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(54) Titre : L'HU-B1.219, UN NOUVEAU RECEPTEUR HUMAIN DE L'HEMATOPOIETINE
(54) Title: HU-B1.219, A NOVEL HUMAN HEMATOPOIETIN RECEPTOR

(57) **Abrégé/Abstract:**

The present invention relates to a novel member of the hematopoietin receptor family, herein referred to as Hu-B1.219. In particular, the invention relates to nucleotide sequences and expression vectors encoding Hu-B1.219 gene product. Genetically engineered host cells that express the Hu-B1.219 coding sequence may be used to evaluate and screen for ligands or drugs involved in Hu-B1.219 interaction and regulation. Since Hu-B1.219 expression has been detected in certain human fetal tissues and cancer cells, molecular probes designed from its nucleotide sequence may be useful for prenatal testing and cancer diagnosis.



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(57) Abstract

The present invention relates to a novel member of the hematopoietin receptor family, herein referred to as Hu-B1.219. In particular, the invention relates to nucleotide sequences and expression vectors encoding Hu-B1.219 gene product. Genetically engineered host cells that express the Hu-B1.219 coding sequence may be used to evaluate and screen for ligands or drugs involved in Hu-B1.219 interaction and regulation. Since Hu-B1.219 expression has been detected in certain human fetal tissues and cancer cells, molecular probes designed from its nucleotide sequence may be useful for prenatal testing and cancer diagnosis.

Hu-B1.219, A NOVEL HUMAN HEMATOPOIETIN RECEPTOR**1. INTRODUCTION**

The present invention relates to a novel member of the
5 hematopoietin receptor family, herein referred to as
Hu-B1.219. In particular, the invention relates to
nucleotide sequences and expression vectors encoding Hu-
B1.219 gene product. Genetically engineered host cells that
express the Hu-B1.219 coding sequence may be used to evaluate
10 and screen for ligands or drugs involved in Hu-B1.219
interaction and regulation. Since Hu-B1.219 expression has
been detected in certain human fetal tissues and cancer
cells, molecular probes designed from its nucleotide sequence
may be useful for prenatal testing and cancer diagnosis.

15

2. BACKGROUND OF THE INVENTION

A variety of diseases, including malignancy and
immunodeficiency, are related to malfunction within the
lympho-hematopoietic system. Some of these conditions could
20 be alleviated and/or cured by repopulating the hematopoietic
system with progenitor cells, which when triggered to
differentiate would overcome the patient's deficiency.
Therefore, the ability to initiate and regulate hematopoiesis
is of great importance (McCune *et al.*, 1988, Science
25 241:1632).

The process of blood cell formation, by which a small
number of self-renewing stem cells give rise to lineage
specific progenitor cells that subsequently undergo
proliferation and differentiation to produce the mature
30 circulating blood cells has been shown to be at least in part
regulated by specific hormones. These hormones are
collectively known as hematopoietic growth factors or
cytokines (Metcalf, 1985, Science 229:16; Dexter, 1987, J.
Cell Sci. 88:1; Golde and Gasson, 1988, Scientific American,
35 July:62; Tabbara and Robinson, 1991, Anti-Cancer Res. 11:81;
Ogawa, 1989, Environ. Health Presp. 80:199; Dexter, 1989, Br.
Med. Bull. 45:337).

With the advent of recombinant DNA technology, the genes encoding a number of these molecules have now been molecularly cloned and expressed in recombinant form (Souza *et al.*, 1986, *Science* 232:61; Gough *et al.*, 1984, *Nature* 309:763; Yokota *et al.*, 1984, *Proc. Natl. Acad. Sci. U.S.A.* 81:1070; Kawasaki *et al.*, 1985, *Science* 230:291). These cytokines have been studied in their structure, biology and even therapeutic potential. Some of the most well characterized factors include erythropoietin (EPO), stem cell factor (SCF), granulocyte macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), and the interleukins (IL-1 to IL-14).

These factors act on different cell types at different stages during blood cell development, and their potential uses in medicine are far-reaching which include blood transfusions, bone marrow transplantation, correcting immunosuppressive disorders, cancer therapy, wound healing, and activation of the immune response. (Golde and Gasson, 1988, *Scientific American*, July:62).

Apart from inducing proliferation and differentiation of hematopoietic progenitor cells, such cytokines have also been shown to activate a number of functions of mature blood cells (Stanley *et al.*, 1976, *J. Exp. Med.* 143:631; Schrader *et al.*, 1981, *Proc. Natl. Acad. Sci. U.S.A.* 78:323; Moore *et al.*, 1980, *J. Immunol.* 125:1302; Kurland *et al.*, 1979, *Proc. Natl. Acad. Sci. U.S.A.* 76:2326; Handman and Burgess, 1979, *J. Immunol.* 122:1134; Vadas *et al.*, 1983, *Blood* 61:1232; Vadas *et al.*, 1983, *J. Immunol.* 130:795), including influencing the migration of mature hematopoietic cells (Weibart *et al.*, 1986, *J. Immunol.* 137:3584).

Cytokines exert their effects on target cells by binding to specific cell surface receptors. A number of cytokine receptors have been identified and the genes encoding them molecularly cloned. Several cytokine receptors have recently been classified into a hematopoietin receptor (HR) superfamily. The grouping of these receptors was based on

the conservation of key amino acid motifs in the extracellular domains (Bazan, 1990, Immunology Today 11:350) (Figure 1). The HR family is defined by three conserved motifs in the extracellular domain of these receptors. The
5 first is a Trp-Ser-X-Trp-Ser (WSXWS box) motif which is highly conserved and located amino-terminal to the transmembrane domain. Most members of the HR family contain this motif. The second consists of four conserved cysteine residues located in the amino-terminal half of the
10 extracellular region. The third is a conserved fibronectin Type III (FN III) domain which is located between the WSXWS box and the cysteines. The members of the HR family include receptors for ligands such as erythropoietin (EPO), granulocyte colony stimulating factor (G-CSF) (Fukunaga,
15 1990, Cell 61:341), granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-3 (IL-3), IL-4, IL-5, IL-6, IL-7, and IL-2 (β -subunit) (Cosman, 1990, TIBS 15:265).

Ligands for the HR are critically involved in the maturation and differentiation of blood cells. For example,
20 IL-3 promotes the proliferation of early multilineage pluripotent stem cells, and synergizes with EPO to produce red cells. IL-6 and IL-3 synergize to induce proliferation of early hematopoietic precursors. GM-CSF has been shown to induce the proliferation of granulocytes as well as increase
25 macrophage function. IL-7 is a bone marrow-derived cytokine that plays a role in producing immature T and B lymphocytes. IL-4 induces proliferation of antigen-primed B cells and antigen-specific T cells. Thus, members of this receptor superfamily are involved in the regulation of the
30 hematopoietic system.

3. SUMMARY OF THE INVENTION

The present invention relates to a novel member of the HR family, referred to as Hu-B1.219. In particular, it
35 relates to the nucleotide sequences, expression vectors, host cells expressing the Hu-B1.219 gene, and proteins encoded by the sequences.

The invention is based, in part, upon Applicants' discovery of a cDNA clone, Hu-B1.219, isolated from a human fetal liver cDNA library. While the nucleotide sequence of this clone shares certain homology with other HR genes, it is also unique in its structure. Three forms of Hu-B1.219 have been identified, and they differ in sequence only at their 3' ends. The sequences are expressed in certain human fetal and tumor cells. Therefore, a wide variety of uses are encompassed by the present invention, including but not limited to, the diagnosis of cancer, the marking of fetal tissues, and the screening of ligands and compounds that bind the receptor molecule encoded by Hu-B1.219.

For the purpose of the present invention, the designation Hu-B1.219 refers to the complete cDNA sequence disclosed in Figure 2A-2G. In addition, Hu-B1.219 also refers to the partial coding sequences within the cDNA sequence of Figure 2A-2G.

4. BRIEF DESCRIPTION OF THE DRAWINGS

- 20 Figure 1. A schematic drawing of conserved regions shared by members of HR family.
- Figure 2A-2G. Nucleotide sequence and deduced amino acid sequence of Hu-B1.219.
- 25 Figure 3A. Comparison of 3' end nucleotide sequences of the three forms of the Hu-B1.219.
- Figure 3B. Comparison of 3' end amino acid sequences of the three forms of Hu-B1.219. The * symbol indicates a stop codon.
- 30 Figure 4. Comparison of the spacing of conserved amino acids in the FN III domain between HR genes and Hu-B1.219.
- Figure 5. Comparison of conserved motifs between HR molecules and Hu-B1.219 in "Block 3".
- 35 Figure 6. Comparison of conserved motifs between HR molecules and Hu-B1.219 in "Block 6".

5. DETAILED DESCRIPTION OF THE INVENTION

5.1. THE Hu-B1.219 CODING SEQUENCE

The present invention relates to nucleic acid and amino acid sequences of a novel member of the HR family. In a specific embodiment by way of example in Section 6, *infra*, a new member of this HR family of receptors was cloned and characterized. The nucleotide coding sequence and deduced amino acid sequence of the novel receptor are unique, and the receptor is referred to as Hu-B1.219. In accordance with the invention, any nucleotide sequence which encodes the amino acid sequence of the Hu-B1.219 gene product can be used to generate recombinant molecules which direct the expression of Hu-B1.219 gene.

Analysis of the Hu-B1.219 sequence revealed significant homology to the FN III domain of the HR family indicating that it was a member of the HR family of receptors. The shared homology between Hu-B1.219 and other known members of the HR family is discussed in Section 6.2, *infra*. However, this receptor also contains regions of previously unreported unique nucleotide sequences.

Northern blot hybridization analysis, indicates that Hu-B1.219 mRNA is highly expressed in cells of hematopoietic origin. In addition, the Hu-B1.219 sequence is expressed in certain tumor cells.

In order to clone the full length cDNA sequence encoding the entire Hu-B1.219 cDNA or to clone variant forms of the molecule, labeled DNA probes made from nucleic acid fragments corresponding to any portion of the partial cDNA disclosed herein may be used to screen the human fetal liver cDNA library. More specifically, oligonucleotides corresponding to either the 5' or 3' terminus of the partial cDNA sequence may be used to obtain longer nucleotide sequences. Briefly, the library may be plated out to yield a maximum of 30,000 pfu for each 150 mm plate. Approximately 40 plates may be screened. The plates are incubated at 37°C until the plaques reach a diameter of 0.25 mm or are just beginning to make contact with one another (3-8 hours). Nylon filters are

placed onto the soft top agarose and after 60 seconds, the filters are peeled off and floated on a DNA denaturing solution consisting of 0.4N sodium hydroxide. The filters are then immersed in neutralizing solution consisting of 1M

5 Tris HCL, pH 7.5, before being allowed to air dry. The filters are prehybridized in casein hybridization buffer containing 10% dextran sulfate, 0.5M NaCl, 50mM Tris HCL, pH 7.5, 0.1% sodium pyrophosphate, 1% casein, 1% SDS, and denatured salmon sperm DNA at 0.5 mg/ml for 6 hours at 60°C.

10 The radiolabeled probe is then denatured by heating to 95°C for 2 minutes and then added to the prehybridization solution containing the filters. The filters are hybridized at 60°C for 16 hours. The filters are then washed in 1X wash mix (10X wash mix contains 3M NaCl, 0.6M Tris base, and 0.02M

15 EDTA) twice for 5 minutes each at room temperature, then in 1X wash mix containing 1% SDS at 60°C for 30 minutes, and finally in 0.3X wash mix containing 0.1% SDS at 60°C for 30 minutes. The filters are then air dried and exposed to x-ray film for autoradiography. After developing, the film is

20 aligned with the filters to select a positive plaque. If a single, isolated positive plaque cannot be obtained, the agar plug containing the plaques will be removed and placed in lambda dilution buffer containing 0.1M NaCl, 0.01M magnesium sulfate, 0.035M Tris HCl, pH 7.5, 0.01% gelatin. The phage

25 may then be replated and rescreened to obtain single, well isolated positive plaques. Positive plaques may be isolated and the cDNA clones sequenced using primers based on the known cDNA sequence. This step may be repeated until a full length cDNA is obtained.

30 It may be necessary to screen multiple cDNA libraries from different tissues to obtain a full length cDNA. In the event that it is difficult to identify cDNA clones encoding the complete 5' terminal coding region, an often encountered situation in cDNA cloning, the RACE (Rapid Amplification of

35 cDNA Ends) technique may be used. RACE is a proven PCR-based strategy for amplifying the 5' end of incomplete cDNAs. 5'-RACE-Ready cDNA synthesized from human fetal liver containing

a unique anchor sequence is commercially available (Clontech). To obtain the 5' end of the cDNA, PCR is carried out on 5'-RACE-Ready cDNA using the provided anchor primer and the 3' primer. A secondary PCR reaction is then carried
5 out using the anchored primer and a nested 3' primer according to the manufacturer's instructions. Once obtained, the full length cDNA sequence may be translated into amino acid sequence and examined for certain landmarks such as a continuous open reading frame flanked by translation
10 initiation and termination sites, a potential signal sequence and transmembrane domain, and finally overall structural similarity to known HR genes.

5.2. EXPRESSION OF Hu-B1.219 SEQUENCE

In accordance with the invention, Hu-B1.219
15 polynucleotide sequence which encodes the Hu-B1.219 protein, peptide fragments of Hu-B1.219, Hu-B1.219 fusion proteins or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of Hu-B1.219 protein, Hu-B1.219 peptide fragment, fusion proteins
20 or a functional equivalent thereof, in appropriate host cells. Such Hu-B1.219 polynucleotide sequences, as well as other polynucleotides which selectively hybridize to at least a part of such Hu-B1.219 polynucleotides or their complements, may also be used in nucleic acid hybridization
25 assays, Southern and Northern blot analyses, etc.

Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence, may be used in the practice of the invention for the cloning and expression
30 of the Hu-B1.219 protein. Such DNA sequences include those which are capable of hybridizing to the human Hu-B1.219 sequences under stringent conditions. The phrase "stringent conditions" as used herein refers to those hybridizing conditions that (1) employ low ionic strength and high
35 temperature for washing, for example, 0.015 M NaCl/0.0015 M sodium citrate/0.1% SDS at 50°C.; (2) employ during hybridization a denaturing agent such as formamide, for

example, 50% (vol/vol) formamide with 0.1% bovine serum albumin/0.1% FicollTM/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM NaCl, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M Sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 g/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC and 0.1% SDS.

Altered DNA sequences which may be used in accordance with the invention include deletions, additions or substitutions of different nucleotide residues resulting in a sequence that encodes the same or a functionally equivalent gene product. The gene product itself may contain deletions, additions or substitutions of amino acid residues within a Hu-B1.219 sequence, which result in a silent change thus producing a functionally equivalent Hu-B1.219 protein. Such amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine, histidine and arginine; amino acids with uncharged polar head groups having similar hydrophilicity values include the following: glycine, asparagine, glutamine, serine, threonine, tyrosine; and amino acids with nonpolar head groups include alanine, valine, isoleucine, leucine, phenylalanine, proline, methionine, tryptophan.

The DNA sequences of the invention may be engineered in order to alter an Hu-B1.219 coding sequence for a variety of ends including but not limited to alterations which modify processing and expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, e.g., site-directed mutagenesis, to insert new restriction sites, to alter glycosylation patterns, phosphorylation, etc.

In another embodiment of the invention, an Hu-B1.219 or a modified Hu-B1.219 sequence may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors or
5 stimulators of Hu-B1.219 activity, it may be useful to encode a chimeric Hu-B1.219 protein expressing a heterologous epitope that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between a Hu-B1.219 sequence and the
10 heterologous protein sequence, so that the Hu-B1.219 may be cleaved away from the heterologous moiety.

In an alternate embodiment of the invention, the coding sequence of a Hu-B1.219 could be synthesized in whole or in part, using chemical methods well known in the art. See, for
15 example, Caruthers et al., 1980, Nuc. Acids Res. Symp. Ser. 7:215-233; Crea and Horn, 1980, Nuc. Acids Res. 9(10):2331; Matteucci and Caruthers, 1980, Tetrahedron Letters 21:719; and Chow and Kempe, 1981, Nuc. Acids Res. 9(12):2807-2817. Alternatively, the protein itself could be produced using
20 chemical methods to synthesize an Hu-B1.219 amino acid sequence in whole or in part. For example, peptides can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography. (e.g., see Creighton, 1983, Proteins
25 Structures And Molecular Principles, W.H. Freeman and Co., N.Y. pp. 50-60). The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, 1983, Proteins, Structures and Molecular Principles, W.H. Freeman
30 and Co., N.Y. , pp. 34-49).

In order to express a biologically active Hu-B1.219, the nucleotide sequence coding for Hu-B1.219, or a functional equivalent, is inserted into an appropriate expression
vector, i.e., a vector which contains the necessary elements
35 for the transcription and translation of the inserted coding sequence. The Hu-B1.219 gene products as well as host cells or cell lines transfected or transformed with recombinant Hu-

B1.219 expression vectors can be used for a variety of purposes. These include but are not limited to generating antibodies (*i.e.*, monoclonal or polyclonal) that competitively inhibit activity of an Hu-B1.219 and neutralize its activity; and antibodies that mimic the activity of Hu-B1.219 ligands in stimulating the receptor to transmit an intracellular signal. Anti-Hu-B1.219 antibodies may be used in detecting and quantifying expression of Hu-B1.219 levels in cells and tissues.

10 5.3. EXPRESSION SYSTEMS

Methods which are well known to those skilled in the art can be used to construct expression vectors containing the Hu-B1.219 coding sequence and appropriate transcriptional/translational control signals. These methods include in vitro recombinant DNA techniques, synthetic techniques and in vivo recombination/genetic recombination. See, for example, the techniques described in Sambrook et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y.

A variety of host-expression vector systems may be utilized to express the Hu-B1.219 coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing the Hu-B1.219 coding sequence; yeast transformed with recombinant yeast expression vectors containing the Hu-B1.219 coding sequence; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the Hu-B1.219 coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the Hu-B1.219 coding sequence; or animal cell systems. The expression elements of these systems vary in their strength and specificities. Depending on the

host/vector system utilized, any of a number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used in the expression vector. For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage λ , plac, ptrp, ptac (ptrp-lac hybrid promoter) and the like may be used; when cloning in insect cell systems, promoters such as the baculovirus polyhedrin promoter may be used; when cloning in plant cell systems, promoters derived from the genome of plant cells (e.g., heat shock promoters; the promoter for the small subunit of RUBISCO; the promoter for the chlorophyll α/β binding protein) or from plant viruses (e.g., the 35S RNA promoter of CaMV; the coat protein promoter of TMV) may be used; when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter) may be used; when generating cell lines that contain multiple copies of the Hu-B1.219 DNA, SV40-, BPV- and EBV-based vectors may be used with an appropriate selectable marker.

In bacterial systems a number of expression vectors may be advantageously selected depending upon the use intended for the Hu-B1.219 expressed. For example, when large quantities of Hu-B1.219 are to be produced for the generation of antibodies or to screen peptide libraries, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include but are not limited to the E. coli expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the Hu-B1.219 coding sequence may be ligated into the vector in frame with the lac Z coding region so that a hybrid AS-lac Z protein is produced; pIN vectors (Inouye & Inouye, 1985, Nucleic acids Res. 13:3101-3109; Van Heeke & Schuster, 1989, J. Biol. Chem. 264:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general,

such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa
5 protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety.

In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel et
10 al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant et al., 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 1987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; and Bitter,
15 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and The Molecular Biology of the Yeast *Saccharomyces*, 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II.

20 In cases where plant expression vectors are used, the expression of the Hu-B1.219 coding sequence may be driven by any of a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV (Brisson et al., 1984, Nature 310:511-514), or the coat protein promoter
25 of TMV (Takamatsu et al., 1987, EMBO J. 6:307-311) may be used; alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al., 1984, EMBO J. 3:1671-1680; Broglie et al., 1984, Science 224:838-843); or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B (Gurley
30 et al., 1986, Mol. Cell. Biol. 6:559-565) may be used. These constructs can be introduced into plant cells using Ti plasmids, Ri plasmids, plant virus vectors, direct DNA transformation, microinjection, electroporation, etc. For reviews of such techniques see, for example, Weissbach &
35 Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp. 421-463; and Grierson &

Corey, 1988, Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9.

An alternative expression system which could be used to express Hu-B1.219 is an insect system. In one such system, 5 Autographa californica nuclear polyhydrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in Spodoptera frugiperda cells. The Hu-B1.219 coding sequence may be cloned into non-essential regions (for example the polyhedrin gene) of the virus and placed under 10 control of an AcNPV promoter (for example the polyhedrin promoter). Successful insertion of the Hu-B1.219 coding sequence will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus 15 lacking the proteinaceous coat coded for by the polyhedrin gene). These recombinant viruses are then used to infect Spodoptera frugiperda cells in which the inserted gene is expressed. (e.g., see Smith et al., 1983, J. Virol. 46:584; Smith, U.S. Patent No. 4,215,051).

In mammalian host cells, a number of viral based 20 expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the Hu-B1.219 coding sequence may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene 25 may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing Hu-B1.219 in infected hosts. (e.g., See Logan & Shenk, 1984, 30 Proc. Natl. Acad. Sci. USA 81:3655-3659). Alternatively, the vaccinia 7.5K promoter may be used. (See, e.g., Mackett et al., 1982, Proc. Natl. Acad. Sci. USA 79:7415-7419; Mackett et al., 1984, J. Virol. 49:857-864; Panicali et al., 1982, Proc. Natl. Acad. Sci. USA 79:4927-4931).

35 Specific initiation signals may also be required for efficient translation of inserted Hu-B1.219 coding sequences. These signals include the ATG initiation codon and adjacent

sequences. In cases where the entire Hu-B1.219 gene, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed.

5 However, in cases where only a portion of the Hu-B1.219 coding sequence is inserted, exogenous translational control signals, including the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the Hu-B1.219 coding sequence to
10 ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription
15 terminators, etc. (see Bittner et al., 1987, Methods in Enzymol. 153:516-544).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific
20 fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. The presence of several consensus N-glycosylation sites in the Hu-B1.219 extracellular domain support the possibility that proper
25 modification may be important for Hu-B1.219 function. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to ensure the correct modification and
30 processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited
35 to CHO, VERO, BHK, HeLa, COS, MDCK, 293, WI38, etc.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell

lines which stably express the Hu-B1.219 may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with the Hu-B1.219 DNA controlled by appropriate expression
5 control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective
10 media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to
15 engineer cell lines which express the Hu-B1.219 on the cell surface. Such engineered cell lines are particularly useful in screening for ligands or drugs that affect Hu-B1.219 function.

A number of selection systems may be used, including but
20 not limited to the herpes simplex virus thymidine kinase (Wigler, et al., 1977, Cell 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 1962, Proc. Natl. Acad. Sci. USA 48:2026), and adenine
phosphoribosyltransferase (Lowy, et al., 1980, Cell 22:817)
25 genes can be employed in tk⁻, hgp^rt⁻ or ap^rt⁻ cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for *dhfr*, which confers resistance to methotrexate (Wigler, et al., 1980, Natl. Acad. Sci. USA
77:3567; O'Hare, et al., 1981, Proc. Natl. Acad. Sci. USA
30 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981), Proc. Natl. Acad. Sci. USA 78:2072); *neo*, which confers resistance to the aminoglycoside G-418 (Colberre-Garapin, et al., 1981, J. Mol. Biol. 150:1); and
hygro, which confers resistance to hygromycin (Santerre, et
35 al., 1984, Gene 30:147) genes. Recently, additional selectable genes have been described, namely *trpB*, which allows cells to utilize indole in place of tryptophan; *hisD*,

which allows cells to utilize histinol in place of histidine (Hartman & Mulligan, 1988, Proc. Natl. Acad. Sci. USA 85:8047); and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue L., 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.).

5.4. IDENTIFICATION OF CELLS THAT EXPRESS Hu-B1.219

10 The host cells which contain the coding sequence and which express the biologically active gene product may be identified by at least four general approaches; (a) DNA-DNA or DNA-RNA hybridization; (b) the presence or absence of "marker" gene functions; (c) assessing the level of
15 transcription as measured by the expression of Hu-B1.219 mRNA transcripts in the host cell; and (d) detection of the gene product as measured by immunoassay or by its biological activity. Prior to the identification of gene expression, the host cells may be first mutagenized in an effort to
20 increase the level of expression of Hu-B1.219, especially in cell lines that produce low amounts of Hu-B1.219.

In the first approach, the presence of the Hu-B1.219 coding sequence inserted in the expression vector can be detected by DNA-DNA or DNA-RNA hybridization using probes
25 comprising nucleotide sequences that are homologous to the Hu-B1.219 coding sequence, respectively, or portions or derivatives thereof.

In the second approach, the recombinant expression vector/host system can be identified and selected based upon
30 the presence or absence of certain "marker" gene functions (e.g., thymidine kinase activity, resistance to antibiotics, resistance to methotrexate, transformation phenotype, occlusion body formation in baculovirus, etc.). For example, if the Hu-B1.219 coding sequence is inserted within a marker
35 gene sequence of the vector, recombinants containing the Hu-B1.219 coding sequence can be identified by the absence of the marker gene function. Alternatively, a marker gene can

be placed in tandem with the Hu-B1.219 sequence under the control of the same or different promoter used to control the expression of the Hu-B1.219 coding sequence. Expression of the marker in response to induction or selection indicates
5 expression of the Hu-B1.219 coding sequence.

In the third approach, transcriptional activity for the Hu-B1.219 coding region can be assessed by hybridization assays. For example, RNA can be isolated and analyzed by Northern blot using a probe homologous to the Hu-B1.219
10 coding sequence or particular portions thereof.

Alternatively, total nucleic acids of the host cell may be extracted and assayed for hybridization to such probes.

In the fourth approach, the expression of the Hu-B1.219 protein product can be assessed immunologically, for example
15 by Western blots, immunoassays such as radioimmuno-precipitation, enzyme-linked immunoassays and the like.

5.5. USES OF Hu-B1.219 ENGINEERED CELL LINES

In an embodiment of the invention, the Hu-B1.219 receptor and/or cell lines that express the Hu-B1.219
20 receptor may be used to screen for antibodies, peptides, or other ligands that act as agonists or antagonists of the Hu-B1.219 receptor. For example, anti-Hu-B1.219 antibodies may be used to inhibit or stimulate receptor Hu-B1.219 function. Alternatively, screening of peptide libraries with
25 recombinantly expressed soluble Hu-B1.219 protein or cell lines expressing Hu-B1.219 protein may be useful for identification of therapeutic molecules that function by inhibiting or stimulating the biological activity of Hu-B1.219. The uses of the Hu-B1.219 receptor and engineered
30 cell lines, described in the subsections below, may be employed equally well for other members of the HR family.

In an embodiment of the invention, engineered cell lines which express most of the Hu-B1.219 coding region or its ligand binding domain or its ligand binding domain fused to
35 another molecule such as the immunoglobulin constant region (Hollenbaugh and Aruffo, 1992, Current Protocols in Immunology, Unit 10.19; Aruffo et al., 1990, Cell 61:1303)

may be utilized to produce a soluble receptor to screen and identify ligand antagonists as well as agonists. The soluble Hu-B1.219 protein or fusion protein may be used to identify a ligand in binding assays, affinity chromatography, immunoprecipitation, Western blot, and the like. Alternatively, the ligand binding domain of Hu-B1.219 may be fused to the coding sequence of the epidermal growth factor receptor transmembrane and cytoplasmic regions. This approach provides for the use of the epidermal growth factor receptor signal transduction pathway as a means for detecting ligands that bind to Hu-B1.219 in a manner capable of triggering an intracellular signal. Synthetic compounds, natural products, and other sources of potentially biologically active materials can be screened in a number of ways.

Random peptide libraries consisting of all possible combinations of amino acids attached to a solid phase support may be used to identify peptides that are able to bind to the ligand binding site of a given receptor or other functional domains of a receptor such as kinase domains (Lam, K.S. et al., 1991, Nature 354: 82-84). The screening of peptide libraries may have therapeutic value in the discovery of pharmaceutical agents that stimulate or inhibit the biological activity of receptors through their interactions with the given receptor.

Identification of molecules that are able to bind to the Hu-B1.219 may be accomplished by screening a peptide library with recombinant soluble Hu-B1.219 protein. Methods for expression and purification of Hu-B1.219 are described in Section 5.2, *supra*, and may be used to express recombinant full length Hu-B1.219 or fragments of Hu-B1.219 depending on the functional domains of interest. For example, the cytoplasmic and extracellular ligand binding domains of Hu-B1.219 may be separately expressed and used to screen peptide libraries.

To identify and isolate the peptide/solid phase support that interacts and forms a complex with Hu-B1.219, it is

necessary to label or "tag" the Hu-B1.219 molecule. The Hu-B1.219 protein may be conjugated to enzymes such as alkaline phosphatase or horseradish peroxidase or to other reagents such as fluorescent labels which may include fluorescein isothiocyanate (FITC), phycoerythrin (PE) or rhodamine. Conjugation of any given label to Hu-B1.219 may be performed using techniques that are routine in the art. Alternatively, Hu-B1.219 expression vectors may be engineered to express a chimeric Hu-B1.219 protein containing an epitope for which a commercially available antibody exist. The epitope specific antibody may be tagged using methods well known in the art including labeling with enzymes, fluorescent dyes or colored or magnetic beads.

The "tagged" Hu-B1.219 conjugate is incubated with the random peptide library for 30 minutes to one hour at 22°C to allow complex formation between Hu-B1.219 and peptide species within the library. The library is then washed to remove any unbound Hu-B1.219 protein. If Hu-B1.219 has been conjugated to alkaline phosphatase or horseradish peroxidase the whole library is poured into a petri dish containing substrates for either alkaline phosphatase or peroxidase, for example, 5-bromo-4-chloro-3-indoyl phosphate (BCIP) or 3,3',4,4'-diaminobenzidine (DAB), respectively. After incubating for several minutes, the peptide/solid phase-Hu-B1.219 complex changes color, and can be easily identified and isolated physically under a dissecting microscope with a micromanipulator. If a fluorescent tagged Hu-B1.219 molecule has been used, complexes may be isolated by fluorescent activated sorting. If a chimeric Hu-B1.219 protein expressing a heterologous epitope has been used, detection of the peptide/Hu-B1.219 complex may be accomplished by using a labeled epitope specific antibody. Once isolated, the identity of the peptide attached to the solid phase support may be determined by peptide sequencing.

In addition to using soluble Hu-B1.219 molecules, in another embodiment, it is possible to detect peptides that bind to cell surface receptors using intact cells. The use

of intact cells is preferred for use with receptors that are multi-subunits or labile or with receptors that require the lipid domain of the cell membrane to be functional. Methods for generating cell lines expressing Hu-B1.219 are described
5 in Section 5.3. The cells used in this technique may be either live or fixed cells. The cells may be incubated with the random peptide library and bind to certain peptides in the library to form a "rosette" between the target cells and the relevant solid phase support/peptide. The rosette can
10 thereafter be isolated by differential centrifugation or removed physically under a dissecting microscope.

As an alternative to whole cell assays for membrane bound receptors or receptors that require the lipid domain of the cell membrane to be functional, the receptor molecules
15 can be reconstituted into liposomes where label or "tag" can be attached.

Various procedures known in the art may be used for the production of antibodies to epitopes of the recombinantly produced Hu-B1.219 receptor. Such antibodies include but are
20 not limited to polyclonal, monoclonal, chimeric, single chain, Fab fragments and fragments produced by an Fab expression library. Neutralizing antibodies i.e., those which compete for the ligand binding site of the receptor are especially preferred for diagnostics and therapeutics.

25 Monoclonal antibodies that bind Hu-B1.219 may be radioactively labeled allowing one to follow their location and distribution in the body after injection. Radioisotope tagged antibodies may be used as a non-invasive diagnostic tool for imaging *de novo* cells of tumors and metastases.

30 Immunotoxins may also be designed which target cytotoxic agents to specific sites in the body. For example, high affinity Hu-B1.219 specific monoclonal antibodies may be covalently complexed to bacterial or plant toxins, such as diphtheria toxin, abrin or ricin. A general method of
35 preparation of antibody/hybrid molecules may involve use of thiol-crosslinking reagents such as SPDP, which attack the primary amino groups on the antibody and by disulfide

exchange, attach the toxin to the antibody. The hybrid antibodies may be used to specifically eliminate Hu-B1.219 expressing tumor cells.

For the production of antibodies, various host animals
5 may be immunized by injection with the Hu-B1.219 protein including but not limited to rabbits, mice, rats, etc. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral gels
10 such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and potentially useful human adjuvants such as BCG (bacilli Calmette-Guerin) and Corynebacterium parvum.

15 Monoclonal antibodies to Hu-B1.219 may be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, (Nature, 1975,
20 256:495-497), the human B-cell hybridoma technique (Kosbor et al., 1983, Immunology Today, 4:72; Cote et al., 1983, Proc. Natl. Acad. Sci., 80:2026-2030) and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). In addition,
25 techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, Proc. Natl. Acad. Sci., 81:6851-6855; Neuberger et al., 1984, Nature, 312:604-608; Takeda et al., 1985, Nature, 314:452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen
30 specificity together with genes from a human antibody molecule of appropriate biological activity can be used. Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce Hu-B1.219-specific single chain
35 antibodies.

Antibody fragments which contain specific binding sites of Hu-B1.219 may be generated by known techniques. For

example, such fragments include but are not limited to: the F(ab')₂ fragments which can be produced by pepsin digestion of the antibody molecule and the Fab fragments which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., 1989, Science, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity to Hu-B1.219.

5.6. USES OF Hu-B1.219 POLYNUCLEOTIDE

An Hu-B1.219 polynucleotide may be used for diagnostic and/or therapeutic purposes. For diagnostic purposes, an Hu-B1.219 polynucleotide may be used to detect Hu-B1.219 gene expression or aberrant Hu-B1.219 gene expression in disease states, e.g., chronic myelogenous leukemia. Included in the scope of the invention are oligonucleotide sequences, that include antisense RNA and DNA molecules and ribozymes, that function to inhibit translation of an Hu-B1.219.

5.6.1. DIAGNOSTIC USES OF AN Hu-B1.219 POLYNUCLEOTIDE

An Hu-B1.219 polynucleotide may have a number of uses for the diagnosis of diseases resulting from aberrant expression of Hu-B1.219. For example, the Hu-B1.219 DNA sequence may be used in hybridization assays of biopsies or autopsies to diagnose abnormalities of Hu-B1.219 expression; e.g., Southern or Northern analysis, including *in situ* hybridization assays. Such techniques are well known in the art, and are in fact the basis of many commercially available diagnostic kits.

5.6.2. THERAPEUTIC USES OF AN Hu-B1.219 POLYNUCLEOTIDE

An Hu-B1.219 polynucleotide may be useful in the treatment of various abnormal conditions. By introducing gene sequences into cells, gene therapy can be used to treat conditions in which the cells do not proliferate or differentiate normally due to underexpression of normal Hu-B1.219 or expression of abnormal/inactive Hu-B1.219. In some instances, the polynucleotide encoding an Hu-B1.219 is

intended to replace or act in the place of a functionally deficient endogenous gene. Alternatively, abnormal conditions characterized by overproliferation can be treated using the gene therapy techniques described below.

- 5 Abnormal cellular proliferation is an important component of a variety of disease states. Recombinant gene therapy vectors, such as viral vectors, may be engineered to express variant, signalling incompetent forms of Hu-B1.219 which may be used to inhibit the activity of the naturally
- 10 occurring endogenous Hu-B1.219. A signalling incompetent form may be, for example, a truncated form of the protein that is lacking all or part of its signal transduction domain. Such a truncated form may participate in normal binding to a substrate but lack signal transduction activity.
- 15 Thus recombinant gene therapy vectors may be used therapeutically for treatment of diseases resulting from aberrant expression or activity of an Hu-B1.219. Accordingly, the invention provides a method of inhibiting the effects of signal transduction by an endogenous Hu-B1.219
- 20 protein in a cell comprising delivering a DNA molecule encoding a signalling incompetent form of the Hu-B1.219 protein to the cell so that the signalling incompetent Hu-B1.219 protein is produced in the cell and competes with the endogenous Hu-B1.219 protein for access to molecules in the
- 25 Hu-B1.219 protein signalling pathway which activate or are activated by the endogenous Hu-B1.219 protein.

- Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery
- 30 of recombinant Hu-B1.219 into the targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors containing an Hu-B1.219 polynucleotide sequence. See, for example, the techniques described in Maniatis et al., 1989, Molecular
- 35 Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience,

N.Y. Alternatively, recombinant Hu-B1.219 molecules can be reconstituted into liposomes for delivery to target cells.

Oligonucleotide sequences, that include anti-sense RNA and DNA molecules and ribozymes that function to inhibit the translation of an Hu-B1.219 mRNA are within the scope of the invention. Anti-sense RNA and DNA molecules act to directly block the translation of mRNA by binding to targeted mRNA and preventing protein translation. In regard to antisense DNA, oligodeoxyribonucleotides derived from the translation initiation site, e.g., between -10 and +10 regions of an Hu-B1.219 nucleotide sequence, are preferred.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. Within the scope of the invention are engineered hammerhead motif ribozyme molecules that specifically and efficiently catalyze endonucleolytic cleavage of Hu-B1.219 RNA sequences.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites which include the following sequences, GUA, GUU and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the target gene containing the cleavage site may be evaluated for predicted structural features such as secondary structure that may render the oligonucleotide sequence unsuitable. The suitability of candidate targets may also be evaluated by testing their accessibility to hybridization with complementary oligonucleotides, using ribonuclease protection assays.

Both anti-sense RNA and DNA molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA

molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors which incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

Various modifications to the DNA molecules may be introduced as a means of increasing intracellular stability and half-life. Possible modifications include but are not limited to the addition of flanking sequences of ribo- or deoxy- nucleotides to the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the oligodeoxyribonucleotide backbone.

Methods for introducing polynucleotides into such cells or tissue include methods for *in vitro* introduction of polynucleotides such as the insertion of naked polynucleotide, *i.e.*, by injection into tissue, the introduction of an Hu-B1.219 polynucleotide in a cell *ex vivo*, *i.e.*, for use in autologous cell therapy, the use of a vector such as a virus, retrovirus, phage or plasmid, etc. or techniques such as electroporation which may be used *in vivo* or *ex vivo*.

6. **EXAMPLE: MOLECULAR CLONING OF A NOVEL HEMATOPOIETIN RECEPTOR COMPLEMENTARY DNA**

6.1. **MATERIALS AND METHODS**

30 6.1.1. **NORTHERN BLOT ANALYSIS**

In order to study the expression of the Hu-B1.219 gene, Northern blots containing RNA obtained from a variety of human tissues (Clontech, Palo Alto, CA) were hybridized with a radiolabeled 530 base pair (bp) DNA probe corresponding to nucleotides #578 through 1107 (see Figure 2A-2G). Briefly, the blots were prehybridized at 42°C for 3-6 hours in a solution containing 5X SSPE, 10X Denhardt's solution, 100

μg/ml freshly denatured, sheared salmon sperm DNA, 50% formamide (freshly deionized), and 2% SDS. The radiolabeled probe was heat denatured and added to the prehybridization mix and allowed to hybridize at 42°C for 18-24 hours with constant shaking. The blots were rinsed in 2X SSC, 0.05% SDS several times at room temperature before being transferred to a wash solution containing 0.1X SSC, 0.1% SDS and agitated at 50°C for 40 minutes. The blots were then covered with plastic wrap, mounted on WhatmanTM paper and exposed to x-ray film at -70°C using an intensifying screen.

6.1.2. REVERSE TRANSCRIPTION/POLYMERASE CHAIN REACTION (RT/PCR)

Total RNA was isolated using standard laboratory procedures (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Approximately 1 μg of total RNA was reverse transcribed and the cDNA was amplified by PCR (Perkin Elmer, Norwalk, CT). The PCR amplification conditions were the same for Hu-B1.219 and Form 1 expression analysis. They were: 94°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec for a total of 40 cycles. The amplified products (224 bp for Hu-B1.219 and 816 bp for Form 1) were resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. The Hu-B1.219 amplimers were GGTTTGCATATGGAAGTC (upper) and CCTGAACCATCCAGTCTCT (lower). The Form 1 specific amplimers were GACTCATTGTGCAGTGTTTCAG (upper) and TAGTGGAGGGAGGGTCAGCAG (lower). The upper amplimer was commonly shared by all 3 forms, whereas the lower amplimer was Form 1-specific.

6.2. RESULTS

A number of cDNA clones were isolated from a human fetal liver cDNA library (Clontech, Palo Alto, CA), and the DNA sequences of several of these clones were determined. These clones (Hu-B1.219 #4, #33, #34, #1, #36, #8, #55, #60, #3, #57, #62) contained overlapping sequences, which were then compiled into a contiguous nucleotide sequence. Both the cDNA sequence and predicted protein sequence from the cDNA are shown in Figure 2A-2G. This cDNA sequence contains two

FN III domains, each containing a "WS box", which are characteristic of genes of the HR family. However, the Hu-B1.219 sequence is not identical to any known gene. Thus, this cDNA represents a novel member of the HR gene family, 5 herein referred to as Hu-B1.219 (Table 1).

Table 1
Cytokine Receptor Gene FN III Domain Sizes (bp)

Gene	Human	Mouse	Rat
Hu-B1.219 (5')	273		
Hu-B1.219 (3')	282		
IL-2R β	291	288	291
IL-2R γ	273		
IL-3R α	246	252	
IL-3R β Aic2a		306 and 273	
IL-3R β Aic2b	306 and 282	303 and 276	
IL-4R	294		291
IL-5R α	276	273	
IL-6R	288	285	
gp130	288	291	288
IL-7R		294	
IL-9R	321	321	
mpl		270	
G-CSFR	300	297	
GM-CSFR	288		
CNTFR	282		285
PRLR			288
EPOR	288	285	288
LIFR-1	321 and 297		

Based on the sequence of Hu-B1.219 presented in Figure 2A-2G, the translation initiation site appears at position #97. The sequence encodes an open reading frame up to and

including nucleotide #2970. It is believed that the sequence between nucleotides #2614 and #2691 encodes a transmembrane domain. The complete sequence encodes a protein of 958 amino acids.

5 However, the sequence in Figure 2A-2G represents only one form of Hu-B1.219 cDNA sequence, herein referred to as Form 1. This is because additional lambda clones were discovered that contained different sequences near the 3' end known as Form 2 and Form 3. All three forms contain the
10 identical sequence up to and including nucleotide #2770, then they diverge at nucleotide #2771 and beyond (Figure 3A). An alignment of deduced amino acid sequences of all three forms corresponding to the 3' end from #2771 until a stop codon is shown in Figure 3B. Two of the originally isolated lambda
15 clones, #36 and #8, contain the 3' end sequences of Form 1 and Form 2, respectively. These three forms of Hu-B1.219 may derive from a common precursor mRNA by an alternative splicing mechanism.

It is noteworthy that the DNA sequence of Form 1 from
20 nucleotide #2771 to the end is 98% identical to a human retrotransposon sequence that is thought to be derived from a human endogenous retroviral DNA sequence (Singer, 1982, Cell 28:433; Weiner et al., 1986, Ann. Rev. Biochem. 55:631; Lower et al., 1993, Proc. Natl. Acad. Sci. USA 90:4480). In order
25 to examine the expression of the different forms of cDNA, RT/PCR was performed using several human cell lines. The results in Table 2 show that Form 1 was expressed as RNA in K-562 cells and in a human fetal liver cDNA preparation. Since Hu-B1.219 was cloned from human fetal liver cDNA
30 library, this served as a positive control. However, with respect to several other human cell lines, Form 1 was not detected, whereas Hu-B1.219 expression was positive. For example, Form 1 was not expressed in KGla cells, but Form 3 was expressed. Thus, it is possible that these three forms
35 of Hu-B1.219 are not expressed simultaneously in the same cells. There may be selective expression of certain forms in particular cell populations.

Table 2
RT/PCR Analysis of Hu-B1.219 Expression

	Cell Lines	Hu-B1.219*	Form 1 Δ	Form 3 Δ
5	MRC5 (Lung fibroblast)	++	+/-	+
	KG1a (lymphoblast)	+	-	++
	Raji (B cell lymphoma)	+	-	+
	Kit 225/K6 (T cell)	+++	-	+
	K562 (myelogenous leukemia)	++++	+++	++++
10	Human Fetal Liver (positive control)	+++	+++	+++

* - Analysis by Northern blots
 Δ - Analysis by RT/PCR

15 Various human tissue RNA were probed with a
 radiolabelled Hu-B1.219 fragment corresponding to nucleotide
 numbers from #578 to #1107 as disclosed in Figure 2A-2G for
 Northern blot analyses. Two different size mRNAs were
 detected. This result suggests that there may be another
 homologous gene or there is alternative splicing of a single
 20 RNA transcript. Hu-B1.219 expression was by far the
 strongest in human fetal tissues, particularly the liver and
 lung. Trace levels were found in several adult tissues.
 Interestingly, a chronic myelogenous leukemia cell line,
 K562, was strongly positive for its expression, while some
 25 expression was also detected in A549 cells, a lung carcinoma
 cell line (Table 3).

30

35

Table 3

SUMMARY OF NORTHERN BLOT ANALYSIS OF
Hu-B1.219 GENE EXPRESSION

	<u>Human Tissues/cell lines</u>	<u>Expression</u>
5	fetal brain	-
	lung	+++
	liver	+++++
	kidney	+
	adult heart	+
	brain	-
10	placenta	+/-
	lung	+
	liver	+
	skeletal muscle	-
	kidney	+/-
	pancreas	-
	spleen	-
15	thymus	-
	prostate	-
	testis	-
	ovary	+
	small intestine	-
	colon	-
	peripheral blood	-
	leukocytes	-
20	cancer HL-60	-
	HeLa	-
	K-562	+++
	MOLT-4	-
	Raji	-
	SW480	-
	A549	+
25	G361	-

Taken together, the data indicates that the Hu-B1.219 cDNA clone represents a new member of the human hematopoietin receptor family. A summary of the data that supports this conclusion is as follows:

1. The Hu-B1.219 DNA and protein sequences do not fully match any known sequences in the corresponding computer data bases.
2. Hu-B1.219 shares certain DNA sequence homology with the IL-6R and IL-4R.
3. It shares certain protein homology with G-CSFR, IL-6R, IL-3R beta chain, gp130, IL-12R, and LIFR.

4. It contains two "WS box" motifs with the correct spacing of conserved amino acids in the FN III domains (see Figure 4).

5. It contains an amphipathic sequence in block 3 of the FN III domains (see Figure 5).

6. It contains alternating hydrophobic and basic amino acids in block 6 of the FN III domains (see Figure 6).

7. It contains conserved cysteines in these cysteine rich regions upstream of the FN III domains.

10 8. It was originally cloned from a hematopoietic tissue, fetal liver.

9. It is expressed by certain fetal tissues.

7. Deposit of Microorganisms

15 The following organisms were deposited with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, Maryland 20852.

Strain Designation Accession No.

	HuB1.219, #1	75885
20	HuB1.219, #4	75886
	HuB1.219, #8	75887
	HuB1.219, #33	75888
	HuB1.219, #34	75889
	HuB1.219, #36	75890
	HuB1.219, #55	75971
	HuB1.219, #60	75973
25	HuB1.219, #3	75970
	HuB1.219, #57	75972
	HuB1.219, #62	75974

The present invention is not to be limited in scope by the exemplified embodiments, which are intended as
 30 illustrations of individual aspects of the invention. Indeed, various modifications for the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to
 35 fall within the scope of the appended claims.

SEQUENCE LISTING

2176463

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Zupancic, Thomas J.
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- (ii) TITLE OF INVENTION: Hu-B1.219, A NOVEL HUMAN HEMATOPOIETIN RECEPTOR
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 - (A) ADDRESSEE: Osler, Hoskin & Harcourt
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 - (C) CITY: Ottawa
 - (D) PROVINCE: Ontario
 - (E) COUNTRY: Canada
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/355,888
 - (B) FILING DATE: 14-DEC-1994
 - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Osler, Hoskin & Harcourt
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (613) 235-7234
 - (B) TELEFAX: (613) 235-2867

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: unknown
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Trp Ser Xaa Trp Ser
1 5

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(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 18 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GGTTTGCATA TGGAAGTC

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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CCTGAACCAT CCAGTCTCT

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(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GACTCATTGT GCAGTGTTC A G

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(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TAGTGGAGGG AGGGTCAGCA G

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(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2991 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..2991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCG CGC GCG ACG CAG GTG CCC GAG CCC CGG CCC GCG CCC ATC TCT GCC	48
Ala Arg Ala Thr Gln Val Pro Glu Pro Arg Pro Ala Pro Ile Ser Ala	
1 5 10 15	
TTC GGT CGA GTT GGA CCC CCG GAT CAA GGT GTA CTT CTC TGA AGT AAG	96
Phe Gly Arg Val Gly Pro Pro Asp Gln Gly Val Leu Leu * Ser Lys	
20 25 30	
ATG ATT TGT CAA AAA TTC TGT GTG GTT TTG TTA CAT TGG GAA TTT ATT	144
Met Ile Cys Gln Lys Phe Cys Val Val Leu Leu His Trp Glu Phe Ile	
35 40 45	
TAT GTG ATA ACT GCG TTT AAC TTG TCA TAT CCA ATT ACT CCT TGG AGA	192
Tyr Val Ile Thr Ala Phe Asn Leu Ser Tyr Pro Ile Thr Pro Trp Arg	
50 55 60	
TTT AAG TTG TCT TGC ATG CCA CCA AAT TCA ACC TAT GAC TAC TTC CTT	240
Phe Lys Leu Ser Cys Met Pro Pro Asn Ser Thr Tyr Asp Tyr Phe Leu	
65 70 75 80	
TTG CCT GCT GGA CTC TCA AAG AAT ACT TCA AAT TCG AAT GGA CAT TAT	288
Leu Pro Ala Gly Leu Ser Lys Asn Thr Ser Asn Ser Asn Gly His Tyr	
85 90 95	

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GAG	ACA	GCT	GTT	GAA	CCT	AAG	TTT	AAT	TCA	AGT	GGT	ACT	CAC	TTT	TCT	336
Glu	Thr	Ala	Val	Glu	Pro	Lys	Phe	Asn	Ser	Ser	Gly	Thr	His	Phe	Ser	
			100					105						110		
AAC	TTA	TCC	AAA	GCA	ACT	TTC	CAC	TGT	TGC	TTT	CGG	AGT	GAG	CAA	GAT	384
Asn	Leu	Ser	Lys	Ala	Thr	Phe	His	Cys	Cys	Phe	Arg	Ser	Glu	Gln	Asp	
		115					120					125				
AGA	AAC	TGC	TCC	TTA	TGT	GCA	GAC	AAC	ATT	GAA	GGA	AGG	ACA	TTT	GTT	432
Arg	Asn	Cys	Ser	Leu	Cys	Ala	Asp	Asn	Ile	Glu	Gly	Arg	Thr	Phe	Val	
	130						135				140					
TCA	ACA	GTA	AAT	TCT	TTA	GTT	TTT	CAA	CAA	ATA	GAT	GCA	AAC	TGG	AAC	480
Ser	Thr	Val	Asn	Ser	Leu	Val	Phe	Gln	Gln	Ile	Asp	Ala	Asn	Trp	Asn	
145					150					155					160	
ATA	CAG	TGC	TGG	CTA	AAA	GGA	GAC	TTA	AAA	TTA	TTC	ATC	TGT	TAT	GTG	528
Ile	Gln	Cys	Trp	Leu	Lys	Gly	Asp	Leu	Lys	Leu	Phe	Ile	Cys	Tyr	Val	
				165					170					175		
GAG	TCA	TTA	TTT	AAG	AAT	CTA	TTC	AGG	AAT	TAT	AAC	TAT	AAG	GTC	CAT	576
Glu	Ser	Leu	Phe	Lys	Asn	Leu	Phe	Arg	Asn	Tyr	Asn	Tyr	Lys	Val	His	
			180					185					190			
CTT	TTA	TAT	GTT	CTG	CCT	GAA	GTG	TTA	GAA	GAT	TCA	CCT	CTG	GTT	CCC	624
Leu	Leu	Tyr	Val	Leu	Pro	Glu	Val	Leu	Glu	Asp	Ser	Pro	Leu	Val	Pro	
		195					200					205				
CAA	AAA	GGC	AGT	TTT	CAG	ATG	GTT	CAC	TGC	AAT	TGC	AGT	GTT	CAT	GAA	672
Gln	Lys	Gly	Ser	Phe	Gln	Met	Val	His	Cys	Asn	Cys	Ser	Val	His	Glu	
	210					215					220					
TGT	TGT	GAA	TGT	CTT	GTG	CCT	GTG	CCA	ACA	GCC	AAA	CTC	AAC	GAC	ACT	720
Cys	Cys	Glu	Cys	Leu	Val	Pro	Val	Pro	Thr	Ala	Lys	Leu	Asn	Asp	Thr	
225					230					235					240	
CTC	CTT	ATG	TGT	TTG	AAA	ATC	ACA	TCT	GGT	GGA	GTA	ATT	TTC	CGG	TCA	768
Leu	Leu	Met	Cys	Leu	Lys	Ile	Thr	Ser	Gly	Gly	Val	Ile	Phe	Arg	Ser	
				245					250					255		
CCT	CTA	ATG	TCA	GTT	CAG	CCC	ATA	AAT	ATG	GTG	AAG	CCT	GAT	CCA	CCA	816
Pro	Leu	Met	Ser	Val	Gln	Pro	Ile	Asn	Met	Val	Lys	Pro	Asp	Pro	Pro	
			260					265					270			
TTA	GGT	TTG	CAT	ATG	GAA	ATC	ACA	GAT	GAT	GGT	AAT	TTA	AAG	ATT	TCT	864
Leu	Gly	Leu	His	Met	Glu	Ile	Thr	Asp	Asp	Gly	Asn	Leu	Lys	Ile	Ser	
		275					280					285				
TGG	TCC	AGC	CCA	CCA	TTG	GTA	CCA	TTT	CCA	CTT	CAA	TAT	CAA	GTG	AAA	912
Trp	Ser	Ser	Pro	Pro	Leu	Val	Pro	Phe	Pro	Leu	Gln	Tyr	Gln	Val	Lys	
	290					295					300					
TAT	TCA	GAG	AAT	TCT	ACA	ACA	GTT	ATC	AGA	GAA	GCT	GAC	AAG	ATT	GTC	960
Tyr	Ser	Glu	Asn	Ser	Thr	Thr	Val	Ile	Arg	Glu	Ala	Asp	Lys	Ile	Val	
305					310				315						320	

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TCA GCT ACA TCC CTG CTA GTA GAC AGT ATA CTT CCT GGG TCT TCG TAT	1008
Ser Ala Thr Ser Leu Leu Val Asp Ser Ile Leu Pro Gly Ser Ser Tyr	
325 330 335	
GAG GTT CAG GTG AGG GGC AAG AGA CTG GAT GGC CCA GGA ATC TGG AGT	1056
Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly Pro Gly Ile Trp Ser	
340 345 350	
GAC TGG AGT ACT CCT CGT GTC TTT ACC ACA CAA GAT GTC ATA TAC TTT	1104
Asp Trp Ser Thr Pro Arg Val Phe Thr Thr Gln Asp Val Ile Tyr Phe	
355 360 365	
CCA CCT AAA ATT CTG ACA AGT GTT GGG TCT AAT GTT TCT TTT CAC TGC	1152
Pro Pro Lys Ile Leu Thr Ser Val Gly Ser Asn Val Ser Phe His Cys	
370 375 380	
ATC TAT AAG AAG GAA AAC AAG ATT GTT CCC TCA AAA GAG ATT GTT TGG	1200
Ile Tyr Lys Lys Glu Asn Lys Ile Val Pro Ser Lys Glu Ile Val Trp	
385 390 395 400	
TGG ATG AAT TTA GCT GAG AAA ATT CCT CAA AGC CAG TAT GAT GTT GTG	1248
Trp Met Asn Leu Ala Glu Lys Ile Pro Gln Ser Gln Tyr Asp Val Val	
405 410 415	
AGT GAT CAT GTT AGC AAA GTT ACT TTT TTC AAT CTG AAT GAA ACC AAA	1296
Ser Asp His Val Ser Lys Val Thr Phe Phe Asn Leu Asn Glu Thr Lys	
420 425 430	
CCT CGA GGA AAG TTT ACC TAT GAT GCA GTG TAC TGC TGC AAT GAA CAT	1344
Pro Arg Gly Lys Phe Thr Tyr Asp Ala Val Tyr Cys Cys Asn Glu His	
435 440 445	
GAA TGC CAT CAT CGC TAT GCT GAA TTA TAT GTG ATT GAT GTC AAT ATC	1392
Glu Cys His His Arg Tyr Ala Glu Leu Tyr Val Ile Asp Val Asn Ile	
450 455 460	
AAT ATC TCA TGT GAA ACT GAT GGG TAC TTA ACT AAA ATG ACT TGC AGA	1440
Asn Ile Ser Cys Glu Thr Asp Gly Tyr Leu Thr Lys Met Thr Cys Arg	
465 470 475 480	
TGG TCA ACC AGT ACA ATC CAG TCA CTT GCG GAA AGC ACT TTG CAA TTG	1488
Trp Ser Thr Ser Thr Ile Gln Ser Leu Ala Glu Ser Thr Leu Gln Leu	
485 490 495	
AGG TAT CAT AGG AGC AGC CTT TAC TGT TCT GAT ATT CCA TCT ATT CAT	1536
Arg Tyr His Arg Ser Ser Leu Tyr Cys Ser Asp Ile Pro Ser Ile His	
500 505 510	
CCC ATA TCT GAG CCC AAA GAT TGC TAT TTG CAG AGT GAT GGT TTT TAT	1584
Pro Ile Ser Glu Pro Lys Asp Cys Tyr Leu Gln Ser Asp Gly Phe Tyr	
515 520 525	
GAA TGC ATT TTC CAG CCA ATC TTC CTA TTA TCT GGC TAC ACA ATG TGG	1632
Glu Cys Ile Phe Gln Pro Ile Phe Leu Leu Ser Gly Tyr Thr Met Trp	
530 535 540	

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ATT	AGG	ATC	AAT	CAC	TCT	CTA	GGT	TCA	CTT	GAC	TCT	CCA	CCA	ACA	TGT	1680
Ile	Arg	Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	Thr	Cys	
545					550					555					560	
GTC	CTT	CCT	GAT	TCT	GTG	GTG	AAG	CCA	CTG	CCT	CCA	TCC	AGT	GTG	AAA	1728
Val	Leu	Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	Ser	Ser	Val	Lys	
				565					570					575		
GCA	GAA	ATT	ACT	ATA	AAC	ATT	GGA	TTA	TTG	AAA	ATA	TCT	TGG	GAA	AAG	1776
Ala	Glu	Ile	Thr	Ile	Asn	Ile	Gly	Leu	Leu	Lys	Ile	Ser	Trp	Glu	Lys	
			580					585					590			
CCA	GTC	TTT	CCA	GAG	AAT	AAC	CTT	CAA	TTC	CAG	ATT	CGC	TAT	GGT	TTA	1824
Pro	Val	Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	Gln	Ile	Arg	Tyr	Gly	Leu	
		595					600					605				
AGT	GGA	AAA	GAA	GTA	CAA	TGG	AAG	ATG	TAT	GAG	GTT	TAT	GAT	GCA	AAA	1872
Ser	Gly	Lys	Glu	Val	Gln	Trp	Lys	Met	Tyr	Glu	Val	Tyr	Asp	Ala	Lys	
	610					615					620					
TCA	AAA	TCT	GTC	AGT	CTC	CCA	GTT	CCA	GAC	TTG	TGT	GCA	GTC	TAT	GCT	1920
Ser	Lys	Ser	Val	Ser	Leu	Pro	Val	Pro	Asp	Leu	Cys	Ala	Val	Tyr	Ala	
625					630					635					640	
GTT	CAG	GTG	CGC	TGT	AAG	AGG	CTA	GAT	GGA	CTG	GGA	TAT	TGG	AGT	AAT	1968
Val	Gln	Val	Arg	Cys	Lys	Arg	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	Ser	Asn	
				645					650					655		
TGG	AGC	AAT	CCA	GCC	TAC	ACA	GTT	GTC	ATG	GAT	ATA	AAA	GTT	CCT	ATG	2016
Trp	Ser	Asn	Pro	Ala	Tyr	Thr	Val	Val	Met	Asp	Ile	Lys	Val	Pro	Met	
			660					665					670			
AGA	GGA	CCT	GAA	TTT	TGG	AGA	ATA	ATT	AAT	GGA	GAT	ACT	ATG	AAA	AAG	2064
Arg	Gly	Pro	Glu	Phe	Trp	Arg	Ile	Ile	Asn	Gly	Asp	Thr	Met	Lys	Lys	
		675					680					685				
GAG	AAA	AAT	GTC	ACT	TTA	CTT	TGG	AAG	CCC	CTG	ATG	AAA	AAT	GAC	TCA	2112
Glu	Lys	Asn	Val	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Met	Lys	Asn	Asp	Ser	
	690					695					700					
TTG	TGC	AGT	GTT	CAG	AGA	TAT	GTG	ATA	AAC	CAT	CAT	ACT	TCC	TGC	AAT	2160
Leu	Cys	Ser	Val	Gln	Arg	Tyr	Val	Ile	Asn	His	His	Thr	Ser	Cys	Asn	
705					710					715					720	
GGA	ACA	TGG	TCA	GAA	GAT	GTG	GGA	AAT	CAC	ACG	AAA	TTC	ACT	TTC	CTG	2208
Gly	Thr	Trp	Ser	Glu	Asp	Val	Gly	Asn	His	Thr	Lys	Phe	Thr	Phe	Leu	
				725					730					735		
TGG	ACA	GAG	CAA	GCA	CAT	ACT	GTT	ACG	GTT	CTG	GCC	ATC	AAT	TCA	ATT	2256
Trp	Thr	Glu	Gln	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Ile	Asn	Ser	Ile	
			740					745					750			
GGT	GCT	TCT	GTT	GCA	AAT	TTT	AAT	TTA	ACC	TTT	TCA	TGG	CCT	ATG	AGC	2304
Gly	Ala	Ser	Val	Ala	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	Met	Ser	
		755				760						765				

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AAA	GTA	AAT	ATC	GTG	CAG	TCA	CTC	AGT	GCT	TAT	CCT	TTA	AAC	AGC	AGT	2352
Lys	Val	Asn	Ile	Val	Gln	Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Asn	Ser	Ser	
770						775					780					
TGT	GTG	ATT	GTT	TCC	TGG	ATA	CTA	TCA	CCC	AGT	GAT	TAC	AAG	CTA	ATG	2400
Cys	Val	Ile	Val	Ser	Trp	Ile	Leu	Ser	Pro	Ser	Asp	Tyr	Lys	Leu	Met	
785					790					795					800	
TAT	TTT	ATT	ATT	GAG	TGG	AAA	AAT	CTT	AAT	GAA	GAT	GGT	GAA	ATA	AAA	2448
Tyr	Phe	Ile	Ile	Glu	Trp	Lys	Asn	Leu	Asn	Glu	Asp	Gly	Glu	Ile	Lys	
				805					810					815		
TGG	CTT	AGA	ATC	TCT	TCA	TCT	GTT	AAG	AAG	TAT	TAT	ATC	CAT	GAT	CAT	2496
Trp	Leu	Arg	Ile	Ser	Ser	Ser	Val	Lys	Lys	Tyr	Tyr	Ile	His	Asp	His	
			820					825					830			
TTT	ATC	CCC	ATT	GAG	AAG	TAC	CAG	TTC	AGT	CTT	TAC	CCA	ATA	TTT	ATG	2544
Phe	Ile	Pro	Ile	Glu	Lys	Tyr	Gln	Phe	Ser	Leu	Tyr	Pro	Ile	Phe	Met	
		835					840					845				
GAA	GGA	GTG	GGA	AAA	CCA	AAG	ATA	ATT	AAT	AGT	TTC	ACT	CAA	GAT	GAT	2592
Glu	Gly	Val	Gly	Lys	Pro	Lys	Ile	Ile	Asn	Ser	Phe	Thr	Gln	Asp	Asp	
850						855					860					
ATT	GAA	AAA	CAC	CAG	AGT	GAT	GCA	GGT	TTA	TAT	GTA	ATT	GTG	CCA	GTA	2640
Ile	Glu	Lys	His	Gln	Ser	Asp	Ala	Gly	Leu	Tyr	Val	Ile	Val	Pro	Val	
865					870					875					880	
ATT	ATT	TCC	TCT	TCC	ATC	TTA	TTG	CTT	GGA	ACA	TTA	TTA	ATA	TCA	CAC	2688
Ile	Ile	Ser	Ser	Ser	Ile	Leu	Leu	Leu	Gly	Thr	Leu	Leu	Ile	Ser	His	
				885					890					895		
CAA	AGA	ATG	AAA	AAG	CTA	TTT	TGG	GAA	GAT	GTT	CCG	AAC	CCC	AAG	AAT	2736
Gln	Arg	Met	Lys	Lys	Leu	Phe	Trp	Glu	Asp	Val	Pro	Asn	Pro	Lys	Asn	
			900					905					910			
TGT	TCC	TGG	GCA	CAA	GGA	CTT	AAT	TTT	CAG	AAG	ATG	CTT	GAA	GGC	AGC	2784
Cys	Ser	Trp	Ala	Gln	Gly	Leu	Asn	Phe	Gln	Lys	Met	Leu	Glu	Gly	Ser	
		915					920					925				
ATG	TTC	GTT	AAG	AGT	CAT	CAC	CAC	TCC	CTA	ATC	TCA	AGT	ACC	CAG	GGA	2832
Met	Phe	Val	Lys	Ser	His	His	His	Ser	Leu	Ile	Ser	Ser	Thr	Gln	Gly	
		930				935					940					
CAC	AAA	CAC	TGC	GGA	AGG	CCA	CAG	GGT	CCT	CTG	CAT	AGG	AAA	ACC	AGA	2880
His	Lys	His	Cys	Gly	Arg	Pro	Gln	Gly	Pro	Leu	His	Arg	Lys	Thr	Arg	
945					950					955					960	
GAC	CTT	TGT	TCA	CTT	GTT	TAT	CTG	CTG	ACC	CTC	CCT	CCA	CTA	TTG	TCC	2928
Asp	Leu	Cys	Ser	Leu	Val	Tyr	Leu	Leu	Thr	Leu	Pro	Pro	Leu	Leu	Ser	
				965					970					975		
TAT	GAC	CCT	GCC	AAA	TCC	CCC	TCT	GTG	AGA	AAC	ACC	CAA	GAA	TGA	TCA	2976
Tyr	Asp	Pro	Ala	Lys	Ser	Pro	Ser	Val	Arg	Asn	Thr	Gln	Glu	*	Ser	
			980					985					990			

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ATA AAA AAA AAA AAA
 Ile Lys Lys Lys Lys
 995

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Arg Ala Thr Gln Val Pro Glu Pro Arg Pro Ala Pro Ile Ser Ala
 1 5 10 15
 Phe Gly Arg Val Gly Pro Pro Asp Gln Gly Val Leu Leu
 20 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 960 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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

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

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Glu	His	Glu	Cys	His	His	Arg	Tyr	Ala	Glu	Leu	Tyr	Val	Ile	Asp	Val	420	425	430	
Asn	Ile	Asn	Ile	Ser	Cys	Glu	Thr	Asp	Gly	Tyr	Leu	Thr	Lys	Met	Thr	435	440	445	
Cys	Arg	Trp	Ser	Thr	Ser	Thr	Ile	Gln	Ser	Leu	Ala	Glu	Ser	Thr	Leu	450	455	460	
Gln	Leu	Arg	Tyr	His	Arg	Ser	Ser	Leu	Tyr	Cys	Ser	Asp	Ile	Pro	Ser	465	470	475	480
Ile	His	Pro	Ile	Ser	Glu	Pro	Lys	Asp	Cys	Tyr	Leu	Gln	Ser	Asp	Gly	485	490	495	
Phe	Tyr	Glu	Cys	Ile	Phe	Gln	Pro	Ile	Phe	Leu	Leu	Ser	Gly	Tyr	Thr	500	505	510	
Met	Trp	Ile	Arg	Ile	Asn	His	Ser	Leu	Gly	Ser	Leu	Asp	Ser	Pro	Pro	515	520	525	
Thr	Cys	Val	Leu	Pro	Asp	Ser	Val	Val	Lys	Pro	Leu	Pro	Pro	Ser	Ser	530	535	540	
Val	Lys	Ala	Glu	Ile	Thr	Ile	Asn	Ile	Gly	Leu	Leu	Lys	Ile	Ser	Trp	545	550	555	560
Glu	Lys	Pro	Val	Phe	Pro	Glu	Asn	Asn	Leu	Gln	Phe	Gln	Ile	Arg	Tyr	565	570	575	
Gly	Leu	Ser	Gly	Lys	Glu	Val	Gln	Trp	Lys	Met	Tyr	Glu	Val	Tyr	Asp	580	585	590	
Ala	Lys	Ser	Lys	Ser	Val	Ser	Leu	Pro	Val	Pro	Asp	Leu	Cys	Ala	Val	595	600	605	
Tyr	Ala	Val	Gln	Val	Arg	Cys	Lys	Arg	Leu	Asp	Gly	Leu	Gly	Tyr	Trp	610	615	620	625
Ser	Asn	Trp	Ser	Asn	Pro	Ala	Tyr	Thr	Val	Val	Met	Asp	Ile	Lys	Val	630	635	640	
Pro	Met	Arg	Gly	Pro	Glu	Phe	Trp	Arg	Ile	Ile	Asn	Gly	Asp	Thr	Met	645	650	655	
Lys	Lys	Glu	Lys	Asn	Val	Thr	Leu	Leu	Trp	Lys	Pro	Leu	Met	Lys	Asn	660	665	670	
Asp	Ser	Leu	Cys	Ser	Val	Gln	Arg	Tyr	Val	Ile	Asn	His	His	Thr	Ser	675	680	685	
Cys	Asn	Gly	Thr	Trp	Ser	Glu	Asp	Val	Gly	Asn	His	Thr	Lys	Phe	Thr	690	695	700	
Phe	Leu	Trp	Thr	Glu	Gln	Ala	His	Thr	Val	Thr	Val	Leu	Ala	Ile	Asn	705	710	715	720

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Ser	Ile	Gly	Ala	Ser	Val	Ala	Asn	Phe	Asn	Leu	Thr	Phe	Ser	Trp	Pro	725	730	735	
Met	Ser	Lys	Val	Asn	Ile	Val	Gln	Ser	Leu	Ser	Ala	Tyr	Pro	Leu	Asn	740	745	750	
Ser	Ser	Cys	Val	Ile	Val	Ser	Trp	Ile	Leu	Ser	Pro	Ser	Asp	Tyr	Lys	755	760	765	
Leu	Met	Tyr	Phe	Ile	Ile	Glu	Trp	Lys	Asn	Leu	Asn	Glu	Asp	Gly	Glu	770	775	780	
Ile	Lys	Trp	Leu	Arg	Ile	Ser	Ser	Ser	Val	Lys	Lys	Tyr	Tyr	Ile	His	785	790	795	800
Asp	His	Phe	Ile	Pro	Ile	Glu	Lys	Tyr	Gln	Phe	Ser	Leu	Tyr	Pro	Ile	805	810	815	
Phe	Met	Glu	Gly	Val	Gly	Lys	Pro	Lys	Ile	Ile	Asn	Ser	Phe	Thr	Gln	820	825	830	
Asp	Asp	Ile	Glu	Lys	His	Gln	Ser	Asp	Ala	Gly	Leu	Tyr	Val	Ile	Val	835	840	845	
Pro	Val	Ile	Ile	Ser	Ser	Ser	Ile	Leu	Leu	Leu	Gly	Thr	Leu	Leu	Ile	850	855	860	
Ser	His	Gln	Arg	Met	Lys	Lys	Leu	Phe	Trp	Glu	Asp	Val	Pro	Asn	Pro	865	870	875	880
Lys	Asn	Cys	Ser	Trp	Ala	Gln	Gly	Leu	Asn	Phe	Gln	Lys	Met	Leu	Glu	885	890	895	
Gly	Ser	Met	Phe	Val	Lys	Ser	His	His	His	Ser	Leu	Ile	Ser	Ser	Thr	900	905	910	
Gln	Gly	His	Lys	His	Cys	Gly	Arg	Pro	Gln	Gly	Pro	Leu	His	Arg	Lys	915	920	925	
Thr	Arg	Asp	Leu	Cys	Ser	Leu	Val	Tyr	Leu	Leu	Thr	Leu	Pro	Pro	Leu	930	935	940	
Leu	Ser	Tyr	Asp	Pro	Ala	Lys	Ser	Pro	Ser	Val	Arg	Asn	Thr	Gln	Glu	945	950	955	960

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

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Ser Ile Lys Lys Lys Lys

5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 241 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..241

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

A GGA CTT AAT TTT CAG AAG ATG CTT GAA GGC AGC ATG TTC GTT AAG	46
Gly Leu Asn Phe Gln Lys Met Leu Glu Gly Ser Met Phe Val Lys	
1 5 10 15	
AGT CAT CAC CAC TCC CTA ATC TCA AGT ACC CAG GGA CAC AAA CAC TGC	94
Ser His His His Ser Leu Ile Ser Ser Thr Gln Gly His Lys His Cys	
20 25 30	
GGA AGG CCA CAG GGT CCT CTG CAT AGG AAA ACC AGA GAC CTT TGT TCA	142
Gly Arg Pro Gln Gly Pro Leu His Arg Lys Thr Arg Asp Leu Cys Ser	
35 40 45	
CTT GTT TAT CTG CTG ACC CTC CCT CCA CTA TTG TCC TAT GAC CCT GCC	190
Leu Val Tyr Leu Leu Thr Leu Pro Pro Leu Leu Ser Tyr Asp Pro Ala	
50 55 60	
AAA TCC CCC TCT GTG AGA AAC ACC CAA GAA TGA TCA ATA AAA AAA AAA	238
Lys Ser Pro Ser Val Arg Asn Thr Gln Glu * Ser Ile Lys Lys Lys	
65 70 75	
AAA	241
Lys	
80	

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 73 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

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

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 6 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ser Ile Lys Lys Lys Lys
 5

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 130 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 2..130

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

A GGA CTT AAT TTT CAG AAG AAA ATG CCT GGC ACA AAG GAA CTA CTG 46
 Gly Leu Asn Phe Gln Lys Lys Met Pro Gly Thr Lys Glu Leu Leu
 1 5 10 15
 GGT GGA GGT TGG TTG ACT TAG GAA ATG CTT GTG AAG CTA CGT CCT ACC 94
 Gly Gly Gly Trp Leu Thr * Glu Met Leu Val Lys Leu Arg Pro Thr
 20 25 30

TCG TGC GCA CCT GCT CTC CCT GAG GTG TGC ACA ATG
 Ser Cys Ala Pro Ala Leu Pro Glu Val Cys Thr Met
 35 40

130

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Leu Asn Phe Gln Lys Lys Met Pro Gly Thr Lys Glu Leu Leu Gly
 1 5 10 15
 Gly Gly Trp Leu Thr
 20

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Glu Met Leu Val Lys Leu Arg Pro Thr Ser Cys Ala Pro Ala Leu Pro
 5 10 15
 Glu Val Cys Thr Met
 20

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 127 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 2..127

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

A	GGA	CTT	AAT	TTT	CAG	AAG	AGA	ACG	GAC	ATT	CTT	TGA	AGT	CTA	ATC	46
	Gly	Leu	Asn	Phe	Gln	Lys	Arg	Thr	Asp	Ile	Leu	*	Ser	Leu	Ile	
	1				5					10					15	
ATG	ATC	ACT	ACA	GAT	GAA	CCC	AAT	GTG	CCA	ACT	TCC	CAA	CAG	TCT	ATA	94
Met	Ile	Thr	Thr	Asp	Glu	Pro	Asn	Val	Pro	Thr	Ser	Gln	Gln	Ser	Ile	
					20				25					30		
GAG	TAT	TAG	AAG	ATT	TTT	ACA	TTC	TGA	AGA	AGG						127
Glu	Tyr	*	Lys	Ile	Phe	Thr	Phe	*	Arg	Arg						
			35						40							

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 11 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gly	Leu	Asn	Phe	Gln	Lys	Arg	Thr	Asp	Ile	Leu
				5					10	

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser	Leu	Ile	Met	Ile	Thr	Thr	Asp	Glu	Pro	Asn	Val	Pro	Thr	Ser	Gln
				5					10					15	
Gln	Ser	Ile	Glu	Tyr											
			20												

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Lys Ile Phe Thr Phe
5

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Glu Pro Tyr Leu Glu Phe Glu Ala Arg Arg Arg Leu Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Glu His Leu Val Gln Tyr Arg Thr Asp Trp Asp His Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Asp His Cys Phe Asn Tyr Glu Leu Lys Ile Tyr Asn Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Thr Thr His Ile Arg Tyr Glu Val Asp Val Ser Ala Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Pro Phe Pro Leu Gln Tyr Gln Val Lys Tyr Gln Val Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gln	Phe	Gln	Ile	Arg	Tyr	Gly	Leu	Ser	Gly	Lys	Glu	Val
1				5					10			

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ser	Thr	Ser	Tyr	Glu	Val	Gln	Val	Arg	Val	Lys	Ala	Gln	Arg	Asn
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Gln	Lys	Arg	Tyr	Thr	Phe	Arg	Val	Arg	Ser	Arg	Phe	Asn	Pro	Leu
1				5					10					15

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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Leu Ser Lys Tyr Asp Val Gln Val Arg Ala Ala Val Ser Ser Met
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Thr Arg Tyr Thr Phe Ala Val Arg Ala Arg Met Ala Pro Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gly Ser Ser Tyr Glu Val Gln Val Arg Gly Lys Arg Leu Asp Gly
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:31:

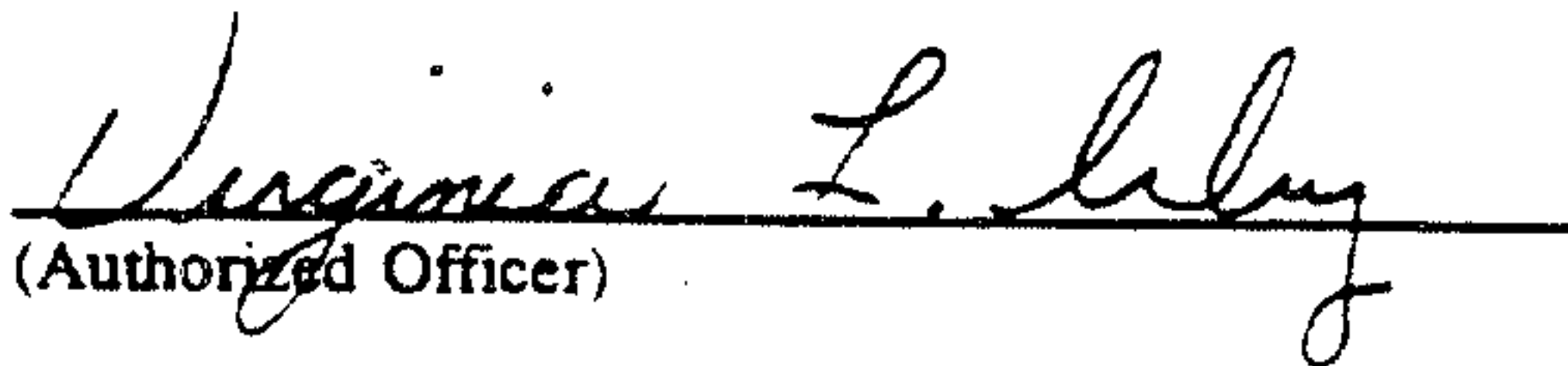
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Ala Val Tyr Ala Val Gln Val Arg Cys Lys Arg Leu Asp Gly
1 5 10 15

International Application No: PCT/

MICROORGANISMS	
Optional Sheet in connection with the microorganism referred to on page 31, lines 15-35 of the description *	
A. IDENTIFICATION OF DEPOSIT *	
Further deposits are identified on an additional sheet *	
Name of depositary institution *	
American Type Culture Collection	
Address of depositary institution (including postal code and country) *	
12301 Parklawn Drive Rockville, MD 20852 US	
Date of deposit * <u>September 14, 1994</u> Accession Number * <u>75885</u>	
B. ADDITIONAL INDICATIONS * (leave blank if not applicable). This information is continued on a separate attached sheet	
C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (if the indications are not all designated States)	
D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")	
E. ☒ This sheet was received with the International application when filed (to be checked by the receiving Office)	
 (Authorized Officer)	
☐ The date of receipt (from the applicant) by the International Bureau *	
was _____	
(Authorized Officer)	

Form PCT/RO/134 (January 1981)

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International Application No: PCT/ /

Form PCT/RO/134 (cont.)

American Type Culture Collection**12301 Parklawn Drive
Rockville, MD 20852
US**Accession No.Date of Deposit

75886	September 14, 1994
75887	September 14, 1994
75888	September 14, 1994
75889	September 14, 1994
75890	September 14, 1994
75970	December 14, 1994
75971	December 14, 1994
75972	December 14, 1994
75973	December 14, 1994
75974	December 14, 1994

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. An isolated polynucleotide, comprising:
 - (a) a nucleotide sequence as shown in SEQ ID NO: 6 from residue #1 to #2770, inclusive;
 - (b) a nucleotide sequence which is complementary to (a); or
 - (c) a nucleotide sequence that hybridizes under stringent conditions to (a) or (b), wherein the stringent conditions comprise either:
 - (i) washing at 50°C with 0.1 x SSC and 0.1% SDS; or
 - (ii) washing at 42°C in 0.2 x SSC and 0.1% SDS,which isolated polynucleotide that hybridizes under stringent conditions to (b), encodes an Hu-B1.219 receptor polypeptide.
2. The isolated polynucleotide of Claim 1 that encodes a soluble receptor polypeptide.
3. The isolated polynucleotide of Claim 1 that encodes a cell membrane-associated receptor polypeptide.
4. An isolated polynucleotide, comprising:
 - (a) a nucleotide sequence as shown in SEQ ID NO: 6;
 - (b) a nucleotide sequence which is complementary to (a); or
 - (c) a nucleotide sequence that hybridizes under stringent conditions to (a) or (b), wherein the stringent conditions comprise:
 - (i) washing at 50°C with 0.1 x SSC and 0.1% SDS; or
 - (ii) washing at 42°C in 0.2 x SSC and 0.1% SDS,

which isolated polynucleotide that hybridizes under stringent conditions to (b), encodes an Hu-B1.219 receptor polypeptide.

5. An isolated polynucleotide, comprising:
 - (a) a nucleotide sequence as shown in SEQ ID NO: 6 in which the nucleotide sequence corresponding to residue #2751 to the 3' end is replaced by the nucleotide sequence of SEQ ID NO: 13;
 - (b) a nucleotide sequence which is complementary to (a);
or
 - (c) a nucleotide sequence that hybridizes under stringent conditions to (a) or (b), wherein the stringent conditions comprise:
 - (i) washing at 50°C with 0.1 x SSC and 0.1% SDS; or
 - (ii) washing at 42°C in 0.2 x SSC and 0.1% SDS,which isolated polynucleotide that hybridizes under stringent conditions to (b), encodes an Hu-B1.219 receptor polypeptide.
6. An isolated polynucleotide, comprising:
 - (a) a nucleotide sequence as shown in SEQ ID NO: 6 in which the nucleotide sequence corresponding to residue #2751 to the 3' end is replaced by the nucleotide sequence of SEQ ID NO: 16;
 - (b) a nucleotide sequence which is complementary to (a);
or
 - (c) a nucleotide sequence that hybridizes under stringent conditions to (a) or (b), wherein the stringent conditions comprise:
 - (i) washing at 50°C with 0.1 x SSC and 0.1% SDS; or
 - (ii) washing at 42°C in 0.2 x SSC and 0.1% SDS,

which isolated polynucleotide that hybridizes under stringent conditions to (b), encodes an Hu-B1.219 receptor polypeptide.

7. An isolated polynucleotide, comprising a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence as shown in SEQ ID NO: 8 from residue #3 to #887, inclusive.
8. The isolated polynucleotide of Claim 7 in which the nucleotide sequence is shown in SEQ ID NO: 6.
9. The isolated polynucleotide of Claim 7 that encodes a naturally-occurring human variant receptor polypeptide having the amino acid sequence as shown in SEQ ID NO: 8 from residue #3 to #887, inclusive, which is immediately followed by the amino acid sequence in SEQ ID NO: 14.
10. The isolated polynucleotide of Claim 9 in which the nucleotide sequence is shown in SEQ ID NO: 6 except that the nucleotide sequence corresponding to residue #2751 to the 3' end is replaced by the nucleotide sequence of SEQ ID NO: 13.
11. The isolated polynucleotide of Claim 7 that encodes a naturally-occurring human variant receptor polypeptide having the amino acid sequence as shown in SEQ ID NO: 8 from residue #3 to #887, inclusive, which is immediately followed by the amino acid sequence in SEQ ID NO: 17.
12. The isolated polynucleotide of Claim 11 in which the nucleotide sequence is shown in SEQ ID NO: 6 except that the nucleotide sequence corresponding to residue #2751 to

the 3' end is replaced by the nucleotide sequence of SEQ ID NO: 16.

13. An isolated polynucleotide, comprising a nucleotide sequence that encodes an extracellular domain of a polypeptide having the amino acid sequence of SEQ ID NO: 25.
14. An isolated polynucleotide, comprising a nucleotide sequence that encodes an extracellular domain of a polypeptide having the amino acid sequence of SEQ ID NO: 30.
15. An isolated polynucleotide, comprising a nucleotide sequence that encodes an extracellular domain of a polypeptide having the amino acid sequence of SEQ ID NO: 31.
16. An isolated polynucleotide, comprising a nucleotide sequence that encodes an extracellular domain of a polypeptide having the amino acid sequence of SEQ ID NO: 8 from residue #266 to #278, inclusive.
17. The isolated polynucleotide of Claim 16, in which the extracellular domain comprises the amino acid sequence of SEQ ID NO: 8 from residue #3 to about #841.
18. The isolated polynucleotide of Claim 16, in which the extracellular domain comprises the amino acid sequence as shown in SEQ ID NO:8.
19. The polynucleotide of any of Claims 1 to 18 which is DNA.

20. The polynucleotide of Claim 19 which is cDNA.
21. The polynucleotide of Claim 19 which exists as a double helix.
22. The polynucleotide of any of Claims 1 to 18 which is RNA.
23. A recombinant vector containing the polynucleotide of any of Claims 1 to 18.
24. A recombinant expression vector containing the polynucleotide of any of Claims 1 to 6 in which the nucleotide sequence of the polynucleotide is operatively associated with a regulatory nucleotide sequence that controls expression of the polynucleotide in a host cell.
25. A recombinant expression vector containing the polynucleotide of any of Claims 7 to 18 in which the nucleotide sequence of the polynucleotide is operatively associated with a regulatory nucleotide sequence that controls expression of the polynucleotide in a host cell.
26. A genetically-engineered host cell containing the polynucleotide of Claim 1 or progeny thereof.
27. A genetically-engineered host cell containing the polynucleotide of any of Claims 1 to 6 or progeny thereof.
28. A genetically-engineered host cell containing the polynucleotide of any of Claims 7 to 18 or progeny thereof.

29. A genetically-engineered host cell containing the polynucleotide of any of Claims 1 to 6 in which the nucleotide sequence of the polynucleotide is operatively associated with a regulatory nucleotide sequence that controls expression of the polynucleotide in the host cell, or progeny thereof.
30. A genetically-engineered host cell containing the polynucleotide of any of Claims 7 to 18 in which the nucleotide sequence of the polynucleotide is operatively associated with a regulatory nucleotide sequence that controls expression of the polynucleotide in the host cell, or progeny thereof.
31. The genetically-engineered host cell of Claim 29 which is a prokaryote.
32. The genetically-engineered host cell of Claim 30 which is a prokaryote.
33. The genetically-engineered host cell of Claim 29 which is an eukaryote.
34. The genetically-engineered host cell of Claim 30 which is an eukaryote.
35. A method for producing a polypeptide, comprising:
 - (a) culturing the genetically-engineered host cell of Claim 31; and
 - (b) recovering the polypeptide from the cultured host cell or its culture medium.
36. A method for producing a polypeptide, comprising:

- (a) culturing the genetically-engineered host cell of Claim 32; and
 - (b) recovering the polypeptide from the cultured host cell or its culture medium.
37. A method for producing a polypeptide, comprising:
- (a) culturing the genetically engineered host cell of Claim 33; and
 - (b) recovering the polypeptide from the cultured host cell or its culture medium.
38. A method for producing a polypeptide, comprising:
- (a) culturing the genetically engineered host cell of Claim 34; and
 - (b) recovering the polypeptide from the cultured host cell or its culture medium.
39. The isolated polynucleotide of Claim 7 that encodes a polypeptide comprising the amino acid sequence shown in SEQ ID NO: 8 from residue #3 to #960, inclusive.
40. An isolated polypeptide comprising the amino acid sequence as shown in SEQ ID NO:8 from residue #3 to #893, inclusive.
41. An isolated polypeptide comprising the amino acid sequence as shown in SEQ ID NO:8 from residue #3 to #960, inclusive.
42. An isolated polypeptide comprising the amino acid sequence as shown in SEQ ID NO:8 from residue #3 to #887, inclusive, which is immediately followed by the amino

acid sequence as shown in SEQ ID NO:14.

43. An isolated polypeptide comprising the amino acid sequence as shown in SEQ ID NO:8 from residue #3 to #887, inclusive, which is immediately followed by the amino acid sequence as shown in SEQ ID NO:17.
44. The polypeptide of Claim 40, 41, 42 or 43 which is a cell membrane-associated receptor.
45. The polypeptide of Claim 40, 41, 42 or 43 which is a naturally-occurring receptor.
46. The polypeptide of Claim 40, 41, 42 or 43 which is a non-naturally-occurring receptor.
47. The polypeptide of Claim 46 which is produced by a recombinant DNA method.
48. The polypeptide of Claim 46 which is produced by a chemical synthetic method.
49. The polypeptide of Claim 40, 41, 42 or 43 which is fused with a second polypeptide.
50. An isolated polypeptide comprising an extracellular domain of a hematopoietin receptor comprising the amino acid sequence as shown in SEQ ID NO:25.
51. An isolated polypeptide comprising an extracellular domain of a hematopoietin receptor comprising the amino acid sequence as shown in SEQ ID NO:30.

52. An isolated polypeptide comprising an extracellular domain of a hematopoietin receptor comprising the amino acid sequence as shown in SEQ ID NO:31.
53. An isolated polypeptide comprising an extracellular domain of a hematopoietin receptor comprising the amino acid sequence from residue #266 to #278, inclusive, of SEQ ID NO:8.
54. The polypeptide of Claim 53 which comprises the amino acid sequence from residue #3 to about #841 of SEQ ID NO:8.
55. The polypeptide of Claim 53 which comprises the amino acid sequence as shown in SEQ ID NO:8.
56. The polypeptide of Claim 50, 51, 52, 53, 54 or 55 which is a cell membrane-associated receptor.
57. The polypeptide of Claim 50, 51, 52, 53, 54 or 55 which is a soluble receptor.
58. The polypeptide of Claim 50, 51, 52, 53, 54 or 55 which is fused with a second polypeptide.
59. An isolated Hu-B1.219 receptor polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a second polynucleotide comprising the complementary sequence that encodes the amino acid sequence as shown in SEQ ID NO:8 from residue #3 to #887, inclusive, wherein the stringent conditions comprise either: (i) washing at 50°C with 0.1 x SSC and 0.1% SDS; or (ii) washing at 42°C

in 0.2 x SSC and 0.1% SDS.

60. An isolated Hu-B1.219 receptor polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a second polynucleotide comprising the complementary sequence that encodes the amino acid sequence as shown in SEQ ID NO:8 from residue #3 to #960, inclusive, wherein the stringent conditions comprise either: (i) washing at 50°C with 0.1 x SSC and 0.1% SDS; or (ii) washing at 42°C in 0.2 x SSC and 0.1% SDS.
61. An isolated Hu-B1.219 receptor polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a second polynucleotide comprising the complementary sequence that encodes the amino acid sequence as shown in SEQ ID NO:8 from residue #3 to #887, inclusive, which is immediately followed by the amino acid sequence as shown in SEQ ID NO:17, wherein the stringent conditions comprise either: (i) washing at 50°C with 0.1 x SSC and 0.1% SDS; or (ii) washing at 42°C in 0.2 x SSC and 0.1% SDS.
62. An isolated Hu-B1.219 receptor polypeptide encoded by a polynucleotide that hybridizes under stringent conditions to a second polynucleotide comprising the complementary sequence that encodes the amino acid sequence as shown in SEQ ID NO:8 from residue #3 to #887, inclusive, which is immediately followed by the amino acid sequence as shown in SEQ ID NO:17, wherein the stringent conditions comprise either: (i) washing at 50°C with 0.1 x SSC and 0.1% SDS; or (ii) washing at 42°C in 0.2 x SSC and 0.1% SDS.

63. The Hu-B1.219 receptor polypeptide of Claim 59 which is encoded by the nucleotide sequence as shown in SEQ ID NO:6 from residue #1 to #2770, inclusive.
64. The Hu-B1.219 receptor polypeptide of Claim 60 which is encoded by the nucleotide sequence as shown in SEQ ID NO:6.
65. The Hu-B1.219 receptor polypeptide of Claim 61 which is encoded by the nucleotide sequence as shown in SEQ ID NO:6 in which the nucleotide sequence corresponding to residue #2751 to the 3' end is replaced by the nucleotide sequence of SEQ ID NO:13.
66. The Hu-B1.219 polypeptide of Claim 62 which is encoded by the nucleotide sequence as shown in SEQ ID NO:6 in which the nucleotide sequence corresponding to residue #2751 to the 3' end is replaced by the nucleotide sequence of SEQ ID NO:16.
67. The polypeptide of any of Claims 59 - 66 which is a cell membrane-associated receptor.
68. The polypeptide of any of Claims 59 - 66 which is a soluble receptor.
69. A polyclonal antibody which specifically binds to the polypeptide of Claim 40.
70. The polyclonal antibody of Claim 69 which recognizes a structurally unique site in the polypeptide.

71. A polyclonal antibody which specifically binds to the polypeptide of Claim 41.
72. The polyclonal antibody of Claim 71 which recognizes a structurally unique site in the polypeptide.
73. A polyclonal antibody which specifically binds to the polypeptide of Claim 42.
74. The polyclonal antibody of Claim 73 which recognizes a structurally unique site in the polypeptide.
75. A polyclonal antibody which specifically binds to the polypeptide of Claim 43.
76. The polyclonal antibody of Claim 75 which recognizes a structurally unique site in the polypeptide.
77. The polyclonal antibody of Claim 69, 71, 73 or 75 which competitively inhibits the binding of the polypeptide to a ligand.
78. An oligonucleotide that comprises a nucleotide sequence that is complementary to at least a region of the isolated polynucleotide according to any one of claims 1 to 6, wherein said oligonucleotide is for use as a hybridization probe or primer.
79. An oligonucleotide that comprises a nucleotide sequence that is complementary or identical to at least a region of a nucleic acid encoding the polypeptide according to any one of claims 59 to 62, wherein said oligonucleotide is for use as a hybridization probe or primer.

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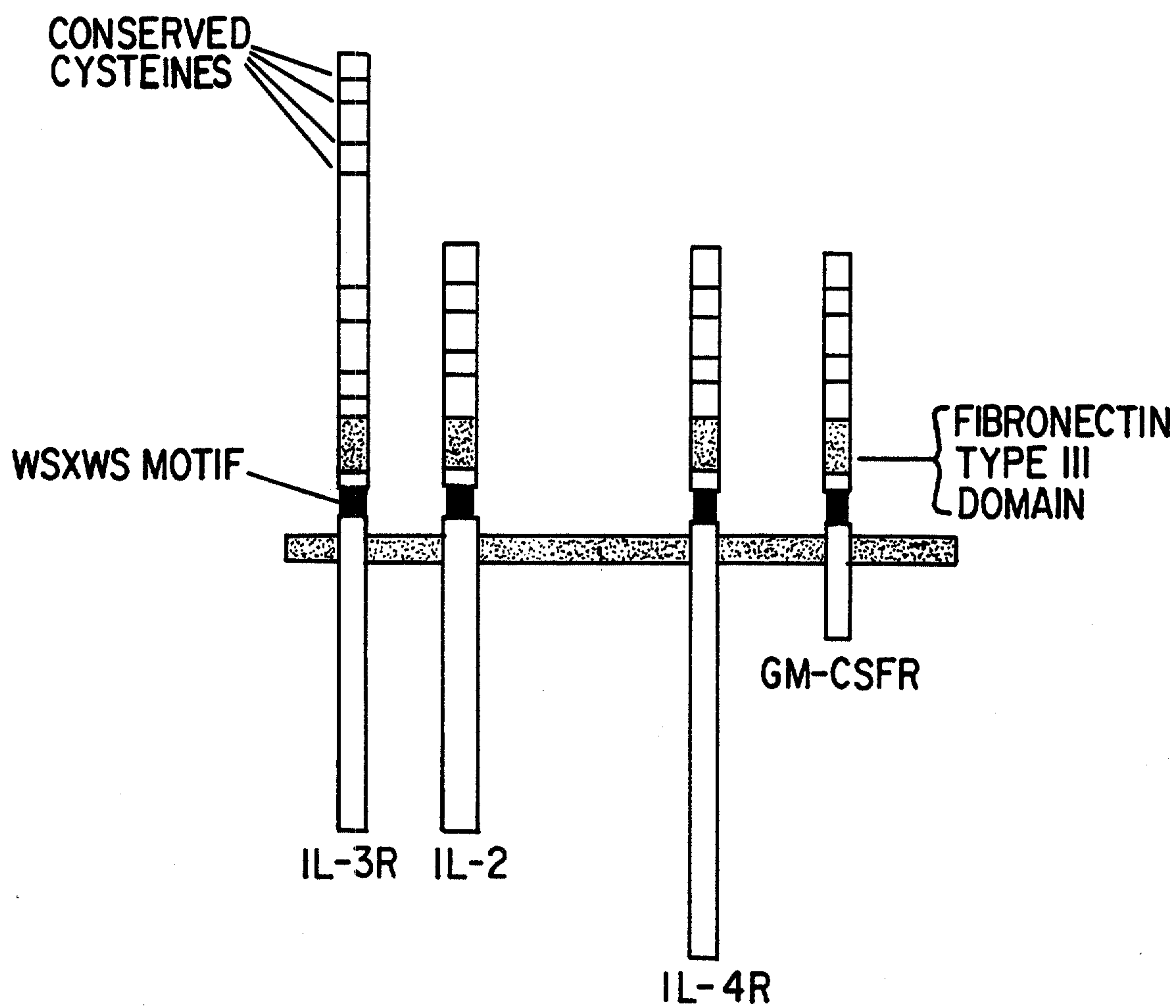


FIG. 1

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9			18			27			36			45			54		
GCG	CGC	GCG	ACG	CAG	GTG	CCC	GAG	CCC	CGG	CCC	GCG	CCC	ATC	TCT	GCC	TTC	GGT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
A	R	A	T	Q	V	P	E	P	R	P	A	P	I	S	A	F	G
63			72			81			90			99			108		
CGA	GTT	GGA	CCC	CCG	GAT	CAA	GGT	GTA	CTT	CTC	TGA	AGT	AAG	ATG	ATT	TGT	CAA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
R	V	G	P	P	D	Q	G	V	L	L	*	S	K	M	I	C	Q
117			126			135			144			153			162		
AAA	TTC	TGT	GTG	GTT	TTG	TTA	CAT	TGG	GAA	TTT	ATT	TAT	GTG	ATA	ACT	GCG	TTT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
K	F	C	V	V	L	L	H	W	E	F	I	Y	V	I	T	A	F
171			180			189			198			207			216		
AAC	TTG	TCA	TAT	CCA	ATT	ACT	CCT	TGG	AGA	TTT	AAG	TTG	TCT	TGC	ATG	CCA	CCA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
N	L	S	Y	P	I	T	P	W	R	F	K	L	S	C	M	P	P
225			234			243			252			261			270		
AAT	TCA	ACC	TAT	GAC	TAC	TTC	CTT	TTG	CCT	GCT	GGA	CTC	TCA	AAG	AAT	ACT	TCA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
N	S	T	Y	D	Y	F	L	L	P	A	G	L	S	K	N	T	S
279			288			297			306			315			324		
AAT	TCG	AAT	GGA	CAT	TAT	GAG	ACA	GCT	GTT	GAA	CCT	AAG	TTT	AAT	TCA	AGT	GGT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
N	S	N	G	H	Y	E	T	A	V	E	P	K	F	N	S	S	G
333			342			351			360			369			378		
ACT	CAC	TTT	TCT	AAC	TTA	TCC	AAA	GCA	ACT	TTC	CAC	TGT	TGC	TTT	CGG	AGT	GAG
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
T	H	F	S	N	L	S	K	A	T	F	H	C	C	F	R	S	E
387			396			405			414			423			432		
CAA	GAT	AGA	AAC	TGC	TCC	TTA	TGT	GCA	GAC	AAC	ATT	GAA	GGA	AGG	ACA	TTT	GTT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Q	D	R	N	C	S	L	C	A	D	N	I	E	G	R	T	F	V

FIG.2A

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441					450			459			468			477			486	
TCA	ACA	GTA	AAT	TCT	TTA	GTT	TTT	CAA	CAA	ATA	GAT	GCA	AAC	TGG	AAC	ATA	CAG	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
S	T	V	N	S	L	V	F	Q	Q	I	D	A	N	W	N	I	Q	
495					504			513			522			531			540	
TGC	TGG	CTA	AAA	GGA	GAC	TTA	AAA	TTA	TTC	ATC	TGT	TAT	GTG	GAG	TCA	TTA	TTT	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
C	W	L	K	G	D	L	K	L	F	I	C	Y	V	E	S	L	F	
549					558			567			576			585			594	
AAG	AAT	CTA	TTC	AGG	AAT	TAT	AAC	TAT	AAG	GTC	CAT	CTT	TTA	TAT	GTT	CTG	CCT	
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K	N	L	F	R	N	Y	N	Y	K	V	H	L	L	Y	V	L	P	
603					612			621			630			639			648	
GAA	GTG	TTA	GAA	GAT	TCA	CCT	CTG	GTT	CCC	CAA	AAA	GGC	AGT	TTT	CAG	ATG	GTT	
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E	V	L	E	D	S	P	L	V	P	Q	K	G	S	F	Q	M	V	
657					666			675			684			693			702	
CAC	TGC	AAT	TGC	AGT	GTT	CAT	GAA	TGT	TGT	GAA	TGT	CTT	GTG	CCT	GTG	CCA	ACA	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
H	C	N	C	S	V	H	E	C	C	E	C	L	V	P	V	P	T	
711					720			729			738			747			756	
GCC	AAA	CTC	AAC	GAC	ACT	CTC	CTT	ATG	TGT	TTG	AAA	ATC	ACA	TCT	GGT	GGA	GTA	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
A	K	L	N	D	T	L	L	M	C	L	K	I	T	S	G	G	V	
765					774			783			792			801			810	
ATT	TTC	CGG	TCA	CCT	CTA	ATG	TCA	GTT	CAG	CCC	ATA	AAT	ATG	GTG	AAG	CCT	GAT	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
I	F	R	S	P	L	M	S	V	Q	P	I	N	M	V	K	P	D	
819					828			837			846			855			864	
CCA	CCA	TTA	GGT	TTG	CAT	ATG	GAA	ATC	ACA	GAT	GAT	GGT	AAT	TTA	AAG	ATT	TCT	
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	
P	P	L	G	L	H	M	E	I	T	D	D	G	N	L	K	I	S	

FIG.2B

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873	882	891	900	909	918
TGG TCC AGC CCA CCA TTG GTA CCA TTT CCA CTT CAA TAT CAA GTG AAA TAT TCA					
W S S P P L V P F P L Q Y Q V K Y S					
927	936	945	954	963	972
GAG AAT TCT ACA ACA GTT ATC AGA GAA GCT GAC AAG ATT GTC TCA GCT ACA TCC					
E N S T T V I R E A D K I V S A T S					
981	990	999	1008	1017	1026
CTG CTA GTA GAC AGT ATA CTT CCT GGG TCT TCG TAT GAG GTT CAG GTG AGG GGC					
L L V D S I L P G S S Y E V Q V R G					
1035	1044	1053	1062	1071	1080
AAG AGA CTG GAT GGC CCA GGA ATC TGG AGT GAC TGG AGT ACT CCT CGT GTC TTT					
K R L D G P G I W S D W S T P R V F					
1089	1098	1107	1116	1125	1134
ACC ACA CAA GAT GTC ATA TAC TTT CCA CCT AAA ATT CTG ACA AGT GTT GGG TCT					
T T Q D V I Y F P P K I L T S V G S					
1143	1152	1161	1170	1179	1188
AAT GTT TCT TTT CAC TGC ATC TAT AAG AAG GAA AAC AAG ATT GTT CCC TCA AAA					
N V S F H C I Y K K E N K I V P S K					
1197	1206	1215	1224	1233	1242
GAG ATT GTT TGG TGG ATG AAT TTA GCT GAG AAA ATT CCT CAA AGC CAG TAT GAT					
E I V W W M N L A E K I P Q S Q Y D					
1251	1260	1269	1278	1287	1296
GTT GTG AGT GAT CAT GTT AGC AAA GTT ACT TTT TTC AAT CTG AAT GAA ACC AAA					
V V S D H V S K V T F F N L N E T K					

FIG.2C

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1305	1314	1323	1332	1341	1350
CCT CGA GGA AAG TTT ACC TAT GAT GCA GTG TAC TGC TGC AAT GAA CAT GAA TGC					
-----	-----	-----	-----	-----	-----
P R G K F T Y D A V Y C C N E H E C					
1359	1368	1377	1386	1395	1404
CAT CAT CGC TAT GCT GAA TTA TAT GTG ATT GAT GTC AAT ATC AAT ATC TCA TGT					
-----	-----	-----	-----	-----	-----
H H R Y A E L Y V I D V N I N I S C					
1413	1422	1431	1440	1449	1458
GAA ACT GAT GGG TAC TTA ACT AAA ATG ACT TGC AGA TGG TCA ACC AGT ACA ATC					
-----	-----	-----	-----	-----	-----
E T D G Y L T K M T C R W S T S T I					
1467	1476	1485	1494	1503	1512
CAG TCA CTT GCG GAA AGC ACT TTG CAA TTG AGG TAT CAT AGG AGC AGC CTT TAC					
-----	-----	-----	-----	-----	-----
Q S L A E S T L Q L R Y H R S S L Y					
1521	1530	1539	1548	1557	1566
TGT TCT GAT ATT CCA TCT ATT CAT CCC ATA TCT GAG CCC AAA GAT TGC TAT TTG					
-----	-----	-----	-----	-----	-----
C S D I P S I H P I S E P K D C Y L					
1575	1584	1593	1602	1611	1620
CAG AGT GAT GGT TTT TAT GAA TGC ATT TTC CAG CCA ATC TTC CTA TTA TCT GGC					
-----	-----	-----	-----	-----	-----
Q S D G F Y E C I F Q P I F L L S G					
1629	1638	1647	1656	1665	1674
TAC ACA ATG TGG ATT AGG ATC AAT CAC TCT CTA GGT TCA CTT GAC TCT CCA CCA					
-----	-----	-----	-----	-----	-----
Y T M W I R I N H S L G S L D S P P					

FIG.2D

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1683	1692	1701	1710	1719	1728
ACA TGT GTC CTT CCT GAT TCT GTG GTG AAG CCA CTG CCT CCA TCC AGT GTG AAA					
T C V L P D S V V K P L P P S S V K					
1737	1746	1755	1764	1773	1782
GCA GAA ATT ACT ATA AAC ATT GGA TTA TTG AAA ATA TCT TGG GAA AAG CCA GTC					
A E I T I N I G L L K I S W E K P V					
1791	1800	1809	1818	1827	1836
TTT CCA GAG AAT AAC CTT CAA TTC CAG ATT CGC TAT GGT TTA AGT GGA AAA GAA					
F P E N N L Q F Q I R Y G L S G K E					
1845	1854	1863	1872	1881	1890
GTA CAA TGG AAG ATG TAT GAG GTT TAT GAT GCA AAA TCA AAA TCT GTC AGT CTC					
V Q W K M Y E V Y D A K S K S V S L					
1899	1908	1917	1926	1935	1944
CCA GTT CCA GAC TTG TGT GCA GTC TAT GCT GTT CAG GTG CGC TGT AAG AGG CTA					
P V P D L C A V Y A V Q V R C K R L					
1953	1962	1971	1980	1989	1998
GAT GGA CTG GGA TAT TGG AGT AAT TGG AGC AAT CCA GCC TAC ACA GTT GTC ATG					
D G L G Y W S N W S N P A Y T V V M					
2007	2016	2025	2034	2043	2052
GAT ATA AAA GTT CCT ATG AGA GGA CCT GAA TTT TGG AGA ATA ATT AAT GGA GAT					
D I K V P M R G P E F W R I I N G D					
2061	2070	2079	2088	2097	2106
ACT ATG AAA AAG GAG AAA AAT GTC ACT TTA CTT TGG AAG CCC CTG ATG AAA AAT					
T M K K E K N V T L L W K P L M K N					

FIG.2E

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2115	2124	2133	2142	2151	2160
GAC TCA TTG TGC AGT GTT CAG AGA TAT GTG ATA AAC CAT CAT ACT TCC TGC AAT					
---	---	---	---	---	---
D S L C S V Q R Y V I N H H T S C N					
2169	2178	2187	2196	2205	2214
GGA ACA TGG TCA GAA GAT GTG GGA AAT CAC ACG AAA TTC ACT TTC CTG TGG ACA					
---	---	---	---	---	---
G T W S E D V G N H T K F T F L W T					
2223	2232	2241	2250	2259	2268
GAG CAA GCA CAT ACT GTT ACG GTT CTG GCC ATC AAT TCA ATT GGT GCT TCT GTT					
---	---	---	---	---	---
E Q A H T V T V L A I N S I G A S V					
2277	2286	2295	2304	2313	2322
GCA AAT TTT AAT TTA ACC TTT TCA TGG CCT ATG AGC AAA GTA AAT ATC GTG CAG					
---	---	---	---	---	---
A N F N L T F S W P M S K V N I V Q					
2331	2340	2349	2358	2367	2376
TCA CTC AGT GCT TAT CCT TTA AAC AGC AGT TGT GTG ATT GTT TCC TGG ATA CTA					
---	---	---	---	---	---
S L S A Y P L N S S C V I V S W I L					
2385	2394	2403	2412	2421	2430
TCA CCC AGT GAT TAC AAG CTA ATG TAT TTT ATT ATT GAG TGG AAA AAT CTT AAT					
---	---	---	---	---	---
S P S D Y K L M Y F I I E W K N L N					
2439	2448	2457	2466	2475	2484
GAA GAT GGT GAA ATA AAA TGG CTT AGA ATC TCT TCA TCT GTT AAG AAG TAT TAT					
---	---	---	---	---	---
E D G E I K W L R I S S S V K K Y Y					
2493	2502	2511	2520	2529	2538
ATC CAT GAT CAT TTT ATC CCC ATT GAG AAG TAC CAG TTC AGT CTT TAC CCA ATA					
---	---	---	---	---	---
I H D H F I P I E K Y Q F S L Y P I					

FIG.2F

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TTT	ATG	²⁵⁴⁷ GAA	GGA	GTG	²⁵⁵⁶ GGA	AAA	CCA	²⁵⁶⁵ AAG	ATA	ATT	²⁵⁷⁴ AAT	AGT	TTC	²⁵⁸³ ACT	CAA	²⁵⁹² GAT	GAT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
F	M	E	G	V	G	K	P	K	I	I	N	S	F	T	Q	D	D
ATT	GAA	²⁶⁰¹ AAA	CAC	²⁶¹⁰ CAG	AGT	GAT	GCA	²⁶¹⁹ GGT	TTA	TAT	²⁶²⁸ GTA	ATT	GTG	²⁶³⁷ CCA	GTA	²⁶⁴⁶ ATT	ATT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
I	E	K	H	Q	S	D	A	G	L	Y	V	I	V	P	V	I	I
TCC	TCT	²⁶⁵⁵ TCC	ATC	²⁶⁶⁴ TTA	TTG	CTT	GGA	²⁶⁷³ ACA	TTA	²⁶⁸² TTA	ATA	TCA	CAC	²⁶⁹¹ CAA	AGA	²⁷⁰⁰ ATG	AAA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
S	S	S	I	L	L	L	G	T	L	L	I	S	H	Q	R	M	K
AAG	CTA	²⁷⁰⁹ TTT	TGG	²⁷¹⁸ GAA	GAT	GTT	CCG	²⁷²⁷ AAC	CCC	²⁷³⁶ AAG	AAT	TGT	TCC	²⁷⁴⁵ TGG	GCA	²⁷⁵⁴ CAA	GGA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
K	L	F	W	E	D	V	P	N	P	K	N	C	S	W	A	Q	G
CTT	AAT	²⁷⁶³ TTT	CAG	²⁷⁷² AAG	ATG	CTT	GAA	²⁷⁸¹ GGC	AGC	²⁷⁹⁰ ATG	TTC	GTT	²⁷⁹⁹ AAG	AGT	CAT	²⁸⁰⁸ CAC	CAC
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L	N	F	Q	K	M	L	E	G	S	M	F	V	K	S	H	H	H
TCC	CTA	²⁸¹⁷ ATC	TCA	²⁸²⁶ AGT	ACC	CAG	GGA	²⁸³⁵ CAC	AAA	²⁸⁴⁴ CAC	TGC	GGA	²⁸⁵³ AGG	CCA	CAG	²⁸⁶² GGT	CCT
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S	L	I	S	S	T	Q	G	H	K	H	C	G	R	P	Q	G	P
CTG	CAT	AGG	AAA	²⁸⁸⁰ ACC	AGA	²⁸⁸⁹ GAC	CTT	TGT	²⁸⁹⁸ TCA	²⁹⁰⁷ CTT	GTT	TAT	²⁹¹⁶ CTG	CTG	ACC	CTC	CCT
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
L	H	R	K	T	R	D	L	C	S	L	V	Y	L	L	T	L	P
CCA	CTA	²⁹²⁵ TTG	TCC	²⁹³⁴ TAT	GAC	CCT	GCC	²⁹⁴³ AAA	TCC	²⁹⁵² CCC	TCT	GTG	²⁹⁶¹ AGA	AAC	ACC	²⁹⁷⁰ CAA	GAA
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
P	L	L	S	Y	D	P	A	K	S	P	S	V	R	N	T	Q	E
TGA	TCA	²⁹⁷⁹ ATA	AAA	²⁹⁸⁸ AAA	AAA	AAA	3'										
---	---	---	---	---	---	---	---										
*	S	I	K	K	K	K											

FIG.2G

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			2760	2770	2780	2790	2800	
HuB1.219	Form	1	2751	AGGACTTAAT	TTTCAGAAGA	TGCTTGAAGG	CAGCATGTTC	GTTAAGAGTC 2800
HuB1.219		2	2751	AGGACTTAAT	TTTCAGAAGA	AAATGCCTGG	CACAAAGGAA	CTACTGGGTG 2800
HuB1.219		3	2751	AGGACTTAAT	TTTCAGAAGA	GAACGGACAT	TCTTTGAAGT	CTAATCATGA 2800
			2810	2820	2830	2840	2850	
HuB1.219	Form	1	2801	ATCACCCTC	CCTAATCTCA	AGTACCCAGG	GACACAAACA	CTGCGGAAGG 2850
HuB1.219		2	2801	GAGGTTGGTT	GACTTAGGAA	ATGCTTGTGA	AGCTACGTCC	TACCTCGTGC 2850
HuB1.219		3	2801	TCACTACAGA	TGAACCCAAT	GTGCCAACTT	CCCAACAGTC	TATAGAGTAT 2850
			2860	2870	2880	2890	2900	
HuB1.219	Form	1	2851	CCACAGGGTC	CTCTGCATAG	GAAAACCAGA	GACCTTTGTT	CACTTGTTTA 2900
HuB1.219		2	2851	GCACCTGCTC	TCCCTGAGGT	GTGCACAATG		 2900
HuB1.219		3	2851	TAGAAGATTT	TTACATTCTG	AAGAAGG...		 2900
			2910	2920	2930	2940	2950	
HuB1.219	Form	1	2901	TCTGCTGACC	CTCCCTCCAC	TATTGTCCTA	TGACCCTGCC	AAATCCCCCT 2950
HuB1.219		2	2901					 2950
HuB1.219		3	2901					 2950
			2960	2970	2980	2990	3000	
HuB1.219	Form	1	2951	CTGTGAGAAA	CACCCAAGAA	TGATCAATAA	AAAAAAAAAA	A..... 3000
HuB1.219		2	2951					 3000
HuB1.219		3	2951					 3000

FIG.3A

			10	20	30	40	50	
HuB1.219	Form	1	1	GLNFQKMLEG	SMFVKSHHHS	LISSTQGHKH	CGRPQGPLHR	KTRDLC SLVY 50
HuB1.219		2	1	GLNFQKKMPG	TKELLGGGWL	T*EMLVKLRP	TSCAPALPEV	CTM..... 50
HuB1.219		3	1	GLNFQKRTDI	L*SLIMITTD	EPNVPTSQQS	IEY*KIFTF*	RR..... 50
			60	70	80	90	100	
HuB1.219	Form	1	51	LLTLPPLLSY	DPAKSPSVRN	TQE*SIKKKK		 100
HuB1.219		2	51					 100
HuB1.219		3	51					 100

FIG.3B

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SPACING OF CONSERVED AMINO ACIDS IN THE EXTRACELLULAR
DOMAINS OF KNOWN CYTOKINE RECEPTOR GENES

...C...C...C...C...A/PP.L/V...W...Y...L...Y..R...G.WSXWS..
(10-11) (28-35) (9-16) (36-48) (2-3) (13-18) (12-20) (26-33) (5-6)(5)(6-11)(2)

CONSERVED AMINO ACIDS IN THE 5' EXTRACELLULAR DOMAINS OF CLONE Hu-B1.219

...C...C...C...C...C...PP.L...W...Y...L...Y..R...G.WSDWS..
(11) (44) (10) (43) (3) (14) (12) (30) (5) (5)(8)(2)

CONSERVED AMINO ACIDS IN THE 3' EXTRACELLULAR DOMAINS OF CLONE Hu-B1.219

...C...C...C...C...C...PP.V...W...Y...P...Y..R...G.WSNWS..
(11) (41) (10) (41) (3) (15) (16) (27) (6) (5)(8)(2)

FIG.4

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* - * - * -

mIL2R β	E P Y L E F E A R R R L L
hIL2R γ	E H L V Q Y R T D W D H S
mIL5R α	D H C F N Y E L K I Y N T
mEPOR	T T H I R Y E V D V S A G
Hu-B1.219(5')	P F P L Q Y Q V K Y Q V K
Hu-B1.219(3')	Q F Q I R Y G L S G K E V

HYDROPHOBIC: "*"

HYDROPHILIC: "-"

FIG.5

* b * b * b

mIL-2R β	S T S Y E V Q V R V K A Q R N
hIL-2R γ	Q K R Y T F R V R S R F N P L
mIL-5R α	L S K Y D V Q V R A A V S S M
mEPOR	G T R Y T F A V R A R M A P S
Hu-B1.219(5')	G S S Y E V Q V R G K R L D G
Hu-B1.219(3')	C A V Y A V Q V R C K R L D G
	Y R

HYDROPHOBIC: "*"

BASIC: "b"

FIG.6