelial		Negative

*Example 43**Cytokine production by human epithelial cell lines cultured with human zcytor17 ligand*

Human disease-state epithelial cell lines (A549, human lung epithelial carcinoma; SkLu1, human lung epithelial adenocarcinoma; DU145, human prostate epithelial carcinoma; PZ-HPV-7, human prostate epithelial HPV transformed; U2OS, human bone epithelial osteosarcoma) were screened for cytokine production in response to zcytor17 ligand *in vitro*. These cell lines have both zcytor17 and OSMR-beta, identified by RT-PCR, and respond to human zcytor17 ligand when assayed with the adenoviral luciferase reporter construct, KZ136 (Example 42). Cytokine production by these cell lines was determined in response to human zcytor17 ligand in a series of three experiments.

A. Cytokine production by human disease-state epithelial cell lines cultured with human zcytor17lig

Cells were plated at a density of 4.5×10^5 cells per well in a 6 well plate (Costar) and cultured in respective growth media. The cells were cultured with test reagents; 100ng/mL zcytor17 ligand, 10ng/mL Interferon gamma (IFN gamma) (R&D Systems, Minneapolis, MN), 10ng/mL Tumor Necrosis Factor alpha (TNF alpha) (R&D Systems, Minneapolis, MN), 10ng/mL IL-1beta (R&D Systems, Minneapolis, MN) or 100ug/mL Lipopolysaccharide (LPS) (Sigma). Supernatants were harvested at 24 and 48 hours and assayed for cytokines; GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor), IL-1b, IL-6, IL-8, MCP-1 (Macrophage Chemoattractant Protein-1) and TNFa. Multiplex Antibody Bead kits from BioSource International (Camarillo, CA) were used to measure cytokines in samples. Assays were read on a Luminex-100 instrument (Luminex, Austin, TX) and data was analyzed using MasterPlex software (MiraiBio, Alameda, CA). Cytokine production (pg/mL) for each cell line in the 24-hour samples is shown below in Table 17.

Table 17

GM-CSF pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	18.80	10.26	16.19	13.26	14.10
IFN-g	16.19	13.36	11.56	16.26	11.81
IL-1b	104.60	126.44	76.77	338.25	27.32
TNFa	106.67	33.20	58.50	107.09	33.79
LPS	17.64	10.62	11.81	25.47	18.34
control	14.81	8.56	13.26	21.67	13.96

IL-1b pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	26.90	30.17	28.77	29.07	28.00
IFN-g	29.07	35.33	21.96	26.90	26.73
IL-1b	1332.88	1256.17	979.02	1107.35	998.60
TNFa	31.11	33.28	35.33	31.24	25.66
LPS	33.28	28.77	29.07	31.11	31.24
control	28.77	28.77	26.73	31.24	29.07

IL-6 pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	20.09	26.89	193.05	19.37	17.30
IFN-g	17.52	33.64	217.58	27.02	17.63
IL-1b	175.44	5920.19	2375.29	304.08	18.44
TNFa	354.16	1002.51	1612.17	103.58	18.33
LPS	18.06	35.65	162.18	22.42	17.30
control	17.63	27.80	71.23	19.32	17.19

IL-8 pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	86.33	150.81	150.61	45.92	6.81
IFN-g	24.07	72.82	163.31	81.78	1.35
IL-1b	1726.24	4083.12	4407.79	5308.83	124.17
TNFa	3068.68	3811.75	2539.39	3324.02	69.65
LPS	20.28	167.13	230.39	115.08	7.95
control	14.92	109.78	107.27	93.44	9.49

MCP-1 pg/mL

	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	8.97	187.29	26.84	105.15	7.20
IFN-g	7.30	267.99	17.05	88.68	7.71
IL-1b	8.11	8039.84	88.78	3723.81	4.70
TNFa	8.50	7100.37	153.26	3826.80	2.80
LPS	9.40	185.83	22.65	61.62	5.61
control	8.16	167.93	13.68	47.78	5.61

TNFa pg/mL	A549	SkLu1	DU145	U2OS	PZ-HPV-7
zcytor17L	16.23	17.52	16.67	15.80	17.09
IFN-g	15.80	17.09	15.80	16.65	15.80
IL-1b	16.66	17.09	15.80	17.95	16.23
TNFa	1639.92	1648.83	2975.07	1348.33	3554.82
LPS	16.87	15.80	15.37	17.09	17.52
control	16.23	15.80	15.80	17.09	16.66

All cell lines tested produced GM-CSF and IL-8 in response to stimulation with control cytokines IL-1b and TNFa. Most cell lines produced IL-6 and MCP-1 in response to IL-1b and TNFa stimulation. Zcytor17 ligand stimulated IL-6 production in the DU145 cell line compared to control (193pg/mL vs. 71pg/mL). Zcytor17 ligand stimulated 3 of 5 cell lines to produce IL-8 with the greatest effect seen in A549 cells (5 fold), and reduced IL-8 production in U2OS cells by 2 fold. There was a slight effect on MCP-1 production by DU145 and U2OS cells when cultured with zcytor17 ligand.

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B. Cytokine production by normal human epithelial cell lines cultured with human zcytor17lig

In addition to the human epithelial cell lines, normal human bronchial epithelial cells (NHBE, Clonetics) were also tested. Cells were plated at a density of 1×10^5 cells per well in a 24 well plate and cultured with test reagents; 1000ng/mL, 100ng/mL and 10ng/mL zcytor17 ligand (A760F), 10ng/mL TNFa, 10ng/mL OSM, 10ng/mL IFNa, 10ng/mL TGFb or 10ng/mL Lymphotactin. Supernatants were harvested at 24 and 48 hours and assayed for cytokines; IL-6, IL-8, MCP-1, MIP-1a, RANTES and Eotaxin. Cytokines were assayed as previously described. Cytokine production (pg/mL) for each cell line in the 48-hour samples is shown below in Table 18.

20

Table 18

IL-6 pg/ml

	A549	DU145	SkLu1	U2OS	NHBE
r17L 1000ng/ml	24.5	56.3	32.1	25.2	64.5
r17IL 100ng/ml	25.0	65.0	31.0	25.4	50.2
r17L 10ng/ml	24.8	51.8	30.2	25.3	54.3
TNFa	272.9	355.4	437.5	36.1	299.3
OSM	26.4	73.5	112.4	25.6	80.4
IFNa	24.6	109.3	33.7	26.4	52.4
TGFb	24.4	102.6	42.7	27.8	268.9
control	24.5	36.3	29.9	25.2	47.9

IL-8 pg/ml

	A549	DU145	SkLu1	U2OS	NHBE
r17L 1000ng/ml	35.0	243.3	45.6	18.6	402.0
r17IL 100ng/ml	31.0	290.7	40.1	21.3	296.0
r17L 10ng/ml	30.4	240.4	33.4	18.9	361.8
TNFa	2809.3	2520.9	1385.2	784.9	1486.3
OSM	37.8	60.6	68.0	22.5	494.6
IFNa	18.9	315.3	39.5	33.1	231.6
TGFb	9.9	77.5	19.6	88.9	246.9
control	10.9	238.0	38.0	39.7	315.8

MCP-1 pg/ml

	A549	DU145	SkLu1	U2OS	NHBE
r17L 1000ng/ml	nd	nd	149.1	81.0	nd
r17IL 100ng/ml	nd	nd	130.6	81.9	nd
r17L 10ng/ml	nd	nd	111.7	49.1	nd
TNFa	nd	22.1	2862.6	1104.7	nd
OSM	nd	17.2	448.2	85.8	nd
IFNa	nd	nd	131.7	10.5	nd
TGFb	nd	1.7	54.5	27.6	nd
control	nd	nd	113.0	1.7	nd

nd = not detected

DU145 cells produced IL-6 in response to zcytor17 ligand, repeating the previous results in Example 43A. However, only A549 and U2OS had similar IL-8 responses as seen Example 43A. SkLu1 and U2OS cells both produced MCP-1 in response to zcytor17 ligand. Cytokine production by NHBE cells was marginal compared to controls.

C. Cytokine production by human disease-state epithelial cell lines co-cultured with human zcytor17lig and IFN gamma

Cells were plated at a density of 2×10^5 cells per well in 24 well plate and co-cultured with 10ng/mL IFN gamma +/- zcytor17 ligand at 100ng/mL, 10ng/mL or 1ng/mL. Supernatants were collected at 24 and 48 hours and assayed for IL-8 and MCP-1 as described above. Cytokine production (pg/mL) for each cell line in the 24-
5 hour samples is shown below in Table 19.

Table 19

		IL-8 pg/ml	MCP-1 pg/ml
A549	10ng/mL IFNg+100ng/mL r17L	86.7	nd
	10ng/mL IFNg+10ng/mL r17L	75.1	nd
	10ng/mL IFNg+1ng/mL r17L	63.6	nd
	10ng/ml IFNg	35.4	nd
	control	36.6	nd
DU145	10ng/mL IFNg+100ng/mL r17L	102.3	nd
	10ng/mL IFNg+10ng/mL r17L	92.9	nd
	10ng/mL IFNg+1ng/mL r17L	79.9	nd
	10ng/ml IFNg	70.7	nd
	control	79.4	nd
SkLu1	10ng/mL IFNg+100ng/mL r17L	152.2	604.9
	10ng/mL IFNg+10ng/mL r17L	194.4	870.7
	10ng/mL IFNg+1ng/mL r17L	138.7	585.4
	10ng/ml IFNg	170.8	652.6
	control	203.0	292.3
U2OS	10ng/mL IFNg+100ng/mL r17L	106.8	357.0
	10ng/mL IFNg+10ng/mL r17L	108.2	347.7
	10ng/mL IFNg+1ng/mL r17L	109.9	293.3
	10ng/ml IFNg	118.8	159.8
	control	146.8	7.0

A549 cells produced IL-8 in response to zcytor17 ligand, however there was no effect of co-culturing cells with the addition of IFN gamma. U2OS cells made
 5 20 fold more MCP-1 when cultured with IFNg and 50 fold more MCP-1 when cultured with IFN gamma + zcytor17 ligand.

Example 44

Zcytor17lig Effects on ³H-TdR Incorporation in DU145 Prostate Epithelial Carcinoma Cells

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Cells were seeded in 96-well tissue clusters (Falcon) at a density of 25,000/well in MEM (Life Technologies) growth medium supplemented with glutamine, pyruvate, non-essential amino acids (Life Technologies) and 10% fetal bovine serum (Hyclone). At confluence (24 hours later), cells were switched to growth
 15 arrest media by substituting 0.1% BSA (Life Technologies) for serum. After 48 hours to achieve cell synchronization, the growth-arrest medium was replaced with fresh medium. Then, human recombinant zcytor17lig (test reagent) was added at various

concentrations (from 0.24 to 60 ng/mL) (see Table 16 below), to test for the effect of the protein on basal DNA replication. Some wells received 2.5% FBS (Hyclone) in addition to zcytor17Ligand, in order to test effect of the protein on elevated levels of TdR incorporation. FBS 10% and 20ng/ml Platelet Derived Growth Factor-BB (PDGF-BB) (R&D) were used as positive control.

Eighteen hours following addition of zcytor17Ligand and the rest of the test reagents, cells were pulsed with 250 nCi/mL [³H]-thymidine (NEN) for 4 hours. Following the 4-hour pulse, media were discarded and 100 μ L trypsin solution (Life Technologies) was added in each well to dislodge the cells. The radioactivity incorporated by DU145 was determined by harvesting the cells with a Packard Filtermate 196 cell harvester and by counting the incorporated label using a Packard TopCount NXT microplate scintillation counter.

As can be seen in Table 20 below, zcytor17lig induced thymidine incorporation in quiescent cells (in 0.1% BSA) in a concentration-dependent manner. This effect reached 2.5-fold of the BSA control at the highest concentration used, 60 ng/mL. In addition, this effect of zcytor17lig was also detectable when the baseline incorporation was elevated by the addition of 2.5% FBS (in this series as potent a mitogen as 10% FBS). These results therefore indicate that under both basal and stimulated conditions zcytor17lig can act as a mitogenic factor for the DU145 carcinoma cells.

Table 16 shows the effects of zcytor17lig on thymidine incorporation by DU145 cells. Results are expressed in cpm/well and numbers are the mean $\pm$ st.dev of triplicate wells.

Table 20

	0.1%BSA	2.5%FBS
BSA Control	1139±336	4228±600
Zcytor17lig (0.24ng/mL)	1430±136	4894±1037
Zcytor17lig (0.74ng/mL)	1657±32	5038±810
Zcytor17lig (2.22ng/mL)	1646±57	5162±808
Zcytor17lig (6.67ng/mL)	2226±189	6385±1613
Zcytor17lig (20ng/mL)	2168±108	5880±1085
Zcytor17lig (60ng/mL)	2512±111	6165±417
PDGF-BB (20ng/mL)	4094±202	5927±360

*Example 45*5 *Expression of huzcytor17Ligand in E. coli*A. *Construction of expression vector pRPS01 that expresses huzcytor17Lig/MBP-6H fusion polypeptide*

An expression plasmid containing a polynucleotide encoding a huzcytor17lig fused C-terminally to Maltose Binding Protein (MBP) was constructed via homologous recombination. The fusion polypeptide contains an N-terminal approximately 388 amino acid MBP portion fused to the huzcytor17Lig described herein. A fragment of huzcytor17lig cDNA was isolated using the PCR method as described herein. Two primers were used in the production of the zcytor17lig fragment in a standard PCR reaction: (1) one containing 40 bp of the vector flanking sequence and 20 bp corresponding to the amino terminus of the huzcytor17lig, and (2) another containing 40 bp of the 3' end corresponding to the flanking vector sequence and 20 bp corresponding to the carboxyl terminus of the huzcytor17lig. Two microliters of the 100 μ l PCR reaction was run on a 1.0% agarose gel with 1 x TBE buffer for analysis, and the expected molecular weight fragment was observed. The remaining PCR

reaction was combined with the second PCR tube and precipitated with 400 μ l of absolute ethanol. The precipitated DNA was used for recombination into the SmaI cut recipient vector pTAP98 to produce the construct encoding the MBP-huzcytor17lig fusion, as described below.

5 The vector pTAP98 was constructed using yeast homologous recombination. One hundred nanograms of EcoR1 cut pMAL-c2 was recombined with 1 μ g PvuI cut pRS316, 1 μ g linker, and 1 μ g ScaI/EcoR1 cut pRS316 were combined in a PCR reaction. PCR products were concentrated via 100% ethanol precipitation. The competent yeast cell (*S. cerevisiae*) strain, SF838-9D α , was combined with 10 μ l of a
10 mixture containing approximately 1 μ g of the huzcytor17lig PCR product (above) and 100 ng of SmaI digested pTAP98 vector, and electroporated at 0.75 kV, 25 μ F and ∞ ohms. The resulting reaction mixture was plated onto URA-D plates and incubated at 30°C.

 After 48 hours, the Ura⁺ yeast transformants from a single plate were
15 selected. DNA was isolated and transformed into electrocompetent *E. coli* cells (e.g., MC1061, Casadaban et. al. *J. Mol. Biol.* 138, 179-207). The resulting *E. coli* cells were plated on MM/CA +AMP 100 mg/L plates (Pryor and Leiting, *Protein Expression and Purification* 10:309-319, 1997) using standard procedures. Four individual clones were harvested from the plates and inoculated into MM/CA with 100 μ g/ml Ampicillin for
20 two hours at 37°C. One milliliter of each of the culture was induced with 1mM IPTG. Approximately 2-4 hours later, 250 μ l of each induced culture was mixed with 250 μ l acid washed glass beads and 250 μ l Thorner buffer with 5% β ME and dye (8M urea, 100 mM Tris pH7.0, 10% glycerol, 2mM EDTA, 5% SDS). Samples were vortexed for one minute and heated to 65°C for 10 minutes. Twenty microliters of each sample was
25 loaded per lane on a 4%-12% PAGE gel (NOVEX). Gels were run in 1X MES buffer. The positive clones were designated pRPS01 and subjected to sequence analysis.

 One microliter of sequencing DNA was used to transform electrocompetent *E. coli* cell strain MC1061. The cells were electropulsed at 2.0 kV, 25 μ F and 400 ohms. Following electroporation, cells were rescued 0.6 ml SOC and
30 grown on LB+Amp plates at 37 °C overnight, with 100 mg/L Ampicillin. Four cultures were induced with ITPG and screened for positives as described above. The positive

clones were expanded for protein purification of the huzcytor17lig/MBP-6H fusion protein using standard techniques.

B. Purification of huzcytor17Lig/MBP-6H from E. coli fermentation

5 Unless otherwise noted, all operations were carried out at 4°C. The following procedure was used to purify recombinant huzcytor17Lig/MBP-6H polypeptide. *E. coli* cells containing the pRPS01 construct and expressing huzcytor17Lig/MBP-6H, were constructed using standard molecular biology methods and cultured in 50.0 g/L SuperBroth II (12 g/L Casien, 24 g/L Yeast Extract, 11.4 g/L
10 di-potassium phosphate, 1.7 g/L Mono-potassium phosphate; Becton Dickenson, Cockeysville, MD), 5 g/L glycerol and 5 mL/L 1M Magnesium Sulfate. Twenty grams of cells were harvested and frozen for protein purification.

The thawed cells were resuspended in 500 mL Amylose Equilibration buffer (20mM Tris, 100mM NaCl, pH 8.0). A French Press cell breaking system
15 (Constant Systems Ltd., Warwick, UK) with a temperature setting of -7°C to -10°C and 30K PSI was used to lyse the cells. The resuspended cells were assayed for breakage by A₆₀₀ readings before and after cycling through the French Press. The processed cell suspension was pelleted at 10,000G for 30 minutes to remove the cellular debris and the supernatant was harvested for protein purification.

20 A 25 ml column of Amylose resin (New England Biolabs, Beverly, MA) (prepared as described below) was poured into a Bio-Rad, 2.5 cm D x 10 cm H glass column. The column was packed and equilibrated by gravity with 10 column volumes (CVs) of Amylose Equilibration buffer. The processed cell supernatant was batch loaded to the Amylose resin overnight, with rocking. The resin was returned to the Bio-
25 Rad column and washed with 10 CV's of Amylose Equilibration buffer by gravity. The column was eluted with ~2 CV of Amylose Elution buffer (Amylose Equilibration buffer + 10 mM Maltose, Fluka Biochemical, Switzerland) by gravity. Ten 5 mL fractions were collected over the elution profile and assayed for Absorbance at 280 and 320 nM. The Amylose resin was regenerated with 1 CV of distilled H₂O, 5 CVs of
30 0.1% (w/v) SDS (Sigma), 5 CVs of distilled H₂O, 5 CVs of Amylose Equilibration buffer and finally 1 CV of Amylose Storage buffer (Amylose Equilibration buffer +

0.02% Sodium Azide). The regenerated column was stored at 4°C.

Elution profile fractions of interest were pooled and dialyzed in a 10K dialysis chamber (Slide-A-Lyzer, Pierce Immunochemicals) against 4 x 4L PBS pH 7.4 (Sigma) over an 8 hour time period to remove low molecular weight contaminants, buffer exchange and desalt. Following dialysis, the material harvested represented the purified hzcytor17Lig/MBP-6H polypeptide. The purified hzcytor17Lig/MBP-6H polypeptide was filter sterilized and analyzed via SDS-PAGE Coomassie staining for an appropriate molecular weight product. The concentration of the hzcytor17Lig/MBP-6H polypeptide was determined by BCA analysis to be 1.28 mg/mL.

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Example 46

Human zcytor17lig Polyclonal Antibody

A. Preparation and Purification

Polyclonal antibodies were prepared by immunizing 2 female New Zealand white rabbits with the purified recombinant protein hzcytor17L/MBP-6H (Example 45). The rabbits were each given an initial intraperitoneal (IP) injection of 200 µg of purified protein in Complete Freund's Adjuvant followed by booster IP injections of 100 µg protein in Incomplete Freund's Adjuvant every three weeks. Seven to ten days after the administration of the second booster injection (3 total injections), the animals were bled and the serum was collected. The animals were then boosted and bled every three weeks.

The hzcytor17L/MBP-6H specific rabbit serum was pre-adsorbed of anti-MBP antibodies using a CNBr-SEPHAROSE 4B protein column (Pharmacia LKB) that was prepared using 10 mg of non-specific purified recombinant MBP-fusion protein per gram of CNBr-SEPHAROSE. The hzcytor17L/MBP-6H-specific polyclonal antibodies were affinity purified from the pre-adsorbed rabbit serum using a CNBr-SEPHAROSE 4B protein column (Pharmacia LKB) that was prepared using 10 mg of the specific antigen purified recombinant protein hzcytor17L/MBP-6H. Following purification, the polyclonal antibodies were dialyzed with 4 changes of 20 times the antibody volume of PBS over a time period of at least 8 hours. HZcytor17-Ligand-specific antibodies were characterized by ELISA using 500 ng/ml of the

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purified recombinant proteins hzcytor17L/MBP-6H or hzcytor17L-CEE produced in a baculovirus expression system as antibody targets. The lower limit of detection (LLD) of the rabbit anti-hzcytor17L/MBP-6H affinity purified antibody is 100 pg/ml on its specific purified recombinant antigen hzcytor17L/MBP-6H and 500 pg/ml on purified recombinant hzcytor17L-CEE produced in a baculovirus expression system.

B. SDS-PAGE and Western blotting analysis of Rabbit Anti-human ZcytoR17lig MBP-6H antibody

Rabbit Anti-human ZcytoR17lig MBP-6H antibody was tested by SDS-PAGE (NuPage 4-12%, Invitrogen, Carlsbad, CA) with coomassie staining method and Western blotting using goat anti-rabbit IgG-HRP. Human and mouse zcytor17lig purified protein (200-25 ng) was electrophoresed using an Invitrogen Novex's Xcell II mini-cell, and transferred to nitrocellulose (0.2 mm; Invitrogen, Carlsbad, CA) at room temperature using Novex's Xcell blot module with stirring according to directions provided in the instrument manual. The transfer was run at 300 mA for one hour in a buffer containing 25 mM Tris base, 200 mM glycine, and 20% methanol. The filter was then blocked with Western A buffer (in house, 50 mM Tris, pH 7.4, 5 mM EDTA, pH 8.0, 0.05% Igepal CA-630, 150 mM NaCl, and 0.25% gelatin) overnight with gentle rocking at 4°C. The nitrocellulose was quickly rinsed, then the rabbit anti-human zcytoR17lig MBP-6H (1:1000) was added in Western A buffer. The blot was incubated for 1.5 hours at room temperature with gentle rocking. The blot was rinsed 3 times for 5 minutes each in Western A, then goat anti-rabbit IgG HRP antibody (1:5000) was added in Western A buffer. The blot was incubated for 1 hour at room temperature with gentle rocking. The blot was rinsed 3 times for 5 minutes each in Western A, then quickly rinsed in H₂O. The blot was developed using commercially available chemiluminescent substrate reagents (ECL Western blotting detection reagents 1 and 2 mixed 1:1; reagents obtained from Amersham Pharmacia Biotech, Buckinghamshire, England) and the blot was exposed to x-ray film for up to 5 minutes.

The purified human zcytor17lig appeared as a large band at about 30 kDa and a smaller band at about 20 kDa under reduced conditions. The mouse zcytor17lig was not detected by the rabbit anti-human zcytor17lig antibody.

*Example 47**Zcytor17lig Effects on U937 Monocyte Adhesion
to Transformed Bone Marrow Endothelial Cell (TRBMEC) Monolayer*

5

Transformed Bone Marrow Endothelial Cells (TRBMEC) were seeded in 96-well tissue clusters (Falcon) at a density of 25,000/well in medium M131 (Cascade Biologics) supplemented with Microvascular Growth Supplement (MVGS) (Cascade Biologics). At confluence (24 hours later), cells were switched to M199
10 (Gibco-Life Technologies) supplemented with 1% Fetal Bovine Serum (Hyclone). Human recombinant zcytor17lig (test reagent) was added at various concentrations (from 0.4 to 10 ng/mL) (see Table 21 below), to test for the effect of the protein on immune cell-endothelial cell interactions resulting in adhesion. Some wells received 0.3ng/ml Tumor Necrosis Factor (TNFalpha R&D Systems), a known pro-
15 inflammatory cytokine, in addition to zcytor17lig, to test an effect of the protein on endothelial cells under inflammatory conditions. TNFalpha at 0.3ng/ml alone was used as positive control and the concentration used represents approximately 70% of the maximal TNFalpha effect in this system, i.e., it does not induce maximal adherence of U937 cells (a human monocyte-like cell line) to the endothelium. For this reason, this
20 setup can detect both upregulation and downregulation of the TNFalpha effects. Basal levels of adhesion both with and without TNFalpha were used as baseline to assess effect of test reagents.

After overnight incubation of the endothelial cells with the test reagents (zcytor17ligand $\pm$ TNFalpha), U937 cells, stained with 5 μ M Calcein-AM fluorescent
25 marker (Molecular Probes), the cells were suspended in RPMI 1640 (no phenol-red) supplemented with 1% FBS and plated at 100,000 cells/well on the rinsed TRBMEC monolayer. Fluorescence levels at excitation/emission wavelengths of 485/538nm (Molecular Devices micro-plate reader, CytoFluor application) were measured 30 minutes later, before and after rinsing the well three times with warm RPMI 1640 (no
30 phenol-red), to remove non-adherent U937. Pre-rinse (total) and post-rinse (adherence-specific) fluorescence levels were used to determine percent adherence (net

adherent/net total x 100 = % adherence).

As can be seen in Table 21 below, zcytor17lig when added alone affected the basal adherence of U937 cells to the endothelial monolayers at the concentration range used (less than 2-fold increases, $p < 0.01$ by ANOVA test). By itself, the positive control, 0.3ng/mL TNFalpha, increased the adherence of U937 cells from a basal 5.8% to 35% (6-fold). In the presence of TNFalpha, zcytor17lig synergized with TNFalpha and further enhanced U937 adhesion in a concentration-dependent manner between 0.4 and 10ng/mL ($p < 0.01$ by ANOVA test). At 10ng/mL, zcytor17lig enhanced the effect of TNFalpha by 62%. These results indicate that zcytor17lig may by itself be a pro-inflammatory agent. Zcytor17lig was able to synergize with sub-maximal concentrations of TNFalpha to increase monocyte adherence to endothelial cells. These results also show that endothelial cells, especially when exposed to pro-inflammatory cytokines such as TNFalpha, are a likely target tissue of zcytor17lig action. The consequence of zcytor17ligand on endothelial cells may be to heighten monocyte or macrophage adhesion to a site of proinflammatory activity. Activated monocytes and macrophages are important in many inflammatory diseases. Therefore inhibition of monocyte/macrophage adhesions may provide a therapeutic rationale for zcytor17ligand antagonists. This data would support the use of zcytor17 ligand antagonists for treatment lung diseases, vascular diseases, autoimmunity, tumor metastasis, disease involving allergic reactions, wound healing and diseases of the skin including contact, allergic or non-allergic dermatistic or psoriasis and inflammatory bowel disease. Table 21 shows the effects of zcytor17lig on U937 monocyte adhesion to TRBMEC endothelial monolayers. Results are expressed in percent adhesion and numbers are the mean $\pm$ st.dev of triplicate wells.

25

Table 21

	<i>Basal</i>	<i>0.3ng/mL TNFalpha</i>
Basal	5.8 $\pm$ 1.2	35 $\pm$ 5.5
zcytor17lig 0.4 ng/mL	9 $\pm$ 0.7	44.7 $\pm$ 2.5
zcytor17lig 1.1 ng/mL	10.4 $\pm$ 0.8	45.2 $\pm$ 0.6
zcytor17lig 3.3 ng/mL	7.9 $\pm$ 1.7	51.1 $\pm$ 4
zcytor17lig 10 ng/mL	9.5 $\pm$ 0.5	56.6 $\pm$ 3.9

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

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

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JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

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THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. An isolated multimeric or heterodimeric cytokine receptor comprising:
a first polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with amino acid residues 20-227 of SEQ ID NO:111 or amino acid residues 20-519 of SEQ ID NO:111; and
a second polypeptide comprising amino acid residues 28-429 of SEQ ID NO:7,
wherein the multimeric or heterodimeric cytokine receptor is capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.
2. An isolated multimeric or heterodimeric cytokine receptor comprising:
a first polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with amino acid residues 20-732 of SEQ ID NO:111 or amino acid residues 20-649 of SEQ ID NO:109; and
a second polypeptide comprising amino acid residues 28-429 of SEQ ID NO:7,
wherein the multimeric or heterodimeric cytokine receptor is capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.
3. The isolated multimeric or heterodimeric cytokine receptor according to Claim 1 or 2, wherein the second polypeptide comprises amino acid residues 28-739 of SEQ ID NO:7.
4. The isolated multimeric or heterodimeric cytokine receptor according to any one of Claims 1, 2 or 3, wherein the second polypeptide comprises amino acid residues 28-979 of SEQ ID NO:7.
5. An isolated multimeric or heterodimeric cytokine receptor comprising a first polypeptide selected from the group consisting of:

- a) a polypeptide comprising amino acids 20-227 of SEQ ID NO:111;
- b) a polypeptide comprising amino acids 20-519 of SEQ ID NO:111;
- c) a polypeptide comprising amino acids 20-732 of SEQ ID NO:111; and
- d) a polypeptide comprising amino acids 20-649 of SEQ ID NO:109;

and a second polypeptide selected from the group consisting of:

- a) a polypeptide comprising amino acid 28-429 of SEQ ID NO:7; and
- b) a polypeptide comprising amino acids 28-739 of SEQ ID NO:7; and
- c) a polypeptide comprising amino acids 28-979 of SEQ ID NO:7,

wherein the multimeric or heterodimeric cytokine receptor is capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.

6. An isolated multimeric or heterodimeric cytokine receptor comprising a first polypeptide comprising amino acid residues 20-227 of SEQ ID NO:111 or comprising amino acid residues 20-519 of SEQ ID NO:111 and a second polypeptide comprising amino acid residues 28-429 of SEQ ID NO:7 or amino acid residues 28-739 of SEQ ID NO:7, wherein the multimeric or heterodimeric cytokine receptor is capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.

7. An isolated multimeric or heterodimeric cytokine receptor comprising a first polypeptide comprising amino acid residues 20-732 of SEQ ID NO:111 or comprising amino acid residues 20-649 of SEQ ID NO:109 and a second polypeptide comprising amino acid residues 28-979 of SEQ ID NO:7, wherein the multimeric or heterodimeric cytokine receptor is capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.

8. A composition comprising the multimeric or heterodimeric cytokine receptor according to any one of Claims 1-7 and a pharmaceutically acceptable vehicle.

9. An isolated polynucleotide comprising:
a first polynucleotide that encodes a first polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with amino acid residues 20-227 of SEQ ID NO:111 or amino acid residues 20-519 of SEQ ID NO:111; and

a second polynucleotide that encodes a second polypeptide comprising amino acid residues 28-429 of SEQ ID NO:7 or comprising amino acid residues 28-739 of SEQ ID NO:7,

wherein the first polypeptide and second polypeptide form a multimeric or heterodimeric cytokine receptor capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.

10. An isolated polynucleotide comprising:

a first polynucleotide that encodes a first polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with amino acid residues 20-732 of SEQ ID NO:111 or amino acid residues 20-649 of SEQ ID NO:109; and

a second polynucleotide that encodes a second polypeptide comprising amino acid residues 28-979 of SEQ ID NO:7,

wherein the first polypeptide and second polypeptide form a multimeric or heterodimeric cytokine receptor capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.

11. An expression vector comprising the following operably linked elements:

a) a first transcription promoter; a first DNA segment encoding a first polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with amino acid residues 20-227 of SEQ ID NO:111 or amino acid residues 20-519 of SEQ ID NO:111; and

b) a second transcription promoter; a second DNA segment encoding a second polypeptide comprising amino acid residues 28-429 of SEQ ID NO:7 or amino acid residues 28-739 of SEQ ID NO:7; and a second transcription terminator;

wherein the first and second polypeptide form a multimeric or heterodimeric cytokine receptor, and

wherein the multimeric or heterodimeric cytokine receptor is capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.

12. An expression vector comprising the following operably linked elements:

a) a first transcription promoter; a first DNA segment encoding a first polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with amino acid residues 20-732 of SEQ ID NO:111 or amino acid residues 20-649 of SEQ ID NO:109; and

b) a second transcription promoter; a second DNA segment encoding a second polypeptide comprising amino acid residues 28-979 of SEQ ID NO:7; and a second transcription terminator;

wherein the first and second polypeptide form a multimeric or heterodimeric cytokine receptor, and

wherein the multimeric or heterodimeric cytokine receptor is capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.

13. A cultured cell comprising the expression vector according to Claim 11 or 12, wherein the cell expresses the first and second polypeptide encoded by the DNA segment or DNA segments.

14. A cultured cell comprising:

a first expression vector comprising:

a) a first transcription promoter;

b) a first DNA segment encoding a first polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with amino acid residues 20-227 of SEQ ID NO:111 or amino acid residues 20-519 of SEQ ID NO:111; and

c) a first transcription terminator; and

a second expression vector comprising:

a) a second transcription promoter;

b) a second DNA segment encoding a second polypeptide comprising amino acid residues 28-429 of SEQ ID NO:7 or amino acid residues 28-739 of SEQ ID NO:7; and

c) a second transcription terminator;

wherein the first polypeptide and the second polypeptide form a multimeric or heterodimeric cytokine receptor capable of binding a ligand comprising amino

acid residues 27-164 of SEQ ID NO:2.

15. A cultured cell comprising:
 - a first expression vector comprising:
 - a) a first transcription promoter;
 - b) a first DNA segment encoding a first polypeptide comprising an amino acid sequence having at least 90 percent sequence identity with amino acid residues 20-732 of SEQ ID NO:111 or amino acid residues 20-649 of SEQ ID NO:109; and
 - c) a first transcription terminator; and
 - a second expression vector comprising:
 - a) a second transcription promoter;
 - b) a second DNA segment encoding a second polypeptide comprising amino acid residues 28-979 of SEQ ID NO:7; and
 - c) a second transcription terminator;

wherein the first polypeptide and the second polypeptide form a multimeric or heterodimeric cytokine receptor capable of binding a ligand comprising amino acid residues 27-164 of SEQ ID NO:2.
16. A method of producing a multimeric or heterodimeric cytokine receptor comprising:
 - culturing a cell according to any one of Claims 13-15; and
 - isolating the multimeric or heterodimeric cytokine receptor produced by the cell.
17. An isolated antibody or antibody fragment that specifically binds to a multimeric or heterodimeric cytokine receptor, wherein the multimeric or heterodimeric cytokine receptor consists of:
 - a first polypeptide consisting of amino acid residues 20-732 of SEQ ID NO:111 or amino acid residues 20-649 of SEQ ID NO:109; and
 - a second polypeptide consisting of amino acid residues 28-979 of SEQ ID NO:7.

18. An isolated antibody or antibody fragment that specifically binds to a multimeric or heterodimeric cytokine receptor, wherein the multimeric or heterodimeric cytokine receptor consists of:

a first polypeptide consisting of amino acid residues 20-732 of SEQ ID NO:111 or amino acid residues 20-649 of SEQ ID NO:109; and

a second polypeptide consisting of amino acid residues 28-979 of SEQ ID NO:7, wherein the antibody or antibody fragment inhibits signaling by a ligand comprising amino acid residues 27-164 of SEQ ID NO:2 through the multimeric or heterodimeric cytokine receptor.

19. The isolated antibody or antibody fragment according to Claim 17 or 18, wherein the antibody or antibody fragment specifically binds the first polypeptide of the multimeric or heterodimeric cytokine receptor.

20. The antibody or antibody fragment according to any one of Claims 17-19, wherein the antibody or antibody fragment further comprises a radionuclide, enzyme, substrate, cofactor, fluorescent marker, chemiluminescent marker, peptide tag, magnetic particle, drug, or toxin.

21. A composition comprising the antibody or antibody fragment according to any one of Claims 17-20 and a pharmaceutically acceptable vehicle.

1/12

	1	15 16	30 31	45	
1 zcytor171ig	---ASHTLPVRLLRP	SDDVQKIVEELQSLS	KMLLKD--VEEEKGV		40
2 mzytor171ig	---ATCSLSFGAPIS	KEDLRTTIDLLKQES	QDLYNNYSIKQASGM		42
3 mIL-3	ASISGRDTHRLTRTL	NCS-SIVKEIIGKL-	--PEP----ELKT--		35
4 hIL-3	APMTQTTPLKTSW-V	NCS-NMIDEIITHLK	QPPLP--LLDFNNLN		41
	46	60 61	75 76	90	
1 zcytor171ig	LVSQNYTLPCLSPDA	QPPNNIHSPAIRAYL	KTIRQLDNKSVIDEI		85
2 mzytor171ig	SADESIQLPCFSLDR	EALTNISVIAHLEK	VKVLSE-NTVDTSWV		86
3 mIL-3	DDEGPSLRNKS----	-----FRRVNLSKFV	ESQGEVDPEDRYVIK		71
4 hIL-3	GEDQDILMENN----	-----LRRPNLEAFN	RAVKSL--QNASAIE		75
	91	105 106	120 121	135	
1 zcytor171ig	IEHLDKLIFQDAPET	NISVPTDTHE---CK	RFILTISQQFSECMD		127
2 mzytor171ig	IRWLTNISC FNPLNL	NISVPGNTDESYDCK	VFVLTVLKQFSNCMA		131
3 mIL-3	SNLQKLNCCLPTSAN	DSALPGVFIRDLD--	DFRKKLRFYMVH-LN		113
4 hIL-3	SILKNLLPCLPLATA	APTRHPIHIKGDWN	EFRRKLTFY----LK		116
	136	150 151	165 166	180	
1 zcytor171ig	LALKSLTSGAQQATT				142
2 mzytor171ig	ELQAKDNTTC				141
3 mIL-3	DLETVLTSRPPQPAS	GSVSPNRGTVEC			140
4 hIL-3	TLENA----QAQQT	LSLAIF			133

FIG. 1

2/12

	10	20	30	40	50	
ZCYTOR	MASHSGPSTSVLFLFCCLGGWLASHTLPVRLLRPSDDVQKIVEELQSLSKMLLKD--VEE					
	:	:	:	:	:	:
M17RL-	MIFHTGTTKPTLVLLCCIGTWLATCSLSFGAPISKEDLRTTIDLLKQESQDLYNNYSIKQ					
	10	20	30	40	50	60
	60	70	80	90	100	110
ZCYTOR	EKGVLVSQNYTLPCLSPDAQPPNNIHSPAIRAYLKTIRQLDNKSVIDE-IIEHLDKLIFQ					
	:	:	:	:	:	:
M17RL-	ASGMSADESIQLPCFSLDREALTNI--SVIIAHLEKVKVLSSENTVDTSWVIRWLTNISC					
	70	80	90	100	110	
	120	130	140	150	160	
ZCYTOR	DAPETNISVPTDTHE---CKRFILTISQQFSECM-DLALKSLTSGAQQATT					
	:	:	:	:	:	:
M17RL-	NPLNLNISVPGNTDESYDCKVFVLTVLKQFSNCMAELQAKDNTTC					
	120	130	140	150	160	

FIG. 2

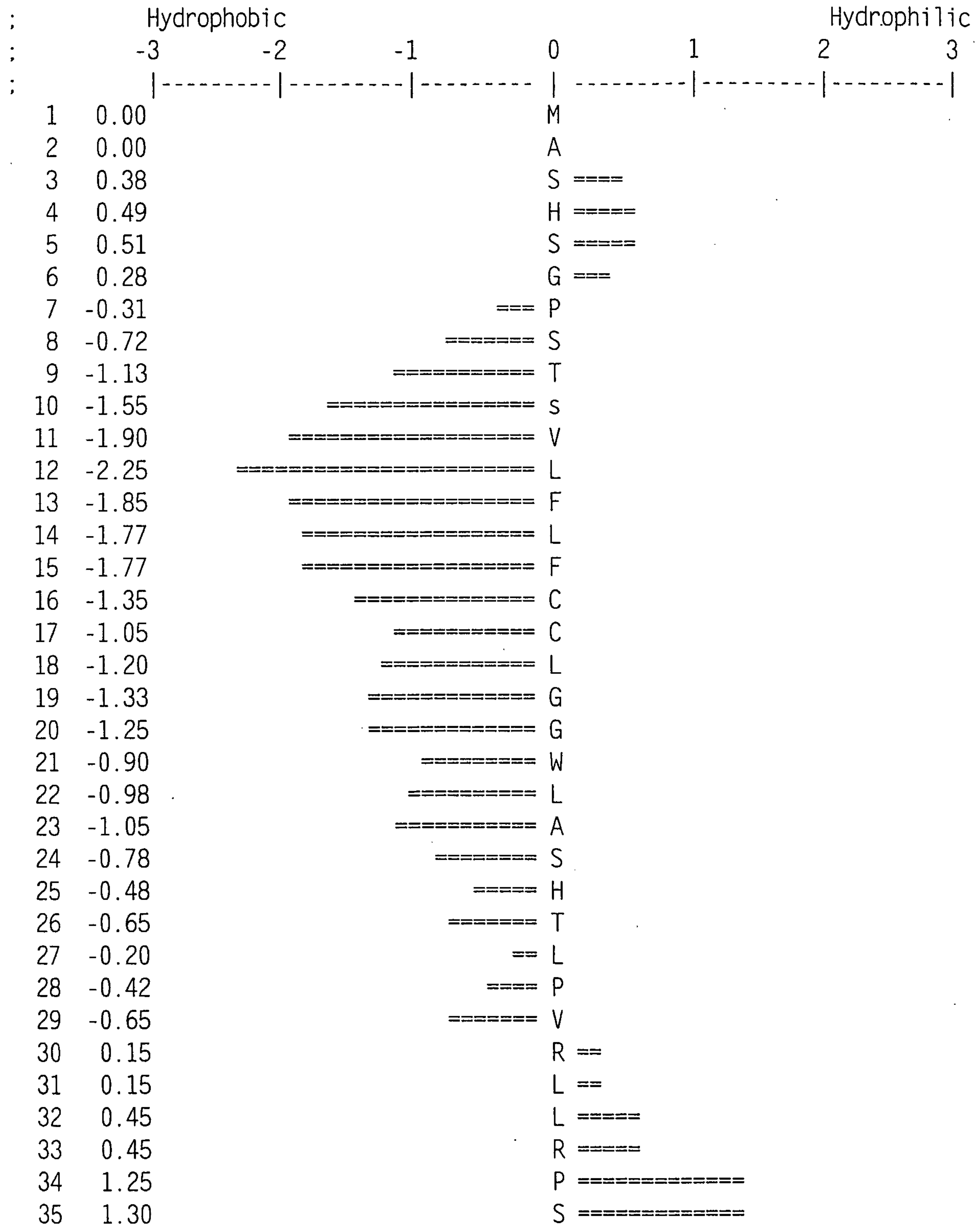


FIG. 3A

4/12

36	0.83	D	=====
37	1.33	D	=====
38	0.98	V	=====
39	0.23	Q	==
40	0.23	K	==
41	0.98	I	=====
42	0.65	V	=====
43	0.18	E	==
44	0.53	E	=====
45	0.48	L	=====
46	0.03	Q	
47	0.03	S	
48	0.12	L	=
49	-0.22	== S	
50	-0.57	===== K	
51	0.23	M	==
52	0.68	L	=====
53	-0.07	= L	
54	0.65	K	=====
55	1.45	D	=====
56	2.25	V	=====
57	2.25	E	=====
58	1.75	E	=====
59	1.75	E	=====
60	0.95	K	=====
61	0.20	G	==
62	-0.25	=== V	
63	-0.72	===== L	
64	-0.68	===== V	
65	-0.42	===== S	
66	-0.18	== Q	
67	-0.23	== N	
68	-0.28	=== y	
69	-0.48	===== T	
70	-0.82	===== L	
71	-0.78	===== P	
72	-0.72	===== C	
73	0.08	L	=
74	0.00	S	

FIG. 3B

5/12

75	0.20	P ==
76	0.50	D =====
77	0.45	A =====
78	0.48	Q =====
79	0.02	P
80	-0.20	== P
81	-0.32	=== N
82	-0.27	=== N
83	-0.27	=== I
84	-0.38	===== H
85	-0.72	===== S
86	0.08	P =
87	0.08	A =
88	-0.35	===== I
89	-0.65	===== R
90	-0.07	= A
91	0.17	Y ==
92	-0.63	===== L
93	-0.05	K
94	0.37	T =====
95	0.37	I =====
96	0.37	R =====
97	0.47	Q =====
98	1.27	L =====
99	0.82	D =====
100	0.53	N =====
101	0.53	K =====
102	0.53	S =====
103	1.00	V =====
104	0.20	I ==
105	-0.15	= D
106	0.60	E =====
107	0.82	I =====
108	0.02	I
109	0.02	E
110	0.82	H =====
111	0.82	L =====
112	0.02	D

FIG. 3C

6/12

113	-0.32	===	K
114	0.02		L
115	0.02		I
116	-0.57	=====	F
117	-0.27	===	Q
118	0.53		D =====
119	0.88		A =====
120	0.88		P =====
121	0.08		E =
122	0.22		T ==
123	-0.03		N
124	-0.53	=====	I
125	-0.53	=====	S
126	-0.07	=	V
127	0.17		P ==
128	0.43		T =====
129	1.18		D =====
130	1.02		T =====
131	1.58		h =====
132	1.58		E =====
133	1.23		C =====
134	0.62		K =====
135	-0.18	==	R
136	-0.58	=====	F
137	-1.38	=====	I
138	-1.83	=====	L
139	-1.38	=====	t
140	-1.05	=====	I
141	-1.17	=====	S
142	-0.55	=====	Q
143	0.25		Q ===
144	0.03		F
145	-0.22	==	S
146	0.25		E ===
147	0.37		C =====
148	0.23		M ==
149	-0.57	=====	D
150	0.10		L =

FIG. 3D

7/12

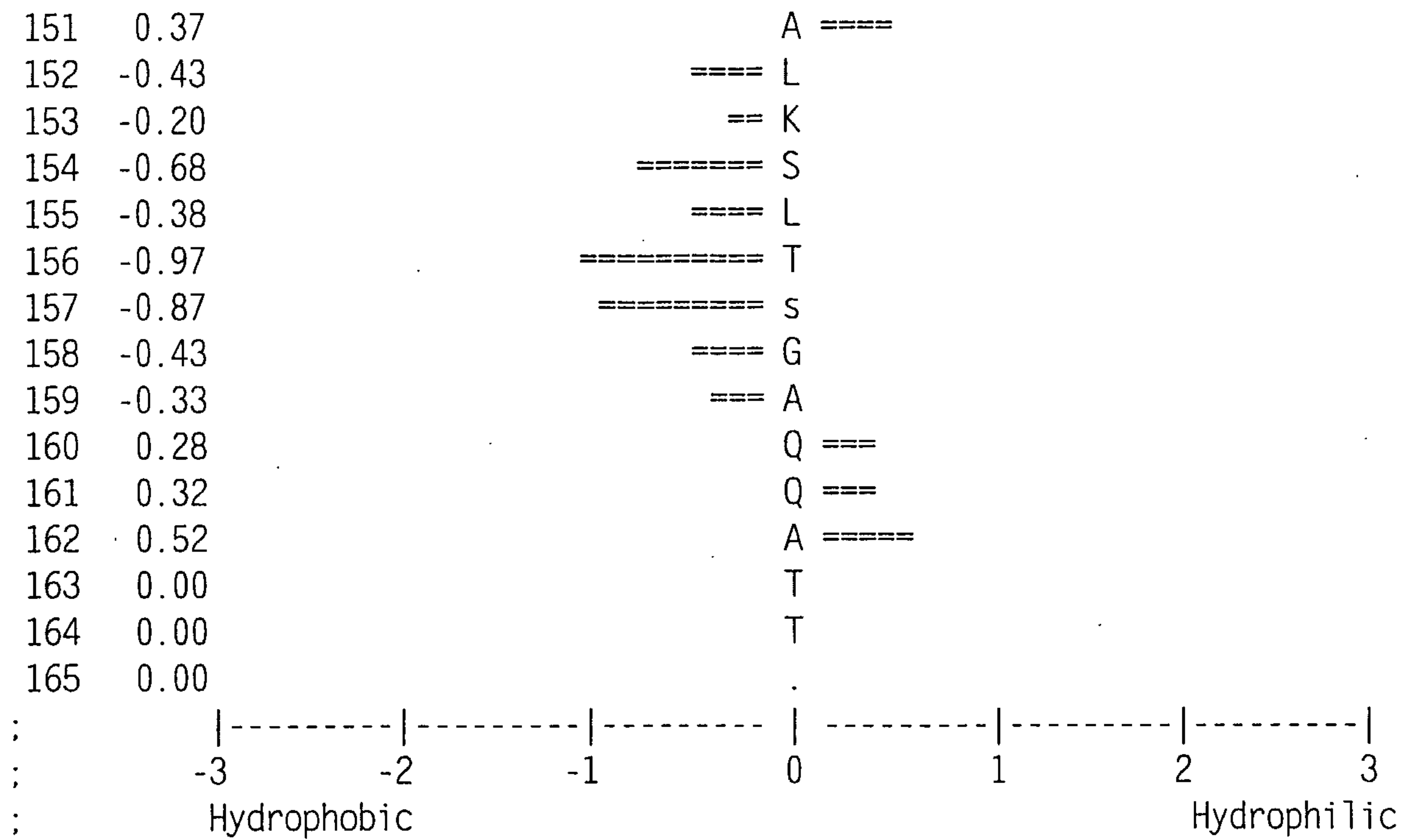


FIG. 3E

8/12

	1		50
SEQ ID NO:109	MMWTWALWML PSLCKFSLAA LPAKPENISC VYYYRKNLTC TWSPGKETS		
SEQ ID NO:113	MMWTWALWML PSLCKFSLAA LPAKPENISC VYYYRKNLTC TWSPGKETS		
SEQ ID NO:111	MMWTWALWML PSLCKFSLAA LPAKPENISC VYYYRKNLTC TWSPGKETS		
SEQ ID NO:115	MMWTWALWML PSLCKFSLAA LPAKPENISC VYYYRKNLTC TWSPGKETS		
	51		100
SEQ ID NO:109	TQYTVKRITYA FGEKHDNCTT NSSTSENRAS CSFFLPRITI PDNYTIEVEA		
SEQ ID NO:113	TQYTVKRITYA FGEKHDNCTT NSSTSENRAS CSFFLPRITI PDNYTIEVEA		
SEQ ID NO:111	TQYTVKRITYA FGEKHDNCTT NSSTSENRAS CSFFLPRITI PDNYTIEVEA		
SEQ ID NO:115	TQYTVKRITYA FGEKHDNCTT NSSTSENRAS CSFFLPRITI PDNYTIEVEA		
	101		150
SEQ ID NO:109	ENGDGVIKSH MTYWRLNIA KTEPPKIFRV KPVLGKIRMI QIEWIKPELA		
SEQ ID NO:113	ENGDGVIKSH MTYWRLNIA KTEPPKIFRV KPVLGKIRMI QIEWIKPELA		
SEQ ID NO:111	ENGDGVIKSH MTYWRLNIA KTEPPKIFRV KPVLGKIRMI QIEWIKPELA		
SEQ ID NO:115	ENGDGVIKSH MTYWRLNIA KTEPPKIFRV KPVLGKIRMI QIEWIKPELA		
	151		200
SEQ ID NO:109	PVSSDLKYTL RFRTVNSTSW MEVNFAKNRK DKNQTYNLTG LQPFTEYVIA		
SEQ ID NO:113	PVSSDLKYTL RFRTVNSTSW MEVNFAKNRK DKNQTYNLTG LQPFTEYVIA		
SEQ ID NO:111	PVSSDLKYTL RFRTVNSTSW MEVNFAKNRK DKNQTYNLTG LQPFTEYVIA		
SEQ ID NO:115	PVSSDLKYTL RFRTVNSTSW MEVNFAKNRK DKNQTYNLTG LQPFTEYVIA		
	201		250
SEQ ID NO:109	LRCVAVKESKF WSDWSQEKMG MTEEEAPCGL ELWRVLKPAE ADGRRPVRL		
SEQ ID NO:113	LRCVAVKESKF WSDWSQEKMG MTEEEAPCGL ELWRVLKPAE ADGRRPVRL		
SEQ ID NO:111	LRCVAVKESKF WSDWSQEKMG MTEEEAPCGL ELWRVLKPAE ADGRRPVRL		
SEQ ID NO:115	LRCVAVKESKF WSDWSQEKMG MTEEEGKL.L PAIPVLSALV ~~~~~		
	251		300
SEQ ID NO:109	WKKARGAPVL EKTLYGNIWY YPESNTNLTE TMNTTNQQL LHLGGESFW		
SEQ ID NO:113	WKKARGAPVL EKTLYGNIWY YPESNTNLTE TMNTTNQQL LHLGGESFW		
SEQ ID NO:111	WKKARGAPVL EKTLYGNIWY YPESNTNLTE TMNTTNQQL LHLGGESFW		
SEQ ID NO:115	~~~~~ ~~~~~ ~~~~~ ~~~~~ ~~~~~		
	301		350
SEQ ID NO:109	SMISYNSLGK SPVATLRIPA IQEKSFCIE VMQACVAEDQ LVVKWQSSAL		
SEQ ID NO:113	SMISYNSLGK SPVATLRIPA IQEK~~~~~ ~~~~~ ~~~~~		
SEQ ID NO:111	SMISYNSLGK SPVATLRIPA IQEKSFCIE VMQACVAEDQ LVVKWQSSAL		
SEQ ID NO:115	~~~~~ ~~~~~ ~~~~~ ~~~~~ ~~~~~		

FIG. 4A

9/12

	351		400
SEQ ID NO:109	DVNTWMIWF PDVDSEPTTL SWESVSQATN WTIQQDKLKP FWCYNISVYP		
SEQ ID NO:113	~~~~~		
SEQ ID NO:111	DVNTWMIWF PDVDSEPTTL SWESVSQATN WTIQQDKLKP FWCYNISVYP		
SEQ ID NO:115	~~~~~		
	401		450
SEQ ID NO:109	MLHDKVGEPY SIQAYAKEGV PSEGPETKVE NIGVKTVTIT WKEIPKSERK		
SEQ ID NO:113	~~~~~		
SEQ ID NO:111	MLHDKVGEPY SIQAYAKEGV PSEGPETKVE NIGVKTVTIT WKEIPKSERK		
SEQ ID NO:115	~~~~~		
	451		500
SEQ ID NO:109	GIICNYTIFY QAEGGKGFSK TVNSSILQYG LESLKRKTSY IVQVMASSTA		
SEQ ID NO:113	~~~~~		
SEQ ID NO:111	GIICNYTIFY QAEGGKGFSK TVNSSILQYG LESLKRKTSY IVQVMASSTA		
SEQ ID NO:115	~~~~~		
	501		550
SEQ ID NO:109	GGTNGTSINF KTLSFSVFEI ILITSLIGGG LLILIIILTVA YGLKKPNKLT		
SEQ ID NO:113	~~~~~		
SEQ ID NO:111	GGTNGTSINF KTLSFSVFEI ILITSLIGGG LLILIIILTVA YGLKKPNKLT		
SEQ ID NO:115	~~~~~		
	551		600
SEQ ID NO:109	HLCWPTVPNP AESSIATWHG DDFKDKLNLK ESDDSVNTED RILKPCSTPS		
SEQ ID NO:113	~~~~~		
SEQ ID NO:111	HLCWPTVPNP AESSIATWHG DDFKDKLNLK ESDDSVNTED RILKPCSTPS		
SEQ ID NO:115	~~~~~		
	601		650
SEQ ID NO:109	DKLVIDKLVV NFGNVLQEIF TDEARTGQEN NLGGEKNGTR ILSSCPTSI~		
SEQ ID NO:113	~~~~~		
SEQ ID NO:111	DKLVIDKLVV NFGNVLQEIF TDEARTGQEN NLGGEKNGYV TCPFRPDCPL		
SEQ ID NO:115	~~~~~		
	651		700
SEQ ID NO:109	~~~~~		
SEQ ID NO:113	~~~~~		
SEQ ID NO:111	GKSFEELPVS PEIPPRKSQY LRSRMPEGTR PEAKEQLLFS GQSLVPDHLC		
SEQ ID NO:115	~~~~~		

FIG. 4B

10/12

	701			733
SEQ ID NO:109	~~~~~	~~~~~	~~~~~	~~~
SEQ ID NO:113	~~~~~	~~~~~	~~~~~	~~~
SEQ ID NO:111	EEGAPNPYLK	NSVTAREFLV	SEKLPEHTKG	EV~
SEQ ID NO:115	~~~~~	~~~~~	~~~~~	~~~

FIG. 4C

11/12

	10	20	30	40
ZCYTOR	MKLSPQPSCVNLGMMWTWALWMLPSLCKFSLAALPAKPENISCVYYY			
	::	::	::	::
M17R-O	MLSSQKGSCSQEPGAAHVQPLGVNAGIMWTLALWAFSFLCKFSLAVLPTKPENISCVFYF			
	10	20	30	40
	50	60	70	80
ZCYTOR	RKNLTCTWSPGKETSQYTVKRTYAFGEKHDNCTTNSSTSENRASCSFFLPRITIPDNY			
	::	::	::	::
M17R-O	DRNLTCTWRPEKETNDTSYIVTLTYSYGK-----SNYSDNATEASYSFPRSCAMPPDIC			
	70	80	90	100
	110	120	130	140
ZCYTOR	TIEVEAENG DGVIKSHMTYWRLENI AKTEPPKIFRVKPV LGIKRMIQIEWIKPELAPVSS			
	::	::	::	::
M17R-O	SVEVQAQNGDGKVKSDITYWHLISIAKTEPP IILSVNPICN--RMFQIQW-KPREKTRGF			
	120	130	140	150
	160	170	180	190
ZCYTOR	DLKYTLRFRTVNSTSWMEVNF AKNRKDNQTYNLTGLQPFTEYVIALRCAVKESKFWSDW			
	::	::	::	::
M17R-O	PLVCMLRFRTVNSSRWTEVNF-ENCK---QVCNLTGLQAFTEYVLALRFRFND SRYWSKW			
	180	190	200	210
	220	230	240	250
ZCYTOR	SQEKMGMT EEEAPCGLELWRVLKPAEADGRRPVRL LWKKARGAPVLEKTLGYNIWYYPES			
	::	::	::	::
M17R-O	SKEETRVTMEEVPHVLDLWRILEPADMNGDRKVRLLWKKARGAPVLEKTFGYHIQYFAEN			
	230	240	250	260
	270	280	290	300
ZCYTOR	NTNLTETMNTTNQQLHLGGESFWVSMISYNSLGKSPVATLRIPAIQKSFQCIEVMQA			
	::	::	::	::
M17R-O	STNLTEINNITTQQYELLMSQAHSVSVTSFNSLGKSQETILRIPDVHEKTFQYIKSMQA			
	290	300	310	320
	330	340	350	360
ZCYTOR	CVAEDQLVVKWQSSALDVNTWMI EWFPDVD-SEPTTLSWESVSQATNWTIQQDKLKPFWC			
	::	::	::	::
M17R-O	YIAEPLL VVNWQSSIPAVDTWIVEWLPEAAMSKFPALSWESVSQVTNWTIEQDKLKPFTC			
	350	360	370	380
	390	400		

Fig. 5A

12/12

	410	420	430	440	450	460
ZCYTOR	YNISVYPMLHDKVGEPYSIQAYAKEGVPSEGPETKVENIGVKT	VTITWKEIPKSERKGII				
						
M17R-O	YNISVYPVLGHRVGEPYSIQAYAKEGTPLKGPETRVENIGLRTATITWKEIPKSARNGFI					
	410	420	430	440	450	460

	470	480	490	500	510	520
ZCYTOR	CNYTIFYQAEGGKGF	SKTVNSSILQYGLESLKRKTSYIVQVMASTSAGGTNGTSINFCTL				
						
M17R-O	NNYTVFYQAEGGKELSKTVNSHALQCDLESLTRRTSYTVWVMASTRAGGTNGVRINFCTL					
	470	480	490	500	510	520

	530	540	550	560	570	580
ZCYTOR	SFSVFEIILITSLIGGGLLILII	ILTVAYGLKKPNKLTHLCWPTVPNPAESSIATWHGDDF				
						
M17R-O	SISVFEIVLLTSLVGGGLLLLSIKTVTFGLRKPNRLTPLCCPDVPNPAESSLATWLGDGF					
	530	540	550	560	570	580

	590	600	610	620	630	640
ZCYTOR	KDKLNLKESDDSVNTEDRILKPCSTPSDKLVIDKL	VVNF	GNVLQEIFTDEARTGQENNLG			
						
M17R-O	K-KSNMKETGNSGNTEDVVLKPCVPAD--LIDKL	VVNF	FENFLEVVLTEEAGKGQASILG			
	590	600	610	620	630	640

	650	660
ZCYTOR	GEKNGTRILSSCPTSI	
		
M17R-O	GEAN-EYILSQEPSCPGHC	
	650	660

Fig 5B