PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [5] :
C12N 15/12, 15/62, 15/85
C07K 13/00, 15/28, A61K 37/02
A61K 48/00, G01N 33/68

A2

(11) International Publication Number: **WO 93/25682**

(43) International Publication Date: 23 December 1993 (23.12.93)

(21) International Application Number: PCT/US93/05569

(22) International Filing Date: 11 June 1993 (11.06.93)

(30) Priority data:
899,075 11 June 1992 (11.06.92) US

(60) Parent Application or Grant
(63) Related by Continuation
US 899,075 (CIP)
Filed on 11 June 1992 (11.06.92)

(71) Applicant *(for all designated States except US):* NEW YORK UNIVERSITY [US/US]; 550 First Avenue, Rm. MSB-153, New York, NY 10016 (US).

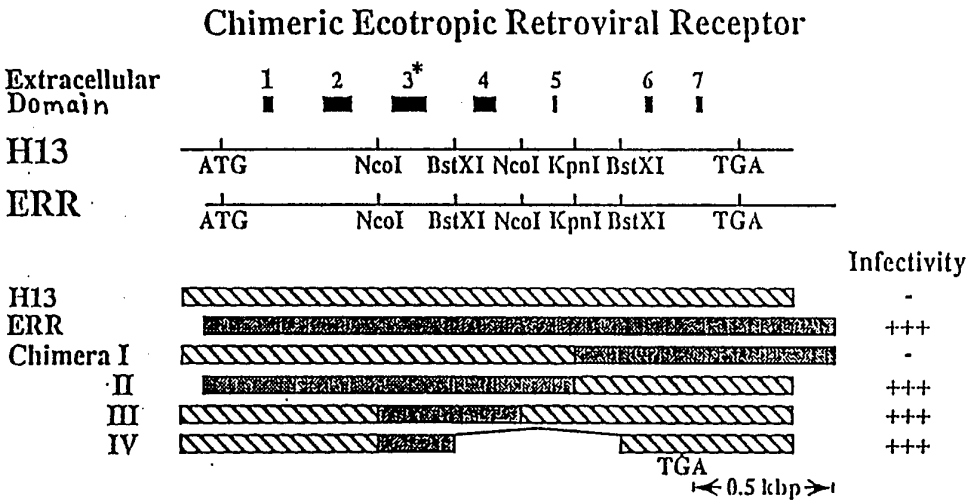
(72) Inventors; and
(75) Inventors/Applicants *(for US only) :* MERUELO, Daniel [US/US]; 9 Country Club Lane, Scarborough, NY 10510 (US). YOSHIMOTO, Takayuki [JP/JP]; 4-6-1 Shiroganedai, Minatoku, Tokyo 108 (JP).

(74) Agent: BROWDY, Roger, L.; Browdy and Neimark, 419 Seventh Street, N.W., Ste. 300, Washington, DC 20004 (US).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: CHIMERIC RECEPTOR POLYPEPTIDES, HUMAN H13 PROTEINS AND USES THEREOF



(57) Abstract

Chimeric receptor polypeptides are disclosed which contain chimeric viral receptors of a first species which bind non-first species specific viruses and/or their envelope protein *env* binding domains, nucleic acid encoding therefore, cells or tissues expressing the chimeric receptor polypeptide, as well as methods of preparing and using thereof. Also disclosed are methods for rendering eukaryotic cells or tissues selectively susceptible to binding and/or infection by recombinant viral vectors or delivery vectors that bind a chimeric receptor of a chimeric receptor polypeptide expressed in target cells or tissues. Also disclosed are gene therapy methods which may be used on selected cells or tissues of a mammal, bird, insect or yeast through the use of transient or constitutive expression or vector association of a chimeric receptor polypeptide having a chimeric receptor binding site. The resulting chimeric receptor cells or tissues are thus rendered susceptible to infection or binding by viral vectors or *env* binding domains thereof which specifically bind the chimeric receptor. The chimeric receptor cells are then susceptible to infection and/or binding by therapeutic and/or diagnostic agents which comprise an *env* binding domain of a non-first species specific virus capable of binding a chimeric receptor polypeptide of the present invention.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FR**	France	**MR**	Mauritania
AU	Australia	**GA**	Gabon	**MW**	Malawi
BB	Barbados	**GB**	United Kingdom	**NL**	Netherlands
BE	Belgium	**GN**	Guinea	**NO**	Norway
BF	Burkina Faso	**GR**	Greece	**NZ**	New Zealand
BG	Bulgaria	**HU**	Hungary	**PL**	Poland
BJ	Benin	**IE**	Ireland	**PT**	Portugal
BR	Brazil	**IT**	Italy	**RO**	Romania
CA	Canada	**JP**	Japan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SK**	Slovak Republic
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SN**	Senegal
CM	Cameroon	**LI**	Liechtenstein	**SU**	Soviet Union
CS	Czechoslovakia	**LK**	Sri Lanka	**TD**	Chad
CZ	Czech Republic	**LU**	Luxembourg	**TG**	Togo
DE	Germany	**MC**	Monaco	**UA**	Ukraine
DK	Denmark	**MG**	Madagascar	**US**	United States of America
ES	Spain	**ML**	Mali	**VN**	Viet Nam
FI	Finland	**MN**	Mongolia		

CHIMERIC RECEPTOR POLYPEPTIDES, HUMAN H13 PROTEINS AND USES THEREOF

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention, in the field of virology and molecular genetics, relates to nucleic acid, methods and proteins involving mammalian mutant viral receptor polypeptides, which bind viruses and/or envelope protein binding domains thereof, as well as to methods of making and using thereof, including diagnostic and therapeutic applications, such as gene therapy.

Description of the Background Art

Viruses infect a cell by first attaching to a cell at a viral receptor. A number of virus-specific cellular receptors have been identified, and most of these receptor molecules have other known cellular functions.

The expression of these virus binding proteins or receptors is a strong determinant of susceptibility to virus infection. Binding is required for fusion of the virus envelope protein (env) to the target cell, an event that may occur at the cell surface or within an acidified endosome after receptor-mediated endocytosis (White et al., *Quant. Rev. Biophys.* 16: 151-195 (1983)). After fusion, the virion core enters the cytoplasm and the viral replication process is initiated. For example, in the case of HIV, recent studies suggested that cell surface molecules other than CD4 may also be important for virus entry into human cells.

Ecotropic and Amphotropic Murine Retrovirus Receptors and Infection

Susceptibility of cells to infection with ecotropic (species specific) or amphotropic (species non-specific) murine leukemia virus (E-MuLV) may also be determined by binding of the virus envelope to a membrane receptor.

Based on viral interference assays, four types of specific MuLV receptors have been postulated: (a) receptors for E-MuLV; (b) receptors for wild-type amphotropic MuLV; (c)

receptors for recombinant viruses derived from E-MuLV, such as the "mink cell focus-inducing" or MCF virus; and (d) receptors for a recombinant virus derived from an amphotropic MuLV (Rein, A. et al., Virology 136:144-152 (1984)).

Recently, a cDNA clone (termed W1) encoding the murine ecotropic retroviral receptor (ERR) (SEQ ID NO:4) was identified (Albritton, L.W. et al., *Cell* 57:659-666 (1989)). This study demonstrated that susceptibility to E-MuLV infection was acquired by the expression of a single mouse gene in human EJ cells.

Viral Receptor Mediated Tissue Specificity

HIV is an example of a virus exhibiting receptor-mediated tissue restriction, apparently based on its use of the CD4 protein as its primary receptor. However, cell-specific receptors are unlikely to be the sole determinant of tissue specificity. The tissue tropism of retroviruses is likely to result from a complex series of factors, such as the tissue specificity of long terminal repeats, variations in viral *env* proteins, cellular factors, and the expression of appropriate cell surface receptors (Kabat, *Curr. Top. Microbiol. Immunol.* 148:1-31 (1989)).

The *tea* (T cell early activation, SEQ ID NO:5) gene, as exemplified by clone 20.5 (MacLeod et al., 1990, *J. Biol. Chem.* 1:271-279), is the first example of a cloned gene or cDNA that has the potential to encode a multiple transmembrane-spanning protein which is induced during T cell activation (Crabtree, *Science* 243:355-361 (1989)). The function of the *tea* gene is not yet known.

The sequence of 20.5 cDNA (SEQ ID NO:5) was found to be strikingly homologous to the murine ERR cDNA clone (SEQ ID NO:3) discussed above (the *Rec-1* gene). Retroviral binding and infection studies are required to determine whether the *tea*-encoded protein functions as a viral receptor (Rein et al., *Virology* 136:144-152 (1984)). Despite the high degree of similarity between *tea* and ERR, the two genes differ in chromosomal location, and their predicted protein products differ in tissue expression patterns.

Gene Therapy and Gene Transfer

One relatively efficient but problematic means for achieving transfer of genes is by amphotropic retrovirus-mediated gene transfer (Gilboa, E., *Bio-Essays* 5:252-258 (1987); Williams, D.A. et al., *Nature* 310:476-480 (1984); Weiss, R.A. et al., *RNA Tumor Viruses*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1985). Recombinant amphotropic retroviruses, have been studied with the possibility of being used as vector for the transfer of genes into human cells (Cone, R.D. et al., *Proc. Natl. Acad. Sci. USA* 81:6349-6353 (1984); Danos, O. et al., *Proc. Natl. Acad. Sci. USA* 85:6460-6464 (1988)) since such amphotropic viruses, such as murine amphotropic E-MuLV can infect human cells. One of the safety problems inherent in this approach, which may preclude progress in the clinic, is the fact that even amphotropic retroviruses that have been rendered replication-defective are sometimes capable of generating wild-type variants through recombinational events which provide replication ability. Such an alteration could lead to the widespread retroviral infection in cells and tissues which were not intended to be genetically modified. This could result in generalized disease such as cancer or other pathologies caused by insertion of the amphotropic virus' nucleic acid with the LTR's into important functioning genes within a cell which disruption could lead to a pathologic state (Mulligan, R. *Science*, 1993). It is to these needs and problems that the present invention is also directed.

Accordingly, there is a need to overcome one or more problems associated with the use of known amphotropic retroviruses for introducing heterologous genes into eukaryotic cells. There is also a need to provide human viral receptor proteins which bind viruses and which can be used in diagnostic and therapeutic applications without the problems immunogenicity and non-specific cell binding found with the use of murine amphotropic recombinant viruses.

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the

present application. Any statement as to content or a date of any document is based on the information available to applicant at the time of filing and does not constitute an admission as to the correctness of such a statement.

SUMMARY OF THE INVENTION

The present invention is intended to overcome one or more deficiencies of the related background art.

The present invention is also intended to provide new and/or superior methods for gene therapy, wherein nucleic acid having a therapeutic and/or diagnostic effect can be delivered to target cells with improved safety and/or selectivity, with reduced nonspecific cell incorporation or association of the nucleic acid.

Such selective gene therapy may be provided according to the present invention by using vectors having viral binding domains which may bind chimeric receptor polypeptides of the present invention; which may be associated with selected target cells. The target cell is made selective for a viral binding domain of a species other than the species of the target cell by association with a chimeric receptor polypeptide. The mutant receptor polypeptide comprises a viral receptor binding site of the same species as the target cell, as a first species, which binding is modified to also bind a non-first species virus.

The present invention is also intended to provide chimeric receptor polypeptides, corresponding to at least the binding portion of a first species-specific virus receptor, which is modified to allow binding of, and/or infection by, a non-first species-specific virus or at least the bindings of the envelope protein (*env*) binding domain thereof. The amino acid sequence of the chimeric receptor polypeptide including the modification in the viral receptor binding site of the chimeric receptor polypeptide, is termed the chimeric receptor binding site. The use of the sequence, regardless of its origin, is embodied in the concept of a chimeric receptor.

Alternatively, a chimeric receptor polypeptide may also contain a binding site sequence of a first species viral

receptor which is modified to provide at least one amino acid substitution, deletion or addition, corresponding to at least one amino acid of a viral receptor binding site of a non-first species specific virus. The substitution, deletion or addition confers the ability of the chimeric receptor polypeptide to bind an *env* binding domain of the non-first species specific virus. Preferably the first species is selected from a human, primate or rodent species.

A chimeric receptor polypeptide is intended to be provided as an isolated, purified, recombinant and/or organically synthesized polypeptide. The resulting chimeric receptor polypeptide, or a chimeric receptor cell expressing, or associated at its surface with, the chimeric receptor polypeptide, is thus capable of binding, and/or being infected by, a non-first species specific virus, or a binding domain thereof, *in vivo*, *in situ*, or *in vitro*.

The present invention is therefore intended to include all compounds, compositions, and methods of making and using such chimeric receptor polypeptides, without undue experimentation, based on the teachings and guidance presented herein.

A chimeric receptor polypeptide of the present invention is further intended to be used to selectively target cells expressing, or associated at their surface with, a chimeric receptor binding site of the chimeric receptor polypeptide for diagnostic, research or therapeutic applications, such as human gene therapy or cancer treatment or diagnosis, as non-limiting examples.

According to one aspect of the present invention, a chimeric receptor polypeptide may be used to provide selective binding of non-first species specific viruses, or an *env* binding domain thereof, to target cells or tissues expressing or associated with a chimeric receptor polypeptide. A non-first species specific virus, or a conjugate containing an *env* binding domain thereof, may be used as a target cell specific delivery vector to deliver at least one diagnostic or therapeutic agent to the target cell or tissue.

The invention also is intended to provide nucleic acid coding for, and/or cells or tissues expressing, such a chimeric receptor polypeptide.

The present invention is thus intended to provide delivery vectors, containing one or more therapeutic and/or diagnostic agents, including vectors suitable for gene therapy, having an improved measure of safety compared to related, art approaches. In particular, the present invention permits the use of cell or tissue specific delivery vectors for therapeutic and/or diagnostic agents which substantially bind only target cells as chimeric receptor cells or tissues expressing cell surface chimeric receptor polypeptides, wherein the delivery vectors may have substantially reduced or substantially no binding to and/or infecting of non-target cells or tissues.

The present invention is also intended to provide gene therapy to selected cells or tissues of a eukaryote, such as a mammal, bird, insect or yeast, *in vitro, in situ*, or *in vivo*, through the use of transient or constitutive expression of a chimeric receptor polypeptide associated with, or on the surface of, resulting chimeric receptor cells of a first species. Such chimeric receptor cells or tissues are thus rendered susceptible to exclusive infection or binding by non-first species specific viral vectors, or *env* binding domains thereof.

The infection of the non-human specific viral vector or binding domain may stably integrate a gene, such as a therapeutic gene, into the selected cells or tissues *in vivo, in vitro, or in situ*, such that the selected cells or tissues express the gene product having the desired effect on the target cell or tissues.

The present invention is also intended to provide gene therapy to specific target cells or tissues, with substantially no or substantially reduced non-target cell viral vector infection and/or binding.

The present invention is further intended to provide gene therapy having substantially no, or substantially reduced reversion or recombination to replication competence or viral

packaging of viral vectors used for delivering the therapeutic agent of the gene therapy. The target cells may express or be associated with a chimeric receptor polypeptide of the present invention.

The present invention is additionally intended to provide a chimeric receptor polypeptide or retroviral polypeptide which can be used to inhibit retroviral infection of a human cell or tissue, *in vivo* or *in vitro* or *in situ*.

Methods of using receptor polypeptides of the present invention are also provided to prevent or treat a viral infection, such as a retroviral infection (e.g. HIV), as a non-limiting example.

A recombinant nucleic acid (SEQ ID NO:8) is also provided, comprising a nucleotide sequence which encodes the H13 molecule, or a sequence substantially corresponding to, or consisting essentially of, the H13 amino acid sequence or mutant thereof, which nucleic acid consists of DNA or RNA.

Other objects, features and advantages of the present invention will become apparent to those skilled in the art from the following detailed description. It should be understood, however, that the detailed description and specific examples, while indicating preferred embodiments of the present invention, are given by way of illustration and not limitation. Many changes and modifications within the scope of the present invention may be made without departing from the spirit thereof, and the invention includes all such modifications.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the H13 nucleic acid sequence (SEQ ID NO:7), including coding and noncoding sequences, and the predicted protein sequence (SEQ ID NO:8) of the H13 protein.

Figure 2 shows a schematic diagram of the alignment of one strand of the H13 and ERR cDNA sequence (SEQ ID NOS:7 and 3, respectively). The sequences were analyzed using the Genetics computer group sequence analysis software package (Devereux, J. et al., *Nucl. Acids Res.* 12:387-395 (1984)).

Figure 3 shows the alignment of H13, ERR and TEA deduced amino acid sequences. Vertical lines indicate sequence homology. Dots indicate lack of homology, with double dots representing conservative amino acid changes. The sequences were analyzed as in Figure 2. Shown in brackets are the sequences of H13 corresponding to Extracellular Domain 3 (residues 210-249) and Extracellular Domain 4 (residues 310-337).

Figure 4 shows an autoradiogram of the hybridization pattern of EcoRI-digested nucleic acid of human (CCL120, CCL119, SupT1, H9, MOLT4), hamster (CHO-K1) and mouse (Balb/c thymocytes, BIOT6R) origin, probed with the KpnI-KpnI fragment (390 bp) of murine ERR cDNA.

Figure 5 shows an autoradiogram of a Southern blot analysis nucleic acid from various species with H13 cDNA (SEQ ID NO:1). Nucleic acid hybridized was EcoRI-digested nucleic acid of human (CCL120, CCL 119, SupT1, H9, MOLT4), hamster (CHO-K1) and mouse thymocytes (Balb/c or BIOT6R) origin.

Figure 6 shows an autoradiogram of H13 gene expression. RNA from the indicated human cell lines was hybridized with the H13 cDNA (SEQ ID NO:1).

Figure 7 shows an autoradiogram of the hybridization pattern of RNA of human (CEM, H9, MOLT4, SupT1, CCL120, CCL119), hamster (CHO K1) and mouse (RL12) origin, probed with the KpnI-KpnI fragment (390 bp) of murine ERR cDNA.

Figure 8 shows a Northern Blot demonstrating the acquisition of susceptibility to infection with murine ecotropic retrovirus by transfection of a resistant cell with ERR cDNA. After transfection of ERR cDNA into hamster CHO K1 cells, the transfectants expressing the murine retroviral receptor gene were infected with murine radiation leukemia virus (RadLV). Two weeks later, Northern blot analysis was performed using a viral probe, and reverse transcriptase (RT) activity of the cell supernatants was measured.

Figure 9 shows hydropathy plots for H13, ERR and TEA predicted proteins. The vertical axis gives the hydropathicity values from the PEPTIDESTRUCTURE program (See, e.g., Jameson et al., *CABIOS* 4: 181-186 (1988)).

Figure 10 shows a graph indicating the antigenicity of H13 predicted protein, analyzed using the PEPTIDESTRUCTURE™ program. One of the highly antigenic peptides (amino acid residues 309-367) was prepared using an AccI-EcoRI fragment as shown in Figure 14.

Figure 11 shows a SDS-PAGE autoradiogram depicting the synthesis of a fusion protein including the H13 protein with glutathione-S-transferase (GST). The fusion protein was prepared by ligating the 180 bp AccI-EcoRI fragment of H13 cDNA to the plasmid pGEX-2T, which expresses antigens as fusion proteins, was induced by addition of isopropyl-beta-thiogalacto-pyranoside (IPTG), and was purified using glutathione-Sepharose chromatography.

Figure 12A-B shows the genetic mapping of the H13 gene to human chromosome 13. The autoradiogram (Figure 12A) shows the hybridization pattern of EcoRI-digested nucleic acid from human-hamster somatic cell hybrids probed with H13 cDNA (SEQ ID NO:1). Lane 1 and 11 contain nucleic acid from human and hamster, respectively. Lanes 2-10 contain nucleic acid which is derived from the chromosomes as designated in the table in Figure 12B.

Figure 13 is a schematic diagram of the genetic structure of the H13 and ERR genes, and four chimeric constructs there between. The infectivity of E-MuLV on human cells transfected with the various constructs is also indicated.

Figure 14 shows a comparison of sequences (nucleotide and amino acid) of the region of H13 and ERR termed Extracellular Domain 3 (as also depicted as part of SEQ ID NO:7, SEQ ID NO:8 and Figure 1). This region of the receptor protein is the most diverse between the human and mouse sequences. The sequences were aligned using Genetics computer group sequence analysis software package (See, e.g., Devereux, J. et al., *Nucl. Acids Res.* 12:387-395 (1984)).

Figure 15 shows a schematic illustration of several

cDNA clones from which the H13 sequence was derived, and their general structural relationship to the murine ERR homolog. Clone 7-2 (H-13.7-2) represents a part of the complete H13 nucleic acid sequence; this was the first H13 clone sequenced, yielding SEQ ID NO:1 and SEQ ID NO:2. Clones 1-1 (H13.1-1) and 3-2 (H13.3-2) each contain parts of the H13 sequence. The combined sequencing of these three clones resulted in the full H13 nucleic acid and amino acid sequences (SEQ ID NO:7 and SEQ ID NO:8, respectively).

Figure 16 shows a schematic illustration of extracellular domains 3 and 4, wherein (*) marks positions which contain critical amino acids for infection by MuLV-E. Mouse-human chimeric receptor molecules (Chimera I-III) were prepared by substitution using common restriction sites in murine ERR and human H13, and their abilities to function as a receptor for MuLV-E was determined using the recombinant MuLV-E, ΨCRE/BAG virions (se, e.g., Price et al. *Proc. Natl. Acad. Sci. USA* 84: 156-160 (1987); Danos et al. *Proc. Natl. Acad. Sci. USA* 15: 6460-6464 (1988)). Black boxes on top of the figure indicate extracellular domains of ERR and H13 (See, e.g., Albritton et al. *Cell* 57: 659-666 (1989); Yoshimoto et al *Virology* 185: 10-17 (1991)), and shaded and striped bars indicate the nucleotide sequences of ERR and H13, respectively. This is one of the representative results of three different experiments.

Figure 17 shows a comparison of nucleotide and amino acid sequences of extracellular domains 3 and 4 in murine ERR and human H13. The alignment was made using the Genetics computer group sequence analysis software package (See, e.g., Devereux et al Nucleic Acids Res. 12:387-395 (1984)). To pinpoint the critical amino acid residues, oligonucleotide-directed mutagenesis was carried out and 13 individual mutant ERR molecules were created which contain one or two amino acids substitutions or insertions as marked by boxes.

Figure 18 shows results demonstrating the acquirement of ability of H13 to function as a receptor for MuLV-E by mutation.

Figure 19 shows the alignment of gp70 amino acid sequences associated with receptor specificity with leukemia. virus sequences. Ecotropic retrovirus sequences: AKv(8); Friend MLV (11); Moloney MLV (12). Non-ecotropic retrovirus sequences: Xenotropic MLV NZB.1U.6(2); polytropic MCF 247 (2); amphotropic MLV 4070A(2), (+) - amino acid identity; (-) - gap in sequence; Caps - conserved amino acid substitutions lower case - non-conserved amino acid substitutions. Number indicate the position of amino acids in the gp70 sequence.

Figure 20 shows an amino acid and nucleic acid comparison of the N-terminal env region of the ecotropic AKv and amphotropic MLV 4070A which contains a 30 amino acid gap within the amphotropic sequence. Nucleotide sequence and corresponding amino acids are shown. AKv sequence (9) Genbank Accession number V01164 Amphotorpic MLB 4070 (2) Genbank Accession number mm469. Numbers above indicate nucleotide positions in reported sequences. Numbers below indicate amino acid positions (see Figure 4). The positions of AKv Sma 1 and Ampho. Rsa 1 restriction sites are also shown. Nucleotide sequences suitable for PCR primers are indicated by arrows.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention relates to new methods of gene therapy and to chimeric receptor polypeptides which are discovered to provide target cell specific binding sites for specific viruses, viral vectors and delivery vectors of therapeutic and/or diagnostic agents. Such chimeric receptor polypeptides of the present invention may be used for diagnostic, research and therapeutic applications, including gene therapy, wherein target cells having such chimeric receptor polypeptides on their surface may be exclusively and/or substantially infected by non-human specific delivery vectors. The present invention thus overcomes one or more problems associated with the use of known viral vectors which are susceptible to non-specific infection of non-target cells, as well as reversion or mutation to replication competence of such viral vectors.

According to the present invention, cells or tissues of humans, or other mammals, may be treated to be associated with, or to transiently or constitutively express a chimeric receptor polypeptide, as a chimeric receptor cell or tissue, *in vivo*, *in situ* or *in vitro*. Animal or human subjects having such chimeric receptor cells or tissues can thus be treated or diagnosed according to methods of the present invention with therapeutic and/or diagnostic agents further comprising an *env* binding domain polypeptide which binds a cell surface chimeric receptor polypeptide of the present invention.

In the context of the present invention, a "chimeric receptor binding site" or "chimeric receptor" refers to a modified first species viral receptor binding site, which modification is comprised of a chimeric receptor polypeptide wherein at least two amino acids are substituted, deleted or added from a non-first species specific viral receptor binding site. The at least two amino acid deletion, substitution or addition confers binding capability on the chimeric receptor for an *env* binding domain of a surface protein of a non-first species specific virus. Preferably, the substitution or addition corresponds to domain 3 of human H13 substituted non human specific virus receptor, such as ERR,, MuLV or GALV. Preferably, the substituted, deleted, or added amino acid residues correspond to residues 200-280 or 220-260, 230-245 or any range or value therein, of H13 (SEQ ID NO: 8), e.g., similarly to the substitutions presented in Table 1 below.

The term "chimeric receptor cell" or "chimeric receptor tissue" refers to a cell or tissue of a first species which has on, or associated with, its cell surface a chimeric receptor polypeptide, such that a non-first species specific virus or *env* binding domain specific for the chimeric receptor polypeptide may bind the chimeric cell or tissue, *in vivo*, *in situ*, or *in vitro*. The chimeric receptor polypeptide may preferably be associated with the chimeric receptor cell via a cell specific receptor associated with a chimeric receptor polypeptide which combination preferably binds to a first species specific cell or tissue type to provide a chimeric receptor cell of the present invention. In another preferred

embodiment, such association is provided by recombinant expression of the specific cell or tissue type of at least one chimeric receptor polypeptide.

The term "associated" in the context of the present invention refers to any type of covalent or non-covalent binding or association such as, but not limited to, a covalent bond, hydrophobic/hydrophilic interaction, Van der Wahls forces, ion pairs, ligand-receptor interaction, epitope-antibody binding site interaction, enzyme-substrate interaction, liposome-hydrophobic interaction, nucleotide base pairing, membrane-hydrophobic interaction, and the like.

The term "chimeric receptor polypeptide" refers to a polypeptide of at least 10 amino acids corresponding to a human viral receptor protein or consensus sequence thereof, wherein the chimeric receptor polypeptide contains a chimeric receptor binding site capable of binding a an *env* binding domain of a non-human specific virus such as 10-700, 10-100, 10-50, 10-30, 20-30, 20-40, 40-60 or any range or value therein.

Alternatively, or additionally, chimeric receptor polypeptides of the present invention may be defined as amino acid sequences of at least 10 residues having at least 80% (such as 81-99%, or any value or range therein, such as 83-85, 87-90, 93-95, 97-98, or 99% or any range or value therein.) homology with the corresponding amino acid sequence of a first species viral receptor, as any ecotropic or amphotropic first species viral receptor, have been modified to include amino acids from a non-first species viral receptor, and which have the biological activity of binding a binding domain of an *env* polypeptide of a non-first species virus. Preferably the first species is human and the second species is rodent, or vice versa. A non-limiting example of such a corresponding human viral receptor sequence is SEQ ID NO: 8, such as corresponding to 10 to 629 amino acids of SEQ ID NO: 8 or any value or range therein.

Additionally or alternatively, a chimeric receptor polypeptide of the present invention may further comprise a hydrophobic amino acid sequence corresponding to at least one

to 20 transmembrane domains of a first species viral receptor protein which is at least 80% (such as 80-100%, or any range or value therein) homologous to the corresponding first species viral receptor. Such transmembrane domains may be analogous to those described, e.g., a human H13 sequence (SEQ ID NO:8) or a murine ERR (SEQ ID NO:4) having 14 potential transmembrane domains (See, e.g., Eisenberg et al., J. Mol. Biol. 179:125-142 (1984)), and which can be determined using hydrophobicity plots according to known method steps, e.g., as referenced therein or herein.

A non-limiting example of a first species viral receptor, as human viral receptor, is the human H13 protein (SEQ ID NO:8), which can be provided as an H13 polypeptide according to the present invention in a non-naturally occurring form, such as purified or chemically synthesized, or recombinantly produced, in either case according to known method steps, e.g., as referenced herein.

Such a non-limiting example of a chimeric receptor polypeptide of the present invention may be, e.g., a modified human H13 amino acid sequence (e.g., such as SEQ ID NO: 8) of at least 10 amino acids which is modified to provide binding capability to a non-human specific virus, such as the non-limiting examples of E-MuLV, gp-70 or ERR receptor protein (SEQ ID NO: 4). Such modifications may preferably include substitution at a receptor binding site of a human viral receptor sequence by at least one non-human viral receptor amino acid, such as a murine ERR amino acid in the corresponding site in H13, to permit infection of a human or non-murine target cell having the chimeric receptor polypeptide, preferably a human cell, with a virus or retrovirus, such as E-MuLV.

Thus, as a further non-limiting subexample, to confer E-MuLV infection susceptibility on a human or non-murine cell, it is preferred to substitute corresponding E-MuLV receptor amino acid residues of domain 3 of H13. Domain 3 comprises residues between positions 210 and 250 (SEQ ID NO:7). Preferred substitution is with 1-10 amino acid residues, or any number or range therein, from the

corresponding domain of ERR, between amino acid residues 210 and 242 (SEQ ID NO:4), preferably amino acids 238, 239 and 242, with more preferably at least 242 being substituted. Substitution of between 1 and 4 residues is preferred. For example, residues and positions which differ in extracellular domain 3 of H13 and ERR are listed below in Table 1. In a more preferred embodiment, at least Pro242 of H13 (SEQ ID NO:8) is replaced by Tyr, and at least one of Gly240 and Val244 (SEQ ID NO:8) is replaced by Val and Glu, respectively, and as presented in Figure 18. Additionally, at least one of H13 amino acid 239 may be preferably replaced by the corresponding ERR amino acid 233.

Table 1

Exemplary Substitutions in H13 Extracellular Domain 3 and Domain 4 for E-MuLV Binding

Domain 3	
Original H13 Residue	Substituting ERR Residue
V 214	I 214
E 222	K 222
E 223	N 223
G 225	S 225
L 233	N 227
E 239	N 232
P 242	Y 235
V 244	E 237

Non-limiting examples of chimeric receptor polypeptides according to the present invention may include Tyr242, Phe242 and/or Trp242 and at least one of Val240, Met240, Leu240, Ile240, Glu244, Gln244, Asp244, or Asn244; and Asn239, Asp239, Glu239 or Gln239 (SEQ ID NO:3), wherein at least Pro242 of H13 (SEQ ID NO:8) is replaced by Tyr, and at least one of Gly240 and Val244 (SEQ ID NO:8) is replaced by Val and Glu, respectively.

Another means for modifying virus binding specificity of H13 is by deletion of one or more of the "extra" amino acid residues in H13 that do not correspond to residues of ERR. Preferred deletions (in extracellular domain 4) are of between one and six residues from H13 positions 326

to 331 (SEQ ID NO:1), most preferably, deletion of all six of these residues.

In another non-limiting example of a chimeric receptor polypeptide of the present invention, an H13 chimeric receptor polypeptide may be provided which confers the binding ability of a human cell, to bind an *env* binding domain of a non-human virus, wherein 1 to 30 amino acids of H13 are substituted, deleted or modified by corresponding amino acids from ERR, in order to confer such binding ability. Preferably, such a chimeric receptor polypeptide may preferably comprises a peptide wherein at least Pro242 of the H13 polypeptide (SEQ ID NO:8) is replaced by Tyr, and at least one of Gly240 and Val244 (SEQ ID NO:8) is replaced by Val and Glu, respectively, or fragments having amino acid sequences substantially corresponding to the amino acid sequence of H13, such that the resulting chimeric receptor is selectively bound by a murine ecotropic retrovirus, which cannot infect other human or non-murine cell or tissue types.

Alternatively, a homologous chimeric receptor polypeptide of an amphotropic first species viral receptor corresponding to H13 may be similarly modified to allow binding of a non-first species specific amphotropic virus, such as a chimeric receptor cell or tissue. Preferably the first species is human or rodent.

Also included are soluble forms of H13 or a chimeric receptor polypeptide, as well as functional derivatives thereof, having similar bioactivity for all the uses described herein. Also intended are all active forms of chimeric receptors or H13 polypeptide derived from the chimeric receptor or H13 transcript, respectively, and all mutants with H13-like activity.

Methods for production of soluble forms of receptors which are normally transmembrane proteins are well known in the art (see, for example, Smith, D.H. et al., Science 238:1704-1707 (1987); Fisher, R.A. et al., Nature 331:76-78 (1988); Hussey, R.E. et al., Nature 331:78-81 (1988); Deen, K.C. et al., Nature 331:82-84 (1988); Traunecker, A. et al., Nature 331:84-86 (1988); Gershoni, J.M. et al., Proc. Natl.

Acad. Sci. USA 85:4087-4089 (1988), which references are hereby incorporated by reference). Such methods are generally based on truncation of the nucleic acid encoding the receptor protein to exclude the transmembrane portion, leaving intact the extracellular domain (or domains) capable of interacting with specific ligands, such as an intact retrovirus or a retroviral protein or glycoprotein. For the purposes of the present invention, it is important that the soluble chimeric receptor polypeptide or H13 polypeptide, comprise elements of the binding site of the chimeric receptor or H13 that permits binding to a virus. A chimeric receptor polypeptide or H13 polypeptide has many amino acid residues, only one or two or more, such as 2-15, or any value or range therein, such as 3-5, 6-9 or 10-15, which are critically involved in virus recognition and binding.

As discussed herein, H13 proteins or chimeric receptor polypeptides of the present invention may be further modified for purposes of drug design, such as, for example, to reduce immunogenicity, to promote solubility or enhance delivery, or to prevent clearance or degradation, according to known method steps. In a further embodiment, the invention provides muteins of a chimeric receptor polypeptide of the present invention. By "mutein" is meant a "fragment," "variant," or "chemical derivative" of an H13 protein or a chimeric receptor polypeptide. A mutein retains at least a portion of the function of the H13 protein which permits its utility in accordance with the present invention.

A "fragment" of the H13 protein or chimeric receptor polypeptide is any subset of the molecule, that is, a shorter peptide.

A "mutein" of the H13 chimeric receptor polypeptide refers to a molecule substantially similar to either the entire peptide or a fragment thereof. Muteins may be conveniently prepared by direct chemical synthesis or recombinant production, including mutagenesis, of the mutein, using methods well-known in the art. See, e.g., Sambrook, *supra*, Ausubel, *supra*, Coligan, *supra*.

Alternatively, muteins of an H13 or chimeric receptor polypeptide can be prepared by mutations in the nucleic acid which encodes the synthesized peptide. Such mutants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence as presented herein. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity. Obviously, the mutations that will be made in the nucleic acid encoding the mutein peptide must not alter the reading frame and preferably will not create complementary regions that could produce secondary mRNA structure (see, e.g., European Patent Publication No. EP 75,444).

At the genetic level, these muteins ordinarily are prepared by site-directed mutagenesis (as exemplified by Adelman et al., *DNA* 2:183 (1983) or Ausubel, *supra*, Sambrook, *supra*) or Coligan et al, eds., *Current Protocols in Immunology*, Greene Publishing Association and Wiley Intersciences N.Y., N.Y., (1992, 1993) of nucleotide in the nucleic acid encoding the peptide molecule, thereby producing nucleic acid encoding the mutant, and thereafter expressing the nucleic acid in recombinant cell culture. The muteins typically exhibit the same qualitative biological activity as a chimeric peptide.

In particular, a chimeric receptor polypeptide or H13 polypeptide having critical amino acid residues derived from other non-human or non-animal specific viruses, such as ERR, may be produced using site-directed mutagenesis.

Another group of muteins are those in which at least one amino acid residue in the protein molecule, and preferably, only one, has been removed and a different residue inserted in its place. For a detailed description of protein chemistry and structure, see Schulz, G.E. et al., *Principles of Protein Structure*, Springer-Verlag, New York, 1978, and Creighton, T.E., *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, 1983, which are hereby entirely incorporated by reference. The types of substitutions which may be made in the protein or peptide

molecule of the present invention may be based on analysis of the frequencies of amino acid changes between a homologous protein of different species, such as those presented in Table 1-2 of Schulz et al., *supra* and Figure 3-9 of Creighton *supra*. Based on such an analysis, conservative substitutions are defined herein as exchanges within one of the following five groups:

1. Small aliphatic, nonpolar or slightly polar residues: ala, ser, thr (pro, gly);
2. Polar, negatively charged residues and their amides: asp, asn, glu, gln;
3. Polar, positively charged residues: his, arg, lys;
4. Large aliphatic, nonpolar residues: met, leu, ile, val (cys); and
5. Large aromatic residues: phe, tyr, trp.

Substantial changes in functional or immunological properties are made by selecting substitutions that are less conservative, such as between, rather than within, the above five groups, which will differ more significantly in their effect on maintaining (a) the structure of the peptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Examples of such substitutions are (a) substitution of gly and/or pro by another amino acid or deletion or insertion of gly or pro; (b) substitution of a hydrophilic residue, e.g., ser or thr, for (or by) a hydrophobic residue, e.g., leu, ile, phe, val or ala; (c) substitution of a cys residue for (or by) any other residue; (d) substitution of a residue having an electropositive side chain, e.g., lys, arg or his, for (or by) a residue having an electronegative charge, e.g., glu or asp; or (e) substitution of a residue having a bulky side chain, e.g., phe, for (or by) a residue not having such a side chain, e.g., gly.

Most deletions and insertions, and substitutions according to the present invention are those which do not

produce radical changes in the characteristics of the protein or peptide molecule. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays, either immunoassays or bioassays. For example, a mutein typically is made by site-specific mutagenesis of the peptide molecule-encoding nucleic acid, expression of the mutant nucleic acid in recombinant cell culture, and, optionally, purification from the cell culture, for example, by immunoaffinity chromatography using a specific antibody on a column (to absorb the mutein by binding to at least one epitope).

The activity of the cell lysate containing H13 or a chimeric receptor polypeptide, or of a purified preparation of H13 or chimeric receptor, can be screened in a suitable screening assay for the desired characteristic. For example, a change in the immunological character of the protein molecule, such as binding to a given antibody, is measured by a competitive type immunoassay (as described herein). Biological activity is screened in an appropriate bioassay, such as virus infectivity, as described herein.

Modifications of such peptide properties as redox or thermal stability, hydrophobicity, susceptibility to proteolytic degradation or the tendency to aggregate with carriers or into multimers are assayed by methods well known to the ordinarily skilled artisan.

Additionally, modified amino acids or chemical derivatives of amino acids of a chimeric receptor polypeptide according to the present invention, may be provided, which polypeptides contain additional chemical moieties or modified amino acids not normally a part of the protein. Covalent modifications of the peptide are thus included within the scope of the present invention. Such modifications may be introduced into a chimeric receptor polypeptide by reacting targeted amino acid residues of the polypeptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues.

Derivatization with bifunctional agents is useful for cross-linking the peptide to a water-insoluble support matrix or to other macromolecular carriers, according to known method steps. Commonly used cross-linking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates described in U.S. Patent Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 (which are herein entirely incorporated by reference), may be employed for protein immobilization.

Other modifications of a chimeric receptor polypeptide of the present invention may include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, *Proteins: Structure and Molecule Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)), acetylation of the N-terminal amine, and, in some instances, amidation of the C-terminal carboxyl groups, according to known method steps.

Such derivatized moieties may improve the solubility, absorption, biological half life, and the like. Such moieties or modifications of a chimeric receptor polypeptide may alternatively eliminate or attenuate any undesirable side effect of the protein and the like. Moieties capable of mediating such effects are disclosed, for example, in *Remington's Pharmaceutical Sciences*, 16th ed., Mack Publishing Co., Easton, PA (1980).

Such chemical derivatives of a chimeric receptor polypeptide also may provide attachment to solid supports, such as for purification, generation of antibodies or cloning; or to provide altered physical properties, such as resistance to enzymatic degradation or increased binding affinity or modulation for a chimeric receptor polypeptide, which is desired for therapeutic compositions comprising a chimeric receptor polypeptide, antibodies thereto or fragments thereof. Such peptide derivatives are known in the art, as well as method steps for making such derivatives.

An "isolated or recombinant H13 polypeptide" refers to a polypeptide having an amino acid sequence substantially corresponding to, or consisting essentially of, at least a 10 amino acid portion of the amino acid sequence of SEQ ID NO:8 and which binds a retrovirus, such as the HIV *env* binding domain. "Substantially corresponding" to an amino acid sequence of a chimeric receptor polypeptide or H13 protein, refers to those amino acid sequences in which at least one amino acid residue in a soluble portion of the protein molecule has been removed and a different residue inserted in its place, the number of substitutions being relatively small and well characterized or conservative, as described herein.

Methods for Making Chimeric receptor Polypeptides or H13 Polypeptides. A preferred use of this invention is the production by chemical synthesis or recombinant nucleic acid technology a chimeric receptor polypeptide or chimeric receptor polypeptide or fragments thereof, where the fragments are small as possible, while still retaining sufficiently high affinity in binding to a non-human specific retrovirus or HIV. Preferred fragments of H13 include extracellular domain 3, as presented herein.

Due to its function as a virus receptor, an extracellular fragment of the H13 or chimeric receptor polypeptide of the present invention may bind to a human or non-human specific virus, respectively. By production of smaller fragments of this peptide, one skilled in the art, using known binding and inhibition assays, will readily be able to identify the minimal peptide capable of binding a

binding domain of a virus or retrovirus with sufficiently high affinity to inhibit infectivity, without undue experimentation based on the teaching and guidance presented herein. Shorter peptides are expected to have two advantages over the larger proteins: (1) greater stability and diffusibility, and (2) less immunogenicity.

As a non-limiting example, due to the presence of amino acid residues from the murine ERR sequence, a mutant chimeric receptor polypeptide of the present invention endows human cells or other non-murine cells expressing the receptor with the ability to be infected by a non-human specific virus, such as murine E-MuLV or HuLV, which may be modified to reduce the possibility of reversion to replication competent forms.

By appropriate substitutions of one or more of the amino acid residues of a viral receptor, chimeric receptor polypeptides of the present invention may be provided without undue experimentation, based on the teaching and guidance presented herein.

The present invention is also intended to encompass binding of a chimeric receptor polypeptide to any non-human ecotropic or amphotropic virus with similar receptor specificity, including retroviruses, such as MuLV or HuLV, and adenoviruses.

One of ordinary skill in the art will be able to determine how to obtain a nucleic acid encoding a viral receptor suitable for use in providing a chimeric receptor polypeptide of the present invention from any species or cell type, without undue experimentation, based on the teaching and guidance presented herein. As a non-limiting example of such a procedure, one will initially screen (using methods routine in the art) a cDNA library of the species or cell type of interest, for example, a human T cell cDNA library, using a probe based on the sequence of a human viral receptor, such as H13. Next, one will clone and sequence the hybridizing nucleic acid to obtain the sequence of the "new" retroviral receptor. By visual inspection or with the aid of a computer program (as described herein) it is possible to identify the regions in which the sequence of the new retroviral receptor

protein differs from ERR or H13. In particular, one will concentrate on domains corresponding to extracellular domain region 3 of H13 or analogous domains.

Based on the sequence differences observed, it is possible, using the teachings provided herein, to create a sequence having one or more amino acid substitutions such that a chimeric receptor between the new receptor and a known receptor is created. The chimeric receptor can then be expressed in a cell of choice and its function can easily be tested using conventional virus binding assays or virus infectivity assays.

Furthermore, according to the present invention, it is possible to modify the receptor attachment site of a virus so that it will not bind to its natural receptor, or bind to a different receptor, based on knowledge of receptor choice determinants in envelope glycoproteins of viruses, such as murine leukemia viruses. See, e.g., Battini et al *J. Virology* 66(3):1468-1475 (1992).

For example, to prevent binding of HIV-1, changes in the sequence of the HIV-1 CD4-binding domain will render this virus non-infective for CD4-bearing cells. Corresponding changes may be introduced into H13, so that this mutant HIV will bind to it. In this way, a safe HIV preparation can be generated which binds only to select cells bearing the appropriate variant receptor, but not to the normal targets of HIV-1.

It is also within the scope of the present invention to express more than one intact or mutant viral receptor polypeptide on the surface of the same cell. Thus, by virtue of a first expressed chimeric receptor polypeptide, e.g. ERR, a human cell can be infected with one virus strain *in vitro* in a transient fashion, and can be manipulated by the judicious use of cytokine growth or differentiation factors. Such cells can be introduced into a recipient. At the desired time, a second virus which binds to a second expressed chimeric receptor polypeptide can be introduced into the individual to infect stably and alter only those introduced cells bearing the second retroviral receptor.

Oligonucleotides representing a portion of the H13 sequence (SEQ ID NO:2) are useful for screening for the presence of homologous genes and for the cloning of such genes. Techniques for synthesizing such oligonucleotides are disclosed by, for example, Wu, R., et al., *Prog. Nucl. Acid. Res. Molec. Biol.* 21:101-141 (1978)), Ausubel et al., *infra*; Sambrook, *infra*. (Belagaje, R., et al., *J. Biol. Chem.* 254:5765-5780 (1979); Maniatis, T., et al., In: *Molecular Mechanisms in the Control of Gene Expression*, Nierlich, D.P., et al., eds., Acad. Press, NY (1976); Wu, R., et al., *Prog. Nucl. Acid Res. Molec. Biol.* 21:101-141 (1978); Khorana, R.G., *Science* 203:614-625 (1979)). Additionally, DNA synthesis may be achieved through the use of automated synthesizers. Techniques of DNA hybridization are disclosed by Sambrook et al. *supra*, and by Haymes, B.D., et al. (In: *DNA Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985)), which references are herein incorporated by reference. Techniques such as, or similar to, those described above have successfully enabled the cloning of genes for human aldehyde dehydrogenases (Hsu, L.C., et al., *Proc. Natl. Acad. Sci. USA* 82:3771-3775 (1985)), fibronectin (Suzuki, S., et al., *Eur. Mol. Biol. Organ. J.* 4:2519-2524 (1985)), the human estrogen receptor gene (Walter, P., et al., *Proc. Natl. Acad. Sci. USA* 82:7889-7893 (1985)), tissue-type plasminogen activator (Pennica, D., et al., *Nature* 301:214-221 (1983)) and human term placental alkaline phosphatase complementary DNA (Kam, W., et al., *Proc. Natl. Acad. Sci. USA* 82:8715-8719 (1985)).

In an alternative way of cloning an H13 gene, receptor mutant or homolog, a library of expression vectors is prepared by cloning DNA or, more preferably, cDNA (from a cell capable of expressing H13, a receptor mutant or homolog) into an expression vector. The library is then screened for members capable of expressing a protein which binds to anti-H13, anti-mutant or anti-homolog antibody, and which has a nucleotide sequence that is capable of encoding polypeptides that have the same amino acid sequence as H13, receptor mutant or homolog proteins or peptides, or fragments thereof.

A nucleic acid sequence encoding the H13, receptor mutant or homolog, polypeptide or protein of the present invention, or a functional derivative thereof, may be recombined with vector DNA in accordance with conventional techniques, including blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases and expressed in an appropriate host cell. Techniques for such manipulations are disclosed by Sambrook, J. et al., *supra*, and are well known in the art.

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene- encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold, L., et al. *Ann. Rev. Microbiol.* 35:365-404 (1981)).

Eukaryotic hosts include yeast, insects, fungi, and mammalian cells either *in vivo*, or in tissue culture. Mammalian cells provide post-translational modifications to protein molecules including correct folding or glycosylation at correct sites. Mammalian cells which may be useful as hosts include cells of fibroblast origin such as VERO or CHO, or cells of lymphoid origin, such as the hybridoma SP2/0-Ag14 or the murine myeloma P3-X63Ag8, and their derivatives. Preferred mammalian cells are cells which are intended to replace the function of the genetically deficient cells *in vivo*. Bone marrow stem cells are preferred for gene therapy of disorders of the hemopoietic or immune system.

For a mammalian cell host, many possible vector systems are available for the expression of a chimeric receptor polypeptide or chimeric receptor polypeptide. A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, Simian virus, or the like, where the regulatory signals are associated with a particular gene which

has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, protein production. When live insects are to be used, silk moth caterpillars and baculoviral vectors are presently preferred hosts for large scale a chimeric receptor polypeptide or chimeric receptor polypeptide production according to the invention. See, e.g., Ausubel, infra, Sambrook, supra.)

If so desired, the expressed a chimeric receptor polypeptide or chimeric receptor polypeptide may be isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis, or the like. For example, the cells may be collected by centrifugation, or with suitable buffers, lysed, and the protein isolated by column chromatography, for example, on DEAE-cellulose, phosphocellulose, polyribocytidylic acid-agarose, hydroxyapatite or by electrophoresis or immunoprecipitation. Alternatively, the chimeric receptor proteins may be isolated by the use of specific antibodies, such as an anti-receptor polypeptide antibody that still reacts with the protein containing ERR-derived amino acid substitutions. Such antibodies may be obtained by well-known methods.

Furthermore, manipulation of the genetic constructs of the present invention allow the grafting of a particular virus-binding domain onto the transmembrane and intracytoplasmic portions of a chimeric receptor polypeptide or chimeric receptor polypeptide, or grafting the retrovirus receptor binding domain of a chimeric receptor polypeptide or chimeric receptor polypeptide onto the transmembrane and intracytoplasmic portions of another molecule, resulting in yet another type of chimeric molecule.

Providing Chimeric Receptor Cells or Tissues

The present invention also relates to a method for rendering a human or other eukaryotic cell or tissue which is susceptible to binding by an *env* binding domain of a non-human virus is provided such as retroviral infection.

The method may optionally first comprise transforming, *in vitro, in vivo or in situ*, a eukaryotic cell or tissue with an expressible nucleic acid encoding a chimeric receptor polypeptide to produce a recombinant chimeric receptor cell or tissue which is capable of expressing a chimeric receptor polypeptide capable of binding an extracellular viral *env* binding domain of a non-specific virus.

Standard reference works setting forth the general principles of recombinant DNA technology include Watson, J.D. et al., *Molecular Biology of the Gene*, Volumes I and II, The Benjamin/Cummings Publishing Company, Inc., publisher, Menlo Park, CA (1987); Darnell, J.E. et al., *Molecular Cell Biology*, Scientific American Books, Inc., publisher, New York, N.Y. (1986); Lewin, B.M., *Genes II*, John Wiley & Sons, publishers, New York, N.Y. (1985); Old, R.W., et al., *Principles of Gene Manipulation: An Introduction to Genetic Engineering*, 2d edition, University of California Press, publisher, Berkeley, CA (1981); Sambrook, J. et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989); and Ausubel et al *Current Protocols in Molecular Biology*, Wiley Interscience, N.Y., (1987, 1993). These references are herein entirely incorporated by reference.

It is preferred in such a method that the *in vivo* transforming is carried out by one selected from: injection of the mutant nucleic acid into the tissue or cell; retroviral infection using a recombinant retrovirus comprising the mutant nucleic acid under control of at least one tissue specific regulatory sequence specific for the tissue or cell; liposome delivery of the mutant nucleic acid to the tissue or cell; antibody delivery of the mutant nucleic acid to the tissue or cell; or contacting a cell or tissue specific antibody conjugated to the mutant nucleic acid to the tissue or cell, according to known method steps.

It is also preferred in such a method that the *in situ* or *in vitro* transforming be carried out by one selected from: injection of the mutant nucleic acid into the tissue or

cell; retroviral infection using a recombinant retrovirus comprising the mutant nucleic acid in expressible form; liposome delivery of the mutant nucleic acid; antibody delivery of the mutant nucleic acid; transfection of the tissue or cell with the mutant nucleic acid; or contacting the cell or tissue specific antibody conjugated to the mutant nucleic acid to the tissue or cell, according to known methods steps. It is additionally preferred that the virus is a recombinant murine ecotropic or hamster amphotropic retrovirus and the chimeric receptor polypeptide is a chimeric receptor polypeptide as presented herein.

A chimeric receptor polypeptide of the present invention can be expressed on the cell surface as an integral membrane protein in a number of cell types, particularly cells of the T lymphocyte and monocyte/macrophage lineages, consistent with *in vitro* tropism of known human retroviruses such as HIV-1 and HTLV-1. Thus, a chimeric receptor polypeptide of the present invention will permit cells of these lineages in the human, which are normally resistant to non-human specific viral infection, to be infected with such viruses.

A major advantage of transfecting non-murine cells with a chimeric receptor polypeptide substituted receptor of the present invention, rather than with the ERR protein, is the major decrease in immunogenicity.

In another embodiment, the present invention includes a modified chimeric receptor cell or tissue produced by a herein-described method, wherein the eukaryotic cell or tissue is selected from, but not limited to mammalian, insect, bird or yeast origin. It is preferred that the mammalian cell or tissue is of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin, but any other mammalian cell may be used.

Transgenic and Chimeric Non-Human Mammals

The present invention is also directed to a transgenic non-human eukaryotic animal (preferably a rodent, such as a mouse) the germ cells and somatic cells of which

contain genomic DNA according to the present invention which codes for the a chimeric receptor polypeptide or chimeric receptor polypeptide capable as serving as a human retrovirus receptor. The a chimeric receptor polypeptide or chimeric receptor polypeptide nucleic acid is introduced into the animal to be made transgenic, or an ancestor of the animal, at an embryonic stage, preferably the one-cell, or fertilized oocyte, stage, and generally not later than about the 8-cell stage. The term "transgene," as used herein, means a gene which is incorporated into the genome of the animal and is expressed in the animal, resulting in the presence of protein in the transgenic animal.

There are several means by which such a gene can be introduced into the genome of the animal embryo so as to be chromosomally incorporated and expressed according to known methods.

Chimeric non-human mammals in which fewer than all of the somatic and germ cells contain the a chimeric receptor polypeptide or chimeric receptor polypeptide nucleic acid of the present invention, such as animals produced when fewer than all of the cells of the morula are transfected in the process of producing the transgenic mammal, are also intended to be within the scope of the present invention.

Chimeric non-human mammals having human cells or tissue engrafted therein are also encompassed by the present invention, which may be used for testing expression of chimeric receptor polypeptides in human tissue and/or for testing the effectiveness of therapeutic and/or diagnostic agents associated with delivery vectors which preferentially bind to a chimeric receptor polypeptide of the present invention. Methods for providing chimeric non-human mammals are provided, e.g, in U.S. serial Nos. 07/508,225, 07/518,748, 07/529,217, 07/562,746, 07/596,518, 07/574,748, 07/575,962, 07/207,273, 07/241,590 and 07/137,173, which are entirely incorporated herein by reference, for their description of how to engraft human cells or tissue into non-human mammals.

The techniques described in Leder, U.S. Patent 4,736,866 (hereby entirely incorporated by reference) for

producing transgenic non-human mammals may be used for the production of the transgenic non-human mammal of the present invention. The various techniques described in Palmiter, R. et al., *Ann. Rev. Genet.* 20:465-99 (1986), the entire contents of which are hereby incorporated by reference, may also be used.

The animals carrying a chimeric receptor polypeptide or chimeric receptor polypeptide gene can be used to test compounds or other treatment modalities which may prevent, suppress or cure a human retrovirus infection or a disease resulting from such infection for those retroviruses which infect the cells using the a chimeric receptor polypeptide or chimeric receptor polypeptide as a receptor. These tests can be extremely sensitive because of the ability to adjust the virus dose given to the transgenic animals of this invention. Such animals will also serve as a model for testing of diagnostic methods for the same human retrovirus diseases. Such diseases include, but are not limited to AIDS, HTLV-induced leukemia, and the like. Transgenic animals according to the present invention can also be used as a source of cells for cell culture.

The transgenic animal model of the present invention has numerous economic advantages over the "SCID mouse" model (McCune, J.M et al., *Science* 241:1632-1639 (1988)) wherein it is necessary to repopulate each individual mouse with the appropriate cells of the human immune system requiring a significantly greater amount of time and experimentation.

Chimeric receptor or H13 Specific Antibodies and Methods

This invention is also directed to an antibody specific for an epitope of a chimeric receptor polypeptide or chimeric receptor polypeptide. In additional embodiments, the antibody of the present invention is used to prevent or treat retrovirus infection, to detect the presence of, or measure the quantity or concentration of, a chimeric receptor polypeptide or chimeric receptor polypeptide in a cell, or in a cell or tissue extract, or a biological fluid. See, generally, Coligan, *supra*, and harlow, *infra*.

The term "antibody" is meant to include polyclonal antibodies, monoclonal antibodies (mAbs), chimeric antibodies, anti-idiotypic (anti-Id) antibodies, and fragments thereof, provided by any known method steps, such as by hybridomas, recombinant techniques or chemical synthesis.

An antibody is said to be "capable of binding" a molecule if it is capable of specifically reacting with the molecule to thereby bind the molecule to the antibody. The term "epitope" is meant to refer to that portion of any molecule capable of being bound by an antibody which can also be recognized by that antibody. Epitopes or "antigenic determinants" usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics.

An "antigen" is a molecule or a portion of a molecule capable of being bound by an antibody which is additionally capable of inducing an animal to produce antibody capable of binding to an epitope of that antigen. An antigen may have one, or more than one epitope. The specific reaction referred to above is meant to indicate that the antigen will react, in a highly selective manner, with its corresponding antibody and not with the multitude of other antibodies which may be evoked by other antigens.

Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen.

Monoclonal antibodies are a substantially homogeneous population of antibodies to specific antigens. MAbs may be obtained by methods known to those skilled in the art. See, for example Kohler and Milstein, *Nature* 256:495-497 (1975) and U.S. Patent No. 4,376,110, see, e.g., Ausubel et al. eds. *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley Interscience, N.Y., (1987, 1992); and Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory (1988); Coligan et al, eds, *Current Protocols in Immunology*, Greene Publishing Associates and Wiley Wiley Interscience, N.Y. (1992, 1993). Such

antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, and any subclass thereof. The hybridoma producing the mAbs of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* production makes this the presently preferred method of production. Briefly, cells from the individual hybridomas are injected intraperitoneally into pristane-primed Balb/c mice to produce ascites fluid containing high concentrations of the desired mAbs. MAbs of isotype IgM or IgG may be purified from such ascites fluids, or from culture supernatants, using column chromatography methods well known to those of skill in the art.

Chimeric antibodies are molecules different portions of which are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region. Chimeric antibodies and methods for their production are known in the art (see, for example, Morrison et al., *Proc. Natl. Acad. Sci. USA* 81:6851-6855 (1984); Neuberger et al., *Nature* 314:268-270 (1985); Sun et al., *Proc. Natl. Acad. Sci. USA* 84:214-218 (1987); Better et al., *Science* 240:1041- 1043 (1988); Better, M.D. International Patent Publication WO 9107494, which references are hereby entirely incorporated by reference).

An anti-idiotypic (anti-Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody can be prepared by immunizing an animal of the same species and genetic type (e.g. mouse strain) as the source of the mAb with the mAb to which an anti-Id is being prepared. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody).

The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. The anti-anti-Id may bear structural similarity to the original mAb which induced the anti-Id. Thus, by using antibodies to

the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

Accordingly, mAbs generated against a chimeric receptor polypeptide or chimeric receptor polypeptide of the present invention may be used to induce anti-Id antibodies in suitable animals, such as Balb/c mice. Spleen cells from such immunized mice are used to produce anti-Id hybridomas secreting anti-Id mAbs. Further, the anti-Id mAbs can be coupled to a carrier such as keyhole limpet hemocyanin (KLH) and used to immunize additional Balb/c mice. Sera from these mice will contain anti-anti-Id antibodies that have the binding properties of the original mAb specific for an a chimeric receptor polypeptide or chimeric receptor polypeptide epitope.

The anti-Id mAbs thus have their own idiotypic epitopes, or "idiotopes" structurally similar to the epitope being evaluated, such as an epitope of a chimeric receptor polypeptide or chimeric receptor polypeptide.

The term "antibody", as presented above, is also meant to include both intact molecules as well as fragments thereof, such as, for example, Fab and $F(ab')_2$, which are capable of binding antigen. Fab and $F(ab')_2$ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)).

Antibody Diagnostic Assays

It will be appreciated that Fab and $F(ab')_2$ and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of a chimeric receptor polypeptide or chimeric receptor polypeptide according to the methods disclosed herein for intact antibody molecules. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments).

The antibodies, or fragments of antibodies, of the present invention may be used to quantitatively or qualita-

tively detect the presence of cells which express a chimeric receptor polypeptide or chimeric receptor polypeptide on their surface or intracellularly. This can be accomplished by immunofluorescence techniques employing a fluorescently labeled antibody (see below) coupled with light microscopic, flow cytometric, or fluorometric detection.

The antibodies of the present invention may be employed histologically, as in immunofluorescence or immunoelectron microscopy, for *in situ* detection of a chimeric receptor polypeptide or chimeric receptor polypeptide. Through the use of such a procedure, it is possible to determine not only the presence of a chimeric receptor polypeptide or chimeric receptor polypeptide but also its distribution on the examined tissue.

Additionally, the antibody of the present invention can be used to detect the presence of soluble a chimeric receptor polypeptide or chimeric receptor polypeptides in a biological sample, such as a means to monitor the presence and quantity of a chimeric receptor polypeptide or chimeric receptor polypeptide used therapeutically.

Such immunoassays for a chimeric receptor polypeptide or chimeric receptor polypeptide typically comprise incubating a biological sample, such as a biological fluid, a tissue extract, freshly harvested cells such as lymphocytes or leukocytes, or cells which have been incubated in tissue culture, in the presence of a detectably labeled antibody capable of identifying H13 protein, and detecting the antibody by any of a number of techniques well-known in the art.

The biological sample may be treated with a solid phase support or carrier (which terms are used interchangeably herein) such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled a chimeric receptor polypeptide- or chimeric receptor polypeptide-specific antibody. The solid phase support may then be washed with the buffer a second time to remove unbound

antibody. The amount of bound label on said solid support may then be detected by conventional means.

By "solid phase support" or "carrier" is intended any support capable of binding antigen or antibodies. Well-known supports, or carriers, include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite.

The binding activity of a given lot of anti-chimeric receptor polypeptide or anti-chimeric receptor polypeptide antibody may be determined according to well-known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

One of the ways in which the chimeric receptor polypeptide- or chimeric receptor polypeptide-specific antibody can be detectably labeled is by linking the same to an enzyme and use in an enzyme immunoassay (EIA) according to known method steps.

Detection may be accomplished using any of a variety of other immunoassays, such as (*Laboratory Techniques and Biochemistry in Molecular Biology*, Work, et al., North Holland Publishing Company, New York (1978) with particular reference to the chapter entitled "An Introduction to Radioimmune Assay and Related Techniques" by T. Chard, incorporated entirely herein by reference.

It is also possible to label the antibody with a fluorescent compound.

The antibody can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the

course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

The antibody molecules of the present invention may be adapted for utilization in an immunometric assay, also known as a "two-site" or "sandwich" assay.

Typical, and preferred, immunometric assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to "extract" the antigen from the sample by formation of a binary solid phase antibody-antigen complex. After a suitable incubation period, the solid support is washed to remove the residue of the fluid sample, including unreacted antigen, if any, and then contacted with the solution containing an unknown quantity of labeled antibody (which functions as a "reporter molecule"). After a second incubation period to permit the labeled antibody to complex with the antigen bound to the solid support through the unlabeled antibody, the solid support is washed a second time to remove the unreacted labeled antibody.

In another type of "sandwich" assay, which may also be useful with the antigens of the present invention, the so-called "simultaneous" and "reverse" assays are used. A simultaneous assay involves a single incubation step as the antibody bound to the solid support and labeled antibody are both added to the sample being tested at the same time. After the incubation is completed, the solid support is washed to remove the residue of fluid sample and uncomplexed labeled antibody. The presence of labeled antibody associated with

the solid support is then determined as it would be in a conventional "forward" sandwich assay.

In the "reverse" assay, stepwise addition first of a solution of labeled antibody to the fluid sample followed by the addition of unlabeled antibody bound to a solid support after a suitable incubation period is utilized. After a second incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labeled antibody. The determination of labeled antibody associated with a solid support is then determined as in the "simultaneous" and "forward" assays.

Therapeutic and Diagnostic Methods Involving Chimeric receptor and/or H13 Polypeptides and/or Chimeric Cells or Tissue

Therapeutic methods are also provided according to the present invention wherein tissue or cells having at least one expressible chimeric receptor polypeptide encoding nucleic acid are subjected to infection by a recombinant non-human specific retroviral vector that recognizes only cells expressing, or bound on their surface, a chimeric receptor polypeptide, and the retroviral vector cannot infect other human cells, due to the non-human specificity of the vector and the relative lack of ability to revert, mutate or recombine to provide replication competence. According to the present invention, a chimeric receptor polypeptide can be selectively associated with a target cell or expressed on the target cell by allowing infection by a recombinant retrovirus having nucleic acid encoding the chimeric receptor polypeptide. This temporary and/or permanent association allows target cells, such as pathologic cells having a particular receptor, to selectively expose or express a chimeric receptor polypeptide, as well as progeny of such pathologic cells in the case of constitutively expressed chimeric receptor polypeptide, making possible specific delivery of therapeutic agents to such target cells and/or their progeny, according to the present invention.

Thus, according to the present invention, a procedure for marking specific types of target cells (and,

opotionally, with recombinant, chromosomal expression of a chimeric receptor polypeptide, their progeny) is provided, by temporarily or permanently associating a chimeric receptor polypeptide with a target cell specific cell surface molecule, such as cell surface receptor, and then administering a recombinant retrovius which binds the chimeric receptor polypeptide and infects the target cell. The infecting virus vector may carry any of a variety of therapeutic nucleic acids or therapeutic genes conferring one or more functions.

In one non-limiting example, a chimeric receptor polypeptide is specifically delivered to a target cell of a first species by association with a target cell specific vector, such as an antibody, liposome or target cell specific receptor ligand, such that the chimeric receptor polypeptide is associated with the target cell for a sufficient time to allow treatment or diagnosis using a therapeutic agent or diagnostic agent associated with the receptor binding or *env* domain of a non-first species specific virus which is capable of binding the chimeric receptor polypeptide. Thus, the therapeutic or diagnostic agent, such as a therapeutic or diagnostic nucleic acid, is delivered preferentially to the target cell, e.g., where the nucleic acid is incorporated into the chromosome of the target cell, to the exclusion of the non-target cells.

As another non-limiting example, the virus vector could carry into the cell a modified retrovirus receptor gene. Once the target cell is infected, the target cell has incorporated the chimeric receptor polypeptide encoding DNA, such that subsequently the target and cell and all progeny will express the chimeric receptor polypeptide on the target cell surface. By marking such target cells and their progeny with a chimeric receptor polypeptide according to the present invention, an animal subject suffering from the pathology can be treated using gene therapy, wherein the gene therapy vector specifically binds the chimeric receptor polypeptide and delivers a therapeutic or diagnostic agent to the target cell. Using such methods of the present invention, specific pathologies may be treated with or without substantially

reduced risk of non-specific retroviral vector infection and gene insertion in to the chromosome.

According to the present invention, a chimeric receptor polypeptide encoding nucleic acid may be combined with a coding sequence for a polypeptide that specifically binds a receptor specific for a particular type of target cell. The expression of such a nucleic acid in a recombinant host provides a fusion protein in recoverable amounts, suitable for therapeutic administration. The pharmaceutically acceptable fusion protein may then be administered to a subject having a pathology such that the fusion protein will specifically bind target cells and will act as an *env* binding domain for a non-human specific virus according to the present invention. The subsequent administration of a non-human specific virus, which has nucleic acid encoding any of a large variety of genes, may confer one or more functions on the infected cell and could have positive or negative therapeutic effects on that cell.

As a further non-limiting example, human tumors may be treated using a chimeric receptor polypeptide as a fusion protein to an antibody fragment of an antibody specific for a human tumor cell surface receptor. Such a fusion protein in pharmaceutically acceptable form may be administered to an animal model or human subject to mark tumor cells for infection by a non-human specific retrovirus which has DNA encoding a chimeric receptor polypeptide. Once the fusion protein has bound the tumor cells as the target cells, a second step may consist of administration of the retrovirus and infection of the tumor cell results in expression of the chimeric receptor polypeptide by subsall of the infected tumor cells, as well as their progeny. Once the tumor cells constitutively express a chimeric receptor polypeptide, then gene therapy can be safely used to deliver a therapeutic agent to the tumor cells as the target cells, substantially without infection into non-target cells. However, it is not necessary that the cell express the receptor via step 2. In fact step 2 can be dispensed with altogether as determined by one skilled in the art.

As a non-limiting sub-example, B3 antibody fragments specific for human tumor cells may be used to provide a fusion protein and gene therapy, wherein a specific pathologic cell specific antibody or binding protein is first expressed as fusion protein, shown to: (a) bind the pathologic cells and allow infection by a non-human specific virus having nucleic acid encoding a chimeric receptor polypeptide *in vitro* and *in vivo*, such that the chimeric receptor polypeptide is expressed on the surface of the virus infected pathologic cell, (b) *in vitro* and *in vivo* killing of the virus infected target cell by at least one therapeutic agent associated with an *env* binding domain as the delivery vector that binds the chimeric receptor polypeptide. Animal model systems may be preferably used before clinical treatment of humans, according to known methods steps.

Selective introduction of a chimeric receptor polypeptide encoding nucleic acid into animal or human cells or tissues, which are to be infected by the recombinant non-human specific virus, such as an ecotropic or amphotropic virus, may be accomplished according to known method steps. Non-limiting examples include *in vitro* transfection of human cells or tissue, such as bone marrow cells (as stem cells or stromal cells), white blood cells, and differentiated or undifferentiated granulocytes, monocytes, macrophages, lymphocytes, erythrocytes, megakaryocytes, cells of the central nervous system, and tissue cells, such as nerve tissue, liver cells, kidney cells, muscle cells, heart cells or myocardial cells, atrial or venus cells or tissue, eye cells, connective tissue or cells, lung tissue or cells, spleen cells or tissue, endocrine tissue or cells, CSF, or cells of the central nervous system, with nucleic acid encoding a chimeric receptor polypeptide, followed by reintroduction into the human subject; or by direct injection of a nucleic acid encoding the chimeric receptor polypeptides into the tissue *in vivo* or *in situ*, such as muscle, heart, liver, kidney, brain, nerve, spleen, pancreas, testes, ovary, pituitary, hypothalamus, gall bladder, eyes, lung, or bone marrow.

Thus, in one aspect of the present invention, a method is provided for transferring at least one therapeutic agent or diagnostic agent to a chimeric receptor bearing cell or tissue. The method comprises providing a chimeric receptor cell or tissue according to the present invention and contacting the chimeric receptor cell or tissue, *in vitro*, *in vivo* or *in situ*, with a delivery vector comprising an *env* binding domain of a non-human virus and at least one therapeutic or diagnostic agent, such that the delivery vector binds the chimeric modified cell and the therapeutic or diagnostic agent has a therapeutic or diagnostic effect on the chimeric receptor cell.

A therapeutic agent or diagnostic agent is selectively delivered according to a method of the present invention to a target cell as a chimeric receptor cell or tissue by a delivery vector which comprises an *env* binding domain of a virus envelope protein which is specific for the chimeric receptor of the chimeric receptor polypeptide expressed extracellularly on the chimeric receptor cell.

Delivery Vectors

The delivery vector may be, but is not limited to, a viral vector, a liposome, or a conjugate of the *env* binding domain associated with diagnostic or therapeutic agent.

The delivery vector may further comprise any diagnostic or therapeutic agent which has a therapeutic or diagnostic effect on the chimeric receptor cell as the target cell for the delivery vector.

Diagnostic or Therapeutic Agent

The diagnostic or therapeutic agent may be, but is not limited to, at least one selected from a nucleic acid, a compound, a protein, an element, a lipid, an antibody, a saccharide, an isotope, a carbohydrate, an imaging agent, a lipoprotein, a glycoprotein, an enzyme, a detectable probe, and antibody or fragment thereof, or any combination thereof, which may be detectably labeled as for labeling antibodies, as described herein. Such labels include, but are not limited to, enzymatic labels, radioisotope or radioactive compounds or elements, fluorescent compounds or metals, chemiluminescent

compounds and bioluminescent compounds. Alternatively, any other known diagnostic or therapeutic agent can be used in a method of the present invention.

Target Cells for Therapeutic Agents

A therapeutic agent used in the present invention may have a therapeutic effect on the target cell as a chimeric receptor cell, the effect selected from, but not limited to: correcting a defective gene or protein, a drug action, a toxic effect, a growth stimulating effect, a growth inhibiting effect, a metabolic effect, a catabolic affect, an anabolic effect, an antiviral effect, an antibacterial effect, a hormonal effect, a neurohumoral effect, a cell differentiation stimulatory effect, a cell differentiation inhibitory effect, a neuromodulatory effect, an antineoplastic effect, an anti-tumor effect, an insulin stimulating or inhibiting effect, a bone marrow stimulating effect, a pluripotent stem cell stimulating effect, an immune system stimulating effect, and any other known therapeutic effects that may be provided by a therapeutic agent delivered to a chimeric receptor cell via a delivery vector according to the present invention.

Therapeutic Nucleic Acids

A therapeutic nucleic acid as a therapeutic agent may have, but is not limited to, at least one of the following therapeutic effects on a chimeric receptor cell: inhibiting transcription of a DNA sequence; inhibiting translation of an RNA sequence; inhibiting reverse transcription of an RNA or DNA sequence; inhibiting a post-translational modification of a protein; inducing transcription of a DNA sequence; inducing translation of an RNA sequence; inducing reverse transcription of an RNA or DNA sequence; inducing a post-translational modification of a protein; transcription of the nucleic acid as an RNA; translation of the nucleic acid as a protein or enzyme; and incorporating the nucleic acid into a chromosome of a chimeric receptor cell for constitutive or transient expression of the therapeutic nucleic acid.

Therapeutic Effects

Such therapeutic effects may include, but are not limited to: turning off a defective gene or processing the expression thereof, such as antisense RNA or DNA; inhibiting viral replication or synthesis; gene therapy as expressing a heterologous nucleic acid encoding a therapeutic protein or correcting a defective protein; modifying a defective or underexpression of an RNA such as an hnRNA, an mRNA, a tRNA, or an rRNA; encoding a toxin in pathological cells; encoding a drug or prodrug, or an enzyme that generates a compound as a drug or prodrug in pathological or normal cells expressing the chimeric receptor; encoding a thymidine kinase varicella-zoster virus thymidine kinase (VZV TK) (see, e.g., Huber et al *Proc. Nat'l Acad. Sci. USA* 88:8039-8042 (1992), the entire contents, including the cited references, are entirely incorporated by reference) in pathogenic cells, such as neoplastic cells to directly or indirectly kill such pathogenic cells; and any other known therapeutic effects. Mutant nucleic acids such as those described above may thus be used in gene therapy.

A therapeutic nucleic acid of the present invention which encodes, or provides the therapeutic effect any known toxin, prodrug or drug gene for delivery to pathogenic cells may also include genes under the control of a tissue specific transcriptional regulatory sequence (TRSs) specific for pathogenic cells, such as neoplastic cells, including α-fetoprotein TRS or liver-associated albumin TRS (see, e.g., Dynan and Tjian *Nature* (London) 316:774-778 (1985)). Such TRSs would further limit the expression of the cell killing toxin, drug or prodrug in the target cell as a cancer cell expressing a chimeric receptor polypeptide of the present invention.

A further example of a therapeutic nucleic acid of the present invention which is delivered and expressed in a chimeric receptor cell, is a therapeutic nucleic acid encoding a thymidine kinase which selectively kills eukaryotic dividing cells, such as brain tumor cells which brain cells surrounding the tumor cells are not dividing. Accordingly, a brain tumor could be injected, transfected or viral vector transformed *in*

vivo with a chimeric receptor polypeptide encoding nucleic acid of the present invention, followed by therapeutic treatment of the chimeric receptor brain tumor cells with a recombinant ecotropic retrovirus encoding a thymidine kinase, such that the brain tumor cells would be selectively killed by expression of the thymidine kinase. See, e.g., F. Anderson et al, *Science*, June, 1992.

Alternatively, an abnormal H13 molecule which results in enhanced susceptibility to disease, may be replaced by infusion of cells of the desired lineage (such as hemopoietic stem cells, for example) transfected with a chimeric receptor polypeptide, such as a mutant H13 protein, under conditions such that the infused cells will preferentially replace the endogenous cell population.

In a method of the present invention, it is also preferred that the delivery vector is a recombinant, non-human specific virus, such that binding of the non-human specific virus to the chimeric receptor cell or tissue results in infection of the modified receptor cell and the therapeutic effect of the therapeutic nucleic acid in the chimeric receptor cell. In another preferred embodiment, the delivery vector includes a complex fusion protein, or nucleic acid encoding therefor, comprising a non-human specific *env* binding domain bound by a linker to a therapeutic or diagnostic agent, such that the binding or contacting results in a desired therapeutic or diagnostic effect. In still another preferred embodiment, the delivery vector may further comprise a liposome, the liposome containing the *env* binding domain and the therapeutic agent, such that the *env* binding domain is capable of binding the chimeric receptor binding site of a chimeric receptor cell.

In another preferred embodiment, the contacting of the delivery vector to the chimeric receptor cell or tissue may result in the chimeric receptor cell or tissue expressing a therapeutically effective amount of the expression product of a therapeutic nucleic acid.

In another preferred embodiment, the therapeutic nucleic acid encodes a toxin which acts to selectively kill the chimeric receptor containing cell or tissue. The pathologic cell may be a cancer cell. In another embodiment, the therapeutic nucleic acid may further encode a growth factor selected from epidermal growth factor, interleukin-2, interleukin-4, interleukin-6, tissue growth factor-α, insulin growth factor-1 or fibroblast growth factor.

The toxin may be a purified or recombinant toxin or toxin fragment comprising at least one functional cytotoxic domain of toxin, e.g., selected from at least one of ricin, *Pseudomonas* exotoxin, diphtheria toxin, endotoxin, a venom toxin, and the like.

The therapeutic nucleic acid may encode at least one member selected from a single chain ribosome inhibitory protein acting to block expression of an abnormal protein in the chimeric receptor cell or tissue; a cytokine; or a growth factor.

According to another aspect of the present invention, a cytotoxic or a chemotherapeutic agent may be attached directly to a delivery vector having an env binding domain or to an antibody or fragment, or a growth factor, that preferentially binds pathologic cells as target chimeric receptor cells. The targets for this type of therapy can also be growth factor receptors, differentiation antigens, or other less characterized cell surface antigens specifically associated with other pathologic cells. It is now established that many cancers overproduce growth factor receptors which can function as oncogenes or in an autocrine way to promote the growth of the cancer cells (Pastan and Fitzgerald, 1991; Velu et al, 1987; Kawano et al, 1988; Hellstrom & Hellstrom, 1989). For example, the epidermal growth factor receptor is present in large amounts (up to 3 x 10^6 receptors per cell) in many squamous cell and epidermoid carcinomas, glioblastomas, and some metastatic ovarian and bladder cancer (Hender et al, 1984; Jones et al, 1990; Lau et al, 1988). By contrast, normal cells can contain a magnitude less receptors per cell (Dunn et al, 1986). In another example the interleukin-2 (IL-

2) receptor is present in substantially higher numbers on the cells of patients with adult T cell leukemia (ATL; 3 x 10^4 receptors per cell) than in normal T cells.

Other differentiation antigens that occur on normal cells such as B lymphocytes are often also present on tumor cell such as B cell lymphomas. Because such antigens are not present on the stem cells that produce B cells, any mature B cells that are killed by targeted therapy will be replaced from the stem cell population from the stem cell population, whereas the cancer cells will not be replaced (Ghetie et al, 1988). Finally, there are antigens preferentially expressed on cancer cells whose functions are not yet understood. Some of these, such as carcinoembryonic antigens (CEA) (Muraro et al, 1985), are fetal antigens, which are either not present or only present in small amounts on normal adult tissues. This group also contains antigens of unknown origin that are only defined by their reactivity with a monoclonal antibody (Fraenkel et al, 1985; Varki et al, 1984; Willingham et al, 1987).

Single-chain antigen-binding proteins which may be used as components of therapeutic or diagnostic delivery vectors of the present invention have numerous advantages in clinical applications because of their small size. These proteins are cleared from serum faster than monoclonal antibodies or Fab fragments. Because they lack the Fc portion of an antibody, which is recognized by cell receptors, they have a lower background for use in imaging applications and they are less immunogenic. They are also expected to penetrate the microcirculation surrounding solid tumors better than monoclonal antibodies.

In such a therapeutic delivery vector, the therapeutic agent is a toxin or toxin fragment or domain; such as a purified or recombinant toxin or toxin fragment comprising at least one functional cytotoxic domain of toxin selected from at least one of ricin, *Pseudomonas* exotoxin, diphtheria toxin, thymidine kinase, and the like. The use of recombinant toxins, e.g., for cancer treatment is known in the art (see, e.g., Pastan and Fitzgerald *Science*, November 22,

1991, pages 1173-1177, and the articles cited therein, which references are herein entirely incorporated by reference).

Obtaining a safer vector for *in vivo* gene therapy.

The use of three different tools can be used to increase the margin of safety *in vivo* gene therapy treatments is provided according to the present invention. These tools are: (a) the use of different packaging cell lines; (b) the use of ecotropic, rather than amphotropic-based vectors, and (c) providing means to target retroviral vectors to the desired cell population *in vivo*.

(a) Use of different packaging cell lines.

Background. Concerns with the safety of exiting amphotropic-virus based vectors has provided an incentive to discover a novel system for the production of high titered ecotropic retrovirus vectors. The strategy relies on the fact that human cells cannot be infected by hamster leukemia virus. (Stenback et al., *Proc. Soc. Exp. Biol. Med.* 122:1219-1223, 1966). Hamster leukemia virus has been reported (Lieber et al., *Science* 182:56-58 91973) to infect only hamster cells, as attempts to infect mouse, rat, cat, monkey and human cells have failed. Replicating murine ecotropic viruses in hamster cells can also be used. This has been achieved by transfecting the murine, or modified human ecotropic receptors into these cells, followed by infection. This strategy takes further advantage of the natural resistance of these cells to replicate amphotropic viruses, and achieve higher titers of ecotropic expression by the repeated amplification of murine ecotropic viral sequences in these cells.

Specific methods involved.

The so called "ping-pong" strategy of Bestwick et al. (*Proc. Natl. Acad. Sci. USA*, 85:5404-5408, 1988), is substantially modified, where two retrovirus are used, neither of which can normally replicate in human cells. A chinese hamster cell expressing Ψ-HaLV; and stably expressing the murine ecotropic virus receptor (cell line A) is used to provide helper virus, and co-cultivated with a second chinese hamster cell expressing Ψ- ecotropic MuLV (cell line B), but not expressing the murine ecotropic virus receptor. In this

manner, virus propagated in cell line A is able to infect cell line B through the HaLV receptor. Virus replicated in this cell now has the murine ecotropic gp70, and is able to infect cell line A. This process is expected to continue until a theoretical maximum number of particle production is achieved (about 10^9 to 10^{10} PFU) (Bodine et al. *Proc. Natl. Acad. Sci. USA* 87:3738-3742, 1990). As discussed herein, it has been shown that amplification of retroviral sequences in mixed packaging line co-cultures is associated with an increased copy number over time in tissue culture (Hesorffer et al. *Hematology/Oncology Clinic of North America* 5:423-432, 1991). Further, this approach is preferred because it appears that retroviral vector DNA is expressed relatively poorly when it is transfected into cells compared to the levels obtained after proviral integration (Bestwick et al., *Proc. Natl. Acad. Sci USA* 85:5404-5408, 1988; Huang and Gilboa *J. Virol* 50:417-424, 1984).

Following this approach, a chinese hamster cell line is provided containing multiple copies of ecotropic virus sequences integrated into genome, and producing at least about 10^7-10^{10}, preferably about 10^9 to 10^{10}, with no particles, recombinant or otherwise, capable of infecting human cells, unless their virus receptors are modified according to the present invention. To insure that this result is obtained, appropriate viral infectivity assays are performed with a variety of human and murine cells lines, normally used to detect viral infectivity. This approach is expected to yield a far safer packaging line than previously available.

HaLV is cloned and a deletion in its packaging signal is produced. The removal of the packaging signal is obtainable by known method steps. Next, transfection and stable expression of the murine ecotropic virus receptor into a CHO hamster cell is provided according to known method steps, e.g., as described in Yoshimoto et al. (1993). Optionally, a cloned helper virus is introduced into these cells as split genomes for added safety. In the case of the murine ecotropic virus helper, plasmids p*gag-pol*gpt and p*env*, derived from the 3PO plasmid representing the ecotropic

Moloney murine leukemia virus with a 134 base pair deletion of the Ψ packaging sequences, are readily obtained from commercial sources or published investigators as presented herein. Such plasmids have been used successfully used to generate PCLs with somewhat greater safety than those using unsplit helper virus genomes.

To clone HaLV, known procedures are used, e.g., the procedure of Anderson et al. (1991), similar to cloning defective retrovirus particles from a recombinant Chinese Hamster ovary cell line. For example, such clones include the pCHOC.ML10 sequence identified by Anderson or endogenous, polytropic murine leukemia virus (MuLV) isolate, MX27 (Stoye and Coffin, 1987). The later probe consists of a 12.3 kb mouse genomic fragment encompassing a complete 9.3 kb provirus genome. Hamster C-type related sequences are isolated from a randomly primed cDNA library of particle RNA in lgt10. If the later probe is used, low stringency hybridization is used to identify plaques of interest. Extracellular particles are prepared from culture fluid recombinant CHO cell subclone, 3-3000-44 (Lasky et al., 1986). This subclone was derived from a dihydrofolate reductase (dhrf)-deficient CHO-DuxB11 cells (Simonsen and Levinson, 1983; Urlab and Chasin, 1980) following transfection with an expression vector containing the genes for murine dhfr and recombinant envelope glycoprotein (gp120) of human immunodeficiency virus type 1 (HIV-1). The CHO-K1 cell line (progenitor of CHO-DUXB11 line) was originally derived from an ovarian biopsy of an adult Chinese Hamster (Puck et al., 1958) and is readily available from commercial sources such as the American Type Culture Collection (ATCC). Alternatively, repair of the hamster sequence with equivalent portion of the murine ecotropic virus is used. As observed by Anderson et al., virus particles are expected to be produced. Since the pCHOC.ML10 sequence is said to contain multiple interruptions of potential coding sequences in all three reading frames of the endonuclease gene, if the clone does not encode an intact endonuclease, the endonuclease region can be replaced with that of a homologous retrovirus genome, such as from ecotropic MuLV. The HaLV

surface envelope proteins is appropriately expressed so that the particles can infect other hamster cells; all other HaLV genes are not essential (LTRs possibly excluded), and replaceable by homologous MuLV sequences. To delete the Ψ region of the clone HaLV, the methodology of Mann et al. is used Cell 33:153-159, 1983).

(b) Use of a Chimeric Receptor Polypeptide.

As a non-limiting example, a modified ecotropic retroviral receptor (MERR) for gene delivery should lower the potential incidence of cancer and related diseases during gene therapy.

There are several advantages to methods of the present invention. First, any recombinant viruses that my arise will not be able to infect human cells, as murine ecotropic viruses cannot replicate in these cells. Furthermore, the gene therapist will be able to limit with exquisite specificity the infection only to those target cells desired to be infected. Potential random insertions of viruses all over the human genome in various types of organs will not be expected or shown to occur, as would be expected whenever human infectable amphotropic-retroviruses-based vectors are used. In addition, because methods of the present invention use a human viral receptor protein which is minimally modified, the possibility of rejection of the infected human cells by the immune system is substantially reduced or eliminated.

(c) Modes of Targeting Vectors

Background: A preferred aspect of the present invention, particularly for in vivo methods, is the method of treating an animal using a delivery vector which permits specific targeting of cells to be infected by recombinant, non-human specific ecotropic viruses which provide a therapeutic effect on a target cell. Non-limiting examples of such an agent include a fusion protein encompassing the V_H and V_L regions of a specific antibody to a cell surface molecule (such as an MHC Class 1 antigen) joined with an appropriate linker peptide and the mouse ecotropic virus receptor or the modified human ecotropic receptor. Alternatively a ligand for a membrane

receptor (such as the epidermal growth factor receptor) fused to the mouse ecotropic virus receptor or the modified human virus ecotropic receptor. The design will be flexible enough so that its specificity can be modified with relative ease.

Pharmaceutical Compositions: Pharmaceutical compositions comprising the proteins, peptides or antibodies of the present invention, such as at least one chimeric receptor polypeptide or antibody include all compositions wherein at least one therapeutic agent is contained in an amount effective to achieve its intended purpose. In addition, pharmaceutical compositions containing at least one therapeutic agent may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically.

Pharmaceutical compositions include suitable solutions for administration by injection or orally, and contain from about 0.001 to 99 percent, preferably from about 20 to 75 percent of active component (i.e., the therapeutic together with the excipient. Pharmaceutical compositions for oral administration include tablets and capsules. Compositions which can be administered rectally include suppositories.

Therapeutic Carriers: The carrier for the active ingredient may be either in sprayable or nonsprayable form. Non-sprayable forms can be semi-solid or solid forms comprising a carrier conducive to topical application and having a dynamic viscosity preferably greater than that of water. Suitable formulations include, but are not limited to, solution, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like. If desired, these may be sterilized or mixed with auxiliary agents, e.g., preservatives, stabilizers, wetting agents, buffers, or salts for influencing osmotic pressure and the like. Preferred vehicles for non-sprayable topical preparations include ointment bases, e.g., polyethylene glycol-1000 (PEG-1000); conventional creams such as HEB cream; gels; as well as petroleum jelly and the like.

Also suitable for systemic or topical application, in particular to the mucus membranes and lungs, are sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier material. The aerosol preparations can contain solvents, buffers, surfactants, perfumes, and/or antioxidants in addition to the proteins or peptides of the present invention. For aerosol administration, the therapeutic agents in accordance with the present invention may be packaged in a squeeze bottle, or in a pressurized container with an appropriate system of valves and actuators. Preferably, metered valves are used with the valve chamber being recharged between actuation or dose, all as is well known in the art.

Therapeutic Administration: A therapeutic agent of the present invention may be administered by any means that achieve its intended purpose, for example, to treat local infection or to treat systemic infection in a subject who has, or is susceptible to, such infection. For example, an immunosuppressed individual is particularly susceptible to retroviral infection and disease.

For example, administration may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, intracranial, transdermal, or buccal routes. Alternatively, or concurrently, administration may be by the oral route. Parenteral administration can be by bolus injection or by gradual perfusion over time.

An additional mode of using of a therapeutic agent of the present invention is by topical application. A therapeutic agent of the present invention may be incorporated into topically applied vehicles such as salves or ointments, which have both a soothing effect on the skin as well as a means for administering the active ingredient directly to the affected area.

For topical applications, it is preferred to administer an effective amount of a therapeutic agent according to the present invention to an infected area, e.g., skin surfaces, mucous membranes, etc. This amount will

generally range from about 0.0001 mg to about 1 g per application, depending upon the area to be treated, whether the use is prophylactic or therapeutic, the severity of the symptoms, and the nature of the topical vehicle employed. A preferred topical preparation is an ointment wherein about 0.001 to about 50 mg of active ingredient is used per cc of ointment base, the latter being preferably PEG-1000.

A typical regimen for treatment or prophylaxis comprises administration of an effective amount over a period of one or several days, up to and including between one week and about six months.

It is understood that the dosage of a therapeutic agent of the present invention administered *in vivo* or *in vitro* will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired. The ranges of effective doses provided below are not intended to be limiting and represent preferred dose ranges. However, the most preferred dosage will be tailored to the individual subject, as is understood and determinable by one of skill in the art.

The total dose required for each treatment may be administered by multiple doses or in a single dose. The therapeutic agent may be administered alone or in conjunction with other therapeutics directed to the viral infection, or directed to other symptoms of the viral disease.

Effective amounts of a therapeutic agent of the present invention are from about 0.001 μg to about 100 mg/kg body weight, and preferably from about 1 μg to about 50 mg/kg body weight.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions, which may contain auxiliary agents or excipients which are known in the art. Pharmaceutical compositions such as tablets and capsules can also be prepared according to routine methods.

Diagnostic Assays: The present invention provides methods for evaluating the presence and the level of normal or chimeric

receptor or H13 protein or mRNA in a subject. Absence, or more typically, low expression of the H13 gene or presence of a mutant H13 in an individual may serve as an important predictor of resistance to retrovirus infection and thus to the development of AIDS or certain types of leukemia or other retrovirus-mediated diseases. Alternatively, over-expression of H13, may serve as an important predictor of enhanced susceptibility to retrovirus infection.

In addition, ERR or H13 mRNA expression is increased in virally-induced tumor cell lines, indicating that the level of mRNA or receptor protein expression may serve as a useful indicator of a viral infection not otherwise detectable. Therefore, by providing a means to measure the quantity of H13 mRNA (see below) or protein (using an immunoassay as described above), the present invention provides a means for detecting a human retrovirus-infected or retrovirus-transformed cell in a subject.

Oligonucleotide probes encoding various portions of a chimeric receptor polypeptide or chimeric receptor polypeptide encoding nucleic acid sequence are used to test cells from a subject for the presence a chimeric receptor polypeptide or chimeric receptor polypeptide DNA or mRNA. A preferred probe would be one directed to the nucleic acid sequence encoding at least 12 and preferably at least 15 nucleotide of a chimeric receptor polypeptide or chimeric receptor polypeptide sequence. Qualitative or quantitative assays can be performed using such probes. For example, Northern analysis (see below) is used to measure expression of a chimeric receptor polypeptide or chimeric receptor polypeptide mRNA in a cell or tissue preparation.

Such methods can be used even with very small amounts of nucleic acid obtained from an individual, for knowing uses of selective amplification techniques. Recombinant nucleic acid methodologies capable of amplifying purified nucleic acid fragments have long been recognized. Typically, such methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of the amplified

nucleic acid fragment. Examples of such methodologies are provided by Cohen et al. (U.S. Patent 4,237,224), Sambrook et al., *supra*, etc.

Recently, an *in vitro* enzymatic method has been described which is capable of increasing the concentration of such desired nucleic acid molecules. This method has been referred to as the "polymerase chain reaction" or "PCR" (Mullis, K. et al., *Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986); Erlich H. et al., EP 50,424; EP 84,796, EP 258,017, EP 237,362; Mullis, K., EP 201,184; Mullis K. et al., US 4,683,202; Erlich, H., US 4,582,788; and Saiki, R. et al., US 4,683,194), which are all entirely incorporated herein by reference.

The polymerase chain reaction provides a method for selectively increasing the concentration of a particular DNA sequence even when that sequence has not been previously purified and is present only in a single copy in a particular sample. The method can be used to amplify either single- or double-stranded DNA. The essence of the method involves the use of two oligonucleotide probes to serve as primers for the template-dependent, polymerase mediated replication of a desired DNA molecule.

Reviews of the PCR are provided by Mullis, K.B. (*Cold Spring Harbor Symp. Quant. Biol.* 51:263-273 (1986)); Saiki, R.K., et al. (*Bio/Technology* 3:1008-1012 (1985)); and Mullis, K.B., et al. (*Meth. Enzymol.* 155:335-350 (1987)).

Having now generally described the invention, the same will be more readily understood through reference to the following example which is provided by way of illustration, and is not intended to be limiting of the present invention.

EXAMPLE I

General Materials and Methods

Cell Lines

The following cell lines were used in the studies described below: CCL120 (ATCC# CCL120), a human B lymphoblastoid cell line; CCL119 (CEM, ATCC# CCL119), a human T lymphoblastoid cell line; SupT1, a human non-Hodgkin's T lymphoma cell line; H9, a single cell clone derived from HUT78,a human cutaneous T cell lymphoma cell line; MOLT4 (ATCC# CRL1582), a human acute lymphoblastic leukemia cell line; HOS (ATCC# CRL1543), a human osteosarcoma cell line; HeLa (ATCC# CCL2), a human epithelioid carcinoma cell line; CHO-K1 (ATCC #61), a Chinese hamster ovary cell line; B10T6R, a radiation-induced thymoma of B10.T(6R) mice; and RL12, a radiation-induced thymoma of C57BL/6Ka mice.

Screening

Human CEM and HUT 78 T-cell cDNA library (lambda gt11) was obtained from Clontech Laboratories Inc. (Palo Alto, California). The human lymphocyte cosmid library (pWE15) was obtained from Stratagene (LaJolla, CA). The libraries were screened by the method of Maniatis et al. (Maniatis, T. et al. *Cell* 15:887-701 (1978)). The BamH1-EcoRI fragment, containing the entire open reading frame of ERR cDNA (pJET) was provided by Drs. Albritton and Cunningham (Harvard Medical School, Boston, MA). This DNA was labelled with ^{32}P by nick translation to a specific activity of about 2 x 10^6 cpm/μg and used as a hybridization probe.

Southern Blot Analysis

High relative mass DNA was prepared from cells as described by Blin, N. et al. (*Nucl. Acids Res.* 3:2303-2308 (1976)) and modified by Pampeno and Meruelo (Pampeno, C. L. et al. *J. Virol.* 58:296-306 (1986)). Restriction endonuclease digestion, agarose gel electrophoresis, transfer to nitrocellulose (Schleicher & Schuell, lnc., Keene, New Hampshire), hybridization and washing was as described (Pampeno, C. L. et al. *supra*; Brown, G. D. et al. *Immunogenetics* 27:239-251 (1988)).

Northern Blot Analysis

Total cellular RNA was isolated from cells by the acid guanidinium thiocyanate-phenol-chloroform method (Chomczynski, P. et al. *Anal. Biochem.* 162:156-159 (1987)). The DNA was electrophoresed in 1% formaldehyde agarose gels and transferred to Nytran filters (Schleicher & Schuell, Inc., Keene, New Hampshire). The hybridization and washing was performed according to Amari, N. M. B. et al. (*Mol. Cell. Biol.* 7:4159-4168 (1987)).

DNA Sequence Analysis

cDNA clones from positive phages were recloned into the EcoRI site of plasmid vector pBluescript (Stratagene). Unidirectional deletions of the plasmids were constructed by using exonuclease III and S1 nuclease, and sequenced by the dideoxy chain termination methods (Sanger, F. S. et al. *Proc. Natl. Acad. Sci. USA* 74:5463- 5467 (1977)) with Sequenase reagents (U.S. Biochemical Corp., Cleveland, Ohio). Restriction maps of positive cosmid inserts were determined using T3 or T7 promoter-specific oligonucleotides to probe partially digested cosmid DNA as described elsewhere (Evans, G.A. et al., *Meth. Enzymol.* 152:604-610 (1987)). EcoRI-EcoRI or EcoRI-HindIII fragments in the cosmids were subcloned into pBluescript or pSport 1 (GIBCO BRL, Gaithersburg, MD). The exons and exon-intron junctions were sequenced using synthetic oligonucleotides as primers. Sequences were compiled and analyzed using the Genetics computer group sequence analysis software package (Devereux, J. et al., *Nucl. Acids Res.* 12:387-395 (1984)).

EXAMPLE II

DNA and Predicted Protein Sequence of H13

The complete nucleotide sequence of H13 (SEQ ID NO:7) including non-coding sequences at the 5' and 3' end of the coding sequence are shown in Figure 1. This sequence includes the partial sequence originally obtained from clone 7-2 (SEQ ID NO:1); nucleotide 1-6 and 1099-1102 of SEQ ID NO:1 were originally incorrectly determined. Figure 1 also shows the complete amino acid sequence predicted from the nucleotide sequence (SEQ ID NO:8). This sequence includes the originally

described partial amino acid sequence (SEQ ID NO:2) with the exception of the N-terminal Pro-Gly and the C-terminal Pro, which were originally incorrectly predicted from the nucleotide sequence.

The nucleotide sequence comparison between H13, and ERR is shown in Figure 2 and the amino acid sequence comparison of H13, ERR and TEA is shown in Figure 3.

The homology between the compared sequences is very high, for example 87.6% homology between H13 and ERR DNA, and 52.3% homology between H13 and TEA amino acids.

EXAMPLE III

Presence and Expression of the H13 Gene in Human Cells

By Southern analysis of DNA taken from cells of various species, it was shown that DNA capable of hybridizing with a murine ERR cDNA probe (Figure 4) and with the H13 cDNA (Figure 5) was present in cells of 5 human cell lines, including CCL120, CCL119, SupT1, H-9 and MOLT-4, and also in hamster cells (CHO-K1) and murine cells (normal Balb/c mouse thymocytes). H13 gene expression was examined using Northern analysis, using the H13 cDNA probe. The probe detected a transcript of approximately 9kb in RNA from HeLa, SupT1, HOS and CCL119 cells (Figure 6). This RNA could also be detected using a murine ERR cDNA probe (Figure 7).

EXAMPLE IV

Transfection of Murine Retroviral Receptor cDNA into Hamster Cells

Murine retroviral receptor (ERR) cDNA was cotransfected into hamster CHO cells, which can not be infected by murine ecotropic retroviruses, with the selectable marker plasmid DNAP, pSV_2Neo, using calcium phosphate (Wigler, M. et al., *Cell* 14: 725-731 (1978)). The transfectant expressing the receptor gene was, then, infected by murine radiation leukemia virus (RadLV). Two weeks later after the infection the reverse transcriptase (RT) activity of the supernatant was measured (Stephenson, J.R. et al., *Virology*

48: 749-756 (1972)), and Northern Blot analysis was performed using a viral probe after preparing its RNA.

As shown in Figure 8, the RT activity detected in untransfected CHO cells which do not express the receptor gene was indistinguishable from the activity of tissue culture medium (background). This indicates that the cells were not infected by MuLV.

Following transfection with the ERR cDNA, the RT activity of the transfected cell supernatant was much higher than background (Figure 8).

The MuLV viral probe detected transcripts in RNA prepared from the transfectant, but not in RNA prepared from untransfected CHO cells. The results indicate that the cells transfected with the ERR cDNA can acquire the susceptibility to ecotropic murine leukemia virus.

EXAMPLE V

Preparation and Use of Antibodies to H-13

It is very difficult to make an H-13-containing fusion protein having the whole predicted protein (SEQ ID NO:2) since the predicted protein is highly hydrophobic, as shown in Figure 9. In order to predict antigenic epitopes present in the protein, therefore, the computer analysis was carried out using the program of PEPTIDESTRUCTURE (Jameson et al., *CABIOS* 4: 181-186 (1988)). Figure 10 shows the antigenicity profile of the H-13 protein sequence.

The DNA sequence encoding a highly antigenic portion (SEQ ID NO:2, amino acid residues 309-367) was prepared by cutting with the restriction enzymes AccI and EcoRI yielding a 180 bp AccI-EcoRI fragment. This fragment of H13 cDNA was ligated to the cloning sites of pGEX-2T plasmid vector (Pharmacia LKB Biotechnology), which can express antigens as fusion proteins with glutathione-S-transferase (GST), in the orientation that permit[s] the expression of the open reading frames (Smith, D.B. et al., *Gene* 67: 31-40 (1988)).

The fusion protein was induced by addition of isopropyl-beta-thiogalactopyranoside (IPTG) to cultures, and was purified using glutathione Sepharose 4B chromatography

(Pharmacia LKB Biotechnology) (see Figure 11). The purified fusion protein injected intramuscularly and subcutaneously into rabbits with Freund's complete adjuvant to obtain antisera.

The antisera are shown to bind specifically to the H-13 protein and epitopic fragments thereof.

Membrane proteins from human cells are prepared according to standard techniques and are separated by polyacrylamide gel electrophoresis, an blotted onto nitrocellulose for Western Blot analysis. The H-13 specific antibodies are shown to bind to proteins on these blots.

EXAMPLE VI

Genetic Mapping of H13

Chromosomal location of the H13 gene was determined using Chromosome Blots (Bios Corp., New Haven, Connecticut) containing DNA from a panel of human-hamster somatic cell hybrids (Kouri, R. E. et al., *Cytogenet. Cell Genet.* 51:1025 (1989)). By comparison of which human chromosomes remained in the human-hamster hybrid cell and the expression of H13 cDNA, the H13 gene was mapped to human chromosome 13 (see Figure 12). Human genes (or diseases caused by mutations therein) linked to chromosome 13 include: retinoblastoma, osteosarcoma, Wilson's disease, Letterer-Siwe disease, Dubin-Johnson syndrome, clotting factor Vii and X, collagen IV α1 and α2 chains, X-ray sensitivity, lymphocyte cytosolic protein-1, carotid body tumor-1, propionyl CoA carboxylase (α subunit), etc.

EXAMPLE VII

A Chimeric receptor Polypeptide Encoded by Chimeric H13/ERR DNA and Protein Molecules

Several chimeric molecules between the mouse ERR sequence and the human H13 sequence were produced, and have been designated Chimera I - Chimera IV. Specifically, four regions in H13 cDNA were substituted based on the use of common restriction sites as shown in Figure 13.

These DNA sequences were transiently transfected into Chinese hamster ovary (CHO) cell lines using pSG5 or pCDM8 expression vectors.

Two days later, these transfectants were tested for their ability to support E-MuLV infection. Cells were infected with a recombinant Moloney E-MuLV designated 2BAG (Price, J. et al., *Proc. Natl. Acad. Sci. USA* 84:156-160 (1987)). This recombinant virus also contained ß-galactosidase and neomycin phosphotransferase (neo^R) genes which provide a selectable marker and a detectable product. The cells were then grown under selective conditions in the presence of the antibiotic G418 at a concentration of 0.6 mg/ml to select neo^R-expressing transfectants. After two weeks, numbers of G418-resistant colonies were counted.

These results indicate that portion of the ERR gene essential for E-MuLV infection is located within NcoI-BstXI restriction sites, and included extracellular Domain 3. Extracellular Domain 3 (as shown in the upper line of Figure 13) is the region of the receptor protein which is most diverse between the human and mouse sequences, as shown in Figure 14. The sequences in Figure 14 (derived from the sequences shown in Figure 1-3) were aligned using Genetics computer group sequence analysis software package (Devereux, J. et al, *Nucl. Acids Res.* 12:387-395 (1984)).

Next, oligonucleotide-directed mutagenesis was employed to produce chimeric molecules containing individual amino acid substitutions within extracellular domain 3. These were transfected as above and the transfectant cells are tested for susceptibility to infection by E-MuLV as shown above.

The results of the above studies show that the human H13 molecule acquires ability to bind to E-MuLV by substituting the native amino acid sequence with between 1 and 4 amino acids from corresponding positions in the murine ERR protein.

EXAMPLE VIII

Chimeric receptor Polypeptides as H13 Derivatives Capable of Providing Infectivity of Ecotropic Murine Leukemic Retrovirus

Human H13 amino acid residues were substituted by murine ERR residues, as described in Figure 18. Mouse-human chimeric receptor molecules were made by substitution using common restriction sites which clarified that the extracellular domains 3 and/or 4 contain the critical amino acid residues. Oligonucleotide-directed mutagenesis was then used to create 13 individual mutant ERR molecules containing one or two amino acids substitutions or insertions within these two extracellular domains. Substitution of at minimum two amino acids, Pro and Val, at the 242 and 244 amino acid residues in human H13 by the corresponding amino acid residues, Tyr and Glu, or substitution of Gly240 and Pro242 in human H13 with Val and Tyr, which correspond to Val233 and Tyr235 of ERR, such that the resulting mutant H13 has the ability to function as a murine ecotropic retroviral receptor. This mutant H13 will be useful in a gene therapy.

To compare the relative abilities of murine ERR and human H13 to function as a receptor for MuLV-E, Chinese hamster ovary (CHO-K1) cell lines were transiently transfected with a vector expressing either murine ERR or human H13 (see legend in Fig. 16). Two days later, these transfectants were infected with the recombinant MuLV-E, ΨCRE/BAG virions, containing the *Escherichia coli lacZ* β-galactosidase and Tn5 neo resistant genes (Price et al. *Proc. Nat'l Acad. Sci. USA* 84:156-160 (1987); Danos et al. *Proc. Nat'l Acad. Sci. USA* 85:6460-6464 (1988)) and selected by G418. After 10-14 d numbers of G418-resistant colonies were counted (Fig. 16). No positive colonies were obtained with H13 transfectants while more than 10^3 colonies were obtained with ERR transfectants. This indicates that H13 molecule without modification by substitution or deletion of amino acids lacks the ability to function as a receptor for MuLV-E.

To identify amino acid residues, modifications, as presented in Figure 18, for MuLV-E infection, H13 modified proteins were prepared by substitution using the common and single restriction site, *Kpn*I, and determined their abilities

to function as a receptor for MuLV-E (Fig. 16). Approximately 10^3 colonies were obtained with transfectants of Chimera I, whose first part is substituted by the corresponding region of ERR, while no colonies were obtained with transfectants of Chimera II, whose last part is substituted by the corresponding region of ERR. This indicates that the critical amino acid residues are located in the first part. To more narrowly define the essential region, Chimera III, whose *Nco*I-*Nco*I fragment is substituted by corresponding region of ERR, was made and its ability to function as the receptor was determined (Fig. 16). Approximately 10^3 colonies were obtained, indicating that the critical region for the infection is located within the *Nco*I-*Nco*I restriction sites.

Figure 17 shows the comparison of sequences of extracellular domains 3 and 4 in murine ERR and human H13, which are aligned using the Genetics computer group sequence analysis software package (Devereaux et al *Nucleic Acid Res.* 12:387-395 (1984)). Extracellular domain 3 is the most diverse region between murine ERR molecules (Mutants 1-11) containing one or two amino acid substitutions or insertions within these two domains (Fig. 17 and Table 2). For each substitution, amino acid residues of ERR were replaced with those found in equivalent position of H13 sequence. For each insertion amino acid residues of H13 were added into equivalent position of ERR sequence which aligned as shown in Fig. 17.

CHO-K1 cells expressing the mutant ERR proteins were tested for their abilities to function as a receptor for MuLV-E. Surprisingly, no colonies were obtained with only Mutant 7 while approximately 500 colonies were obtained with the other mutants, indicating that of these 11 mutants only Mutant 7 abrogates the ability to function as the receptor (Table 2). Mutant 7 has two amino acid substitutions, Tyr (235 amino acid residue in ERR) to Pro (corresponding to amino acid residue 242 in H13) and Glu (corresponding to amino acid residue 244 in ERR) to Val (244 amino acid residue in H13). Therefore, Mutants 7A and B, which contain just one amino acid substitution (Mutant 7A: Tyr to Pro and Mutant 7B: Glu to

Val), were prepared and tested for their abilities to function as the receptor. Although Mutant 7B has almost the same ability to function as the receptor as the intact ERR, Mutant 7A was found to almost completely abrogate the ability (Table 2). These results suggest that the Tyr located at 235 amino acid residue in ERR sequence is very important to function as a receptor protein for MuLV-E and substitution of this amino acid residue leads ERR to lose its ability to function as the receptor.

To determine whether the H13 molecule would acquire the ability to function as the receptor if certain amino acid residues in the H13 are substituted by the corresponding amino acid residues in ERR, eight mutants of H13 were made as shown in Fig. 18, and their abilities to function as the receptor were determined. The H13 mutants were created by the method of altered site-directed mutagenesis using a phagemid vector, pSELECT-1 (Promega), e.g., as presented in Lewis and Thompson, *Nucleic Acids Res.* 18:3439-3443 (1990). The mutagenesis is based on the use of single stranded DNA and two primers, one mutagenic and a second correction primer which corrects a defect in the vector to ampicillin resistance.

The insert of pSG5H13 was completely digested with BamHI and partially with EcoRI, and subcloned into the BamHI-EcoRI site of pSELECT-1 to obtain pSELECT-1 anti-sense H13. The insert of PSG5H13 Mutants 5 and 8 were excised with EcoRI and subcloned into the EcoRI site of pSELECT-1 to obtain pSELECT-1 antisense H13 Mutants 5 and 8. H13 mutants 1-3 and 5 were prepared using the pSELECT-1 antisense H13 as a template and oligonucleotides:

AAAGAAGGGAAGTACGGRGRRGGRGG (SEQ ID NO:9) (H13 Mutant 1);
ACACAAAAGAAGTGAAGTACGGTGTTGGTGG (SEQ ID NO:10) (H13 Mutant 2);
ATGACACAAAAAACGTGAAGTACGGTGTTGGTGG (SEQ ID NO:11) (H13 Mutant 3); and
AAAGAAGGGAAGTACGGTGAGGGTGGATTCATG (SEQ ID NO:12) (H13 Mutant 5). H13 Mutant 4 was prepared using pSELECT-1 antisense H13 Mutant 8 and oligonucleotide TGAAGTACGGTGTTGGTGGATTCATG (SEQ ID NO:13). H13 Mutants 6-8 were prepared using pSELECT-1 antisense H13 Mutant 5 and oligonucleotides

ACACAAAAGAAGTGAAGTACGGTGA (SEQ ID NO:14) (H13 Mutant 6); AATGACACAAAAAACGTGAAGTACGGTGA (SEQ ID NO: 15) (H13 Mutant 7);, and AACAATGACACAAACGTGAAGTACGGTGAGGGTGGATTCATG (SEQ ID NO: 16) (H13 Mutant 8).

The ERR mutants were also created by the method of altered site-directed mutagenesis using a phagemid vector, pSELECT-1 (Promega), e.g., as presented in Lewis and Thompson, *Nucleic Acids Res.* 18:3439-3443 (1990). The mutagenesis is based on the use of single stranded DNA and two primers, one mutagenic and a second correction primer which corrects a defect in the vector to ampicillin resistance.

The insert of pSG5ERR was partially digested with BamHI and EcoRI, and subcloned to the BamHI and EcoRI sites in pSELECT-1 to obtain pSELECT-1 sense and anti-sense ERR. Single stranded DNA was prepared from pSELECT-1 sense (for preparation of Mutants 2 and 6) and antisense (for preparation of the other mutants) ERR and mutagenesis was carried out according to the manufacturer's directions (Promega). The correctly mutated clones were selected by directly sequencing using Sequenase (USB) and two ERR specific antisense oligonucleotides GGTGGCGATGCAGTCAA (SEQ ID NO: 17) for mutants 1-7 and TCAGCCATGGCATAGATA (SEQ ID NO:18) for Mutants 8-11) as primers. Mutated inserts of the phagemids prepared by mini-preps were excised with EcoRI and subcloned into the EcoRI site of pSG5. The presence of mutations was confirmed by sequencing each plasmid using the same primers as used above. Each mutant was then transiently transfected into CHO-K1 cells by the method using Lipofectin reagent and their susceptibilities to infection by MuLV-E were determined as above for results shown in Figure 16.

Accordingly, it was shown that mutant H13 polypeptides according to the present invention containing Tyr242 and at least one of Val 240 and Glu244 provide a mutant H13 receptor binding region that is functionally recognized by ecotropic murine retroviruses, such as MuLV-E, such that expression of such a chimeric receptor polypeptide on the extracellular surface of a human cells allows binding and infection by a murine ecotropic retrovirus. Such a method can

thus be used according to the present invention as a method for gene therapy *in vitro*, *in vitro* or *in situ*. Alternatively, such a method of the present invention can be used to introduce heterologous or exogenous genes into human cells or tissues using a murine ecotropic retroviral vector. Thus, the use of an H13 mutant according to the present invention provides a much safer means for gene therapy than the use of amphotropic retroviral vectors, which overcomes the problems of unintended infection of non-target cells by amphotropic retroviruses, as well as immunogenicity reduction for the use of relatively lower dosages of recombinant ecotropic virus.

In summary, the cloning of the human ecotropic retrovirus receptor and the realization that murine ecotropic viruses cannot normally infect human cells, excepting for the introduction of a molecular modification, sets the state for improving dramatically the safety of gene therapy involving retroviruses. In the first step the modified gene for the ecotropic retroviral receptor will be delivered to target cells and transiently expressed. The murine ecotropic-virus-vector would then be used to infect, stably integrate, direct the expression of the desired therapeutic gene. Zero to some time may elapse between the first step of the infection.

The ecotropic virus vector to be used will carry deletions of the structural genes and be propagated in "safe" packaging cell lines for added safety. The construction of novel packaging lines producing virus titers in the 10^8 to 10^{10} particles/ml range is expected and devoid of recombinant viruses capable of infecting human cells. Furthermore, modification of the viral envelope glycoprotein will eliminate any determinants which would interfere with virus infectivity *in vivo* or diminish virus titers. The human ecotropic virus receptor homolog also is studied to determine its normal gene function and gain sufficient understanding of the protein to eliminate the likelihood that gene therapy protocols would affect its normal function in a deleterious manner.

EXAMPLE IX

CLONING OF A HUMAN AMPHOTROPIC VIRUS RECEPTOR.

The amphotropic receptor is cloned for development of gene therapy vectors. The receptor for amphotropic-MLV are cloned by a similar strategy to that used to clone the receptors for Gibbon ape leukemia virus and mouse ecotropic virus (E-MLV) according to known method steps (e.g., Brown et al, 1990; Anderson et al, 1991). The strategy relies on the fact that human cells can be infected by A-MLV but hamster cells cannot. The inability to infect hamster cells result from their lack of a suitable receptor, making possible the transfection the human gene into hamster cells, rendering them infectable by A-MLV. Isolating these cells by infection with antibiotic-resistant recombinant viruses followed by selection of antibiotic containing media is then performed. The receptor gene is accessible to cloning by virtue of its association with human repetitive DNA. When the amphotropic receptor gene is cloned, its similarities and dissimilarities to the ecotropic virus receptor is studied in a variety of assays and by a variety of techniques (e.g., Yoshimoto et al, 1993).

Methods CHO cells are plated on the morning of transfection. Sheared human genomic DNA (50 μg) and pSV_{2gpt} DNA (1 μg) are coprecipitated with calcium phosphate by the method of Wigler et al. (1978) and applied to CHO cells. The next day, transfected cells are passaged at 2 x 10^5 cells per plate in gpt selection medium (DMEM/10% FCS, hypoxanthine 15 μg, xanthine μg/ml, thymidine 10 μg/ml, glycine 10 μg/ml, methotrexate 0.1 μM, and mycophenolic acid 25 μg/ml). After 21 days under selection, colonies are dispersed by brief exposure to trypsin/EDTA and replaced prior to exposure to viruses, allowing for enrichment of cells that have acquired human DNA.

PA317/LNL6 amphotropic retrovirus producer fibroblasts are grown to confluence and refed with fresh medium. Twelve to twenty hours later, the culture medium is filtered (0.45 μm, Nalge), brought to 8 μg/ml of polybrene, and incubated with the transfected CHO cells. After 4-12 hours fresh medium is added, and the infection protocol

repeated the next day. Three days later, these cells are replated at 2 x 10^5 cells per 82 mm plate in DMEM/10% FCS containing 1 mg/ml of G418, and selection medium is replaced every 3 days for 15 days. It is expected that out of 20,000 transfectants, only a few (10-20) will develop into g418 resistant colonies. To authenticate that clones are infectable by this amphotropic virus indeed express the amphotropic virus receptor, they are also infected by a second amphotropic virus (Ψ-2-AM-ZIP-DHFR). It is possible for cells to acquire the virus through a low efficiency pathway not involving the virus receptor. G418-resistant colonies are isolated with cell cloning cylinders and each is exposed to Ψ-2-AM-ZIP-DHFR virus as described above for the neomycin virus. Following exposure to the virus, cells are selected in DMEM/10% dialyzed FCS containing methotrexate (150 nM). After 14 days, plates are stained for the presence of methotrexate-resistant colonies with 1% crystal violet.

DNA is then prepared from this primary transfected cell line (1°TF) and used in a second cycle of the transfection/infection protocol. To identify the receptor gene is the secondary transfectant cell lines, Southern blots using a panel of human repetitive sequences as probes are made. Because of the low efficiency of DNA transfection (0.1% genome/cycle), cycles of transfection/selection are adequate to segregate the receptor gene away from the remainder of the murine genome (Murray et al., 1981). To isolate the desired fragment, a lambda phage library is prepared from secondary transfectants DNA and hybridized with the radiolabeled repetitive probe that seems most appropriate from the Southern blot screening. To identify molecular clones that contain the protein encoding portion of an amphotropic receptor gene, RNA transcript present in the 2°TFs growing the amphotropic viruses are identified. The specific transfer of the gene in question is expected to transfer susceptibility to amphotropic virus infection in a consistent manner, and a large panel of cells infectable by these viruses express this molecule is provided.

EXAMPLE X:

Expression of Therapeutic Delivery Vector According to the Present Invention

A complementary DNA (cDNA) from the antibody B3 (e.g., Brinkmann et al, 1991) is used to construct an Fv fragment that is fused to a chimeric receptor polypeptide of the present invention. This single-chain recombinant receptor, then is used to allow retrovirus infection of targeted human cells. Antibody to B3, which binds to a carbohydrate antigen expressed on the surface of many carcinomas, has been used to make a single-chain recombinant toxin that causes the complete regression of human tumors in mice (Brinkamnn et al, 1991). A single-chain Fv and two different (B3(Fv) immunotoxins, B3(Fv)-PE40 and B3(Fv)PE38KDEL vectors are used via standard recombinant DNA technologies to insert into the chimeric receptor polypeptide encoding nucleic acid, or substitute its recombinant toxin with the virus binding domain of the gene encoding the modified region of the human ecotropic virus receptor. The resulting plasmid (B3(Fv)-mH13) as well as the immunotoxin vector B3(Fv)PE38KDEL are expressed in a host, such as *E. coli*, and the single chain immunoreceptor and single-chain immunotoxin are purified to homogeneity as known in the art.

The antitumor activity of the B3(Fv)-mH13 is determined first *in vitro*. The B3 antibody reacts uniformly with the surface of many mucinous carcinomas of the colon, stomach, and ovary and with normal tissues, such as glands of the stomach, epithelia of the trachea and bladder, differentiated epithelium of the esophagus, and small bowel mucin. (Pastan et al. (*Cancer Res.* 51:3781-3787, 1991)). The B3 antibody also reacts uniformly with many human tumor cell lines, including MCF7, MDA-MB-468, and HTB20 (breast), A431 (epidermoid), TH29 (colon), HTB33 (cervical), and DU145 (prostrate). Infection in some or all of these cells is expected, such as A431, of a non-human specific recombinant retroviral vector, such as a murine ecotropic retrovirus vector carrying the neomycin resistant gene, after first delivering to those cells the modified receptor peptide by use

of the fusion protein derived from B3(Fv)-mH13. The viral vector having an *env* binding domain which binds the chimeric receptor polypeptides and a therapeutic agent, such as a murine ecotropic retrovirus vector carrying the thymidine kinase gene, is used preliminarily to cause cell death of cultured tumor cells, such as A431 cells, by the addition of ganciclovir to the cell cultures.

When such cell is shown to work in culture, then pathological cell killing in animal model systems is used, such as a rabbit model or a rat model. Thus, murine ecotropic virus-based vectors of the present invention are expected to be incapable of infecting these cells, unless a chimeric receptor polypeptide or the corresponding region of the murine ecotropic virus receptor is expressed on the selected animal model cell surface via the delivery vector presented herein. At various intervals after the fusion protein injection, a viral vector carrying the thymidine kinase gene is used to infect the animal models expressing the chimeric receptor polypeptide on the selected target cells. This expression is followed by the administration of ganciclovir to the animals. This protocol is expected to achieve tumor reduction as the ganciclovir is phosphorylated within tumor cells to its toxic form and in conjunction with the associated "bystander effect".

EXAMPLE XI

Construction of a vector for the expression of the fusion protein of a chimeric receptor polypeptide and a single-chain antigen-binding protein recognizing tumor or pathologic cells or tissues.

An example of a suitable methodology for introducing a is illustrated in Figure 4. The expression plasmid pUL1 contains the gene for the immunotoxin B3(Fv)-PE40, which is a fusion protein including an antibody fragment to an antibody to B3 specific of carcinoma cells, conjugated to the toxin PE40. The pUL1 expression plasmid is modified to replace the PE40 toxin encoding portion with a chimeric receptor polypeptide of the present invention or the corresponding

region of the murine ecotropic virus receptor to provide a delivery vector that transforms carcinoma cells *in vivo*, *in situ* or *in vitro*, to express a chimeric receptor polypeptide.

B3(Fv) is a single chain antigen-binding protein derived from a monoclonal antibody to B3. The B3 antibody fragment recognizes a carbohydrate antigen which is found on the surface of many mucinous carcinomas. However, the antibody fragment reacts with only a limited number of normal tissues, such that the antibody fragment will preferentially bind carcinoma cells *in vivo*. PE40 is a truncated derivative of *Pseudomonas* exotoxin. The PE40 coding region has a Hind III restriction site at the 5' end, the point of connection to the DNA encoding B3(Fv), and an EcoRI site just beyond 3' end. This Hind III-EcoRI fragment encoding PE40 is removed from pUL1 and both termini of the linearized pUL1 are partially filled-in with dATP, yielding cohesive ends -AA. The -TTCGA at 3' end of B3(Fv) coding region and AATTC- at the other terminus of the linearized plasmid are similarly modified to complement the chimeric receptor polypeptide encoding DNA, as follows.

Nru 1-Pst 1 fragment of a chimeric receptor polypeptide the present invention, as a modified H13 cDNA, which contains whole coding region of modified H13, is digested with Tfi I and the 850 bp fragment, which contains the region encoding the third extracellular domain of the modified H13, is purified on a 1.5% agarose gel. The purified 850 bp Tfi 1 fragment is then digested with BsrI and 85 bp Bsr 1-Tfi I fragment, which encodes the whole third extracellular domain of the modified H13 designated Ex3mH13. The resulting restriction fragment EX3m13 is purified on 2.0% agarose gel. The purified 85 bp Bsr 1-Tfi 1 fragment has cohesive ends AGC- at 5' end and -GG at '3 end.

CGTCG- -CCTAA.

This 3' end is partially filled-in with dATP, making the 3' end

-GGA -CCTAA.

After partial filling-in, the 85 bp Bsr 1-Tfi fragment is be ligated to the partially filled-in Hind III-EcoRI site of the linearized pUL1 using adapters
CGCTTTCAACTGGC (SEQ ID NO:19)
AAGTTGAC
and TTCTAATTAG (SEQ ID NO:20)
GATTAATCTT (SEQ ID NO:21)

which are specially designed to prevent any frame shift. The resulting plasmid is designated pBH30.

The gene encoding B3(Fv) has a Ndel site at 5' end. The resulting plasmid pBH30 is then digested with Ndel and the termini are filled in with dATP and dTTP to become blunt ends. Then the linearized and filed-up plasmid is digested with EcoRI and the fragment containing B3(Fv)-Ex3mH13 coding region is purified on an agarose gel.

The purified fragment is integrated into an expression vector pTrcHisB at the BgIII-EcoRI site, which is positioned downstream of the series of a Trc promoter, an ATG initiation codon, a polyhistidine coding region and an enterokinase-cleavable site coding region, using an adaptor
GATCCCCGGG (SEQ ID NO:22)
GGGCCC

so that any frameshift should be prevented.

The resulting plasmid is designated pBH3.

Expression and purification of B3(Fv)-Ex3mH13 fusion protein.

The expression plasmid pBH3 allows B3(Fv)-Ex3MH13 to be expressed as a fusion protein composed of a polyhistidine metal binding domain, an enterokinase-cleavable site and B3(Fv)-Ex3mH13.

E. coli HB101 is then transformed with pBH3. The expression is induced with isopropyl β-d-thiogalactoside and the cells are harvested and resuspended in a buffer solution. The suspension is sonicated and the supernatant is loaded on a

Ni^{2}+ metal affinity resin column. The protein bound to the resin is eluted by competition with glycine.

The eluted protein is then treated with enterokinase for the polyhistidine sequence to be removed.

The resulting fusion protein is expected to specifically bind the pathologic cells described and is suitable for providing a chimeric cell as a target for therapy as described herein.

EXAMPLE XII

Increasing the efficiency of *in vivo* virus replication through definition of critical regions of the viral envelope which binds to a receptor, such as a chimeric receptor polypeptide of the present invention.

Background

Equally important to the goal of developing improved vectors is understanding the critical regions of the viral envelope which binds to the receptor. Engineering of new vectors is dramatically improved according to the present invention and their *in vivo* titers effectively increased. Consequently, the present invention provides for the dissection of viral envelope elements required for binding to both receptors' as well as the characterization of the degree of modification that these proteins will tolerate without abrogating their capacity to bind the receptors, as well as to examine how the limited differences between the human and mouse ecotropic receptors responsible for allowing binding of the virus. A second aim is to eliminate any potential complement binding region on the viral envelope which might

lead to virus lysis in humans, a problem which has been argued by some investigators to probably limit the infectious potential *in vivo* of therapeutic vectors based on murine retroviruses.

The finding of nonspecific inactivation and lysis of murine, feline, and simian C-type viruses was originally published by Welsh et al. (1975, 1976). The lysis is due to antibody-independent binding of the human C1q complement component to gp70, leading to the activation of the classic complement pathway (Cooper et al., 1976). It is believed that C1q recognizes gp70 because the gp70 molecules have a domain resembling the C1q recognition site on the Fc fragment of immunoglobulins. It was suggested (Welsh et al., 1975; Cooper et al., 1976) that the nonspecific lysis of retroviruses by human complements is an adaptive defense system that may protect against viremia and cause the lysis of cells expressing gp70 on the surface. However, preliminary findings suggest that complement-deficient patients do not exhibit elevated levels of cross-reacting antibodies to primate retroviruses, indicating no greater susceptibility to retrovirus infection in these patients (Kurth et al., 1979b). Further, Gallagher et al. (1978) have shown a similar lytic activity of gibbon ape sera for retroviral envelopes, yet some of the same gibbons were infected with GALV and synthesized anti-GALV antibodies. The protective effect of the complement-dependent lysis of retrovirus, therefore, remains controversial.

Sequence of the murine ecotropic viral envelope which binds to the virus receptor.

One way of defining the binding parameters of Murine leukemia virus (MuLV) surface glycoprotein ($gp70^{SU}$) is to determine which regions of $gp70^{SU}$ participate in the specific interactions with cell surface receptors. A variety of studies have suggested that the determinants for receptor specificity lie in the N-terminal two thirds of $gp70^{SU}$ (Ott and Rein J. Virol 66:4632-4638, 1992 -- INSERT REFS 13, 16). In fact, Heard and Danos (1991) have recently shown than an Env fragment containing most of this region of Friend MULV $gp70^{SU}$ can bind to the ecotropic receptor in NIH 3T3 cells. Recently Ott and Rein have attempted to map receptor specificity in $gp70^{SU}$ by constructing a series of chimeric env genes, using Moloney MCF (Mo-MCF), 10A1, and amphotropic $gp70^{SU}$ sequences. The analysis of MuLVs containing these chimeric $gp70^{SU}$ gave both simple and complex results. In some cases, receptor specificity could be mapped to a single region of $gp70^{SU}$. Finally, some combinations seemed to be capable of fully functional interactions with one receptor but also a partial or abortive interaction with one or two receptors.

Heard and Danos (*J. Virol.* 65:4026-4032, 1991) have shown, using an interference assay, that the gp70 amino-terminal domain folds into a structure which recognizes the ecotropic receptor regardless of the carboxy-terminal part of the molecule. They have argued that it may be difficult to interpret the functional consequences of structural modifications, introduced in envelope glycoprotein by using virus entry assays. On the other hand, in cells constitutively expressing envelope glycoproteins, the interference phenomenon results from envelope-receptor

interactions alone. Therefore, the delineation of cell resistance to further entry of virus particles that bind the same receptor provides a functional receptor binding assay.

Experimentation

Interference assays are used to more precisely define the region of the murine ecotropic envelope glycoprotein (MuLVE-gp70) which is critical for binding to its receptor. To establish more precisely the structural requirements for binding to the ecotropic receptor the MuLVE-gp70 is modified by in-frame deletions within the amino-terminal domain, and by oligonucleotide directed mutagenesis. A comparison of envelope sequences shows that MLVgp70s differ in two limited regions in their amino-terminal domains. These are amino acids 50 to 116 and 170 to 183. They also differ in the proline-rich segment, amino acids 244 to 283. By analogy with the avian sarcoma and leukemia virus envelope glycoproteins, in which determinants for receptor interactions have been ascribed to short hypervariable sequences (Heard and Danos *J. Virol.* 65:4026-4032, 1991;), one or more of these three hypervariable regions are expected to include receptor binding cells. Defective retroviral vector transducing a modified E. coli lacZ gene is used to infect cells expressing wild-type or modified MuLVE-gp70s. Susceptibilities to infection are determined by counting X-Gal-positive foci.

The ecotropic envelope N-terminal domain vectors is transiently transfected into simian Cos-7 cells that express the exogenous murine ecotropic retroviral receptor gene (Albritton et al, 1989). Since the pSG5 vector utilizes the early SV40 eukaryotic promoter, the Cos cells (expressing to T

antigen gene) permits a high level of env gene expression (Gluzman, 1981). Transfected cells are infected with the ecotropic pseudotype Ψ CRE/BAG (ATCC CRL 1850) virions (Price et al, 1987) and the number of β-galactosidase (gal) foci are assayed. Modified env fragments which decrease the number of β-gal foci are further examined for their ability to interact with the ERR.

1. Transfection of the ERR gene into Cos-7 cells.

The ERR gene was cloned into the pcDNA/neo expression vector (Invitrogen) and transfected into Cos-7 cells by the CaPO4 method (Stratagene). Cos-7 cells have been shown to be capable of producing high-titers of MuLV retroviruses (Landau and Littman, 1992). Transfected cells are selected for neomycin resistance to 500 μg/ml G418. Resistant clones are tested for their ability to be infected by Ψ CRE/BAG virions (Price et al, 1987). Cells are fixed with 0.5% glutaraldehyde in physiological buffered saline and stained with a histochemical solution containing 1 mg/ml X-gal (Sanes et al, 1986). Those cells having the highest number of β-gal foci are used for interference assays.

2. Construction of modified env genes to further delineate receptor binding sequences.

This experiment determines the smallest N-terminal fragment that will block ecotropic virus infection. The gp70 gene from the Akv endogenous murine leukemia virus is used in these studies; the Akv genome is capable of producing active virions and the env sequence has been determined (Lenz et al, 1982).

The entire gp70 gene, contained between AccI and XbaI restriction sites is cloned into the psG5 eukaryotic expression vector (Stratagene). Fragments of the env gene are also cloned to produce the following N-terminal peptides: Acc1/Alu1 247 aa fragment produces the results of Heard and Danos (1991) and functionally block infection. Of particular interest is the 122aa Acc 1/Sma 1 fragment which contains the region thought to determine receptor binding specificity (Battini et al, 1992).

3. Interference assays of modified env gene constructs.

Recombinant env gene constructs are transiently transfected into Cos-7 cells containing the murine ERR. This system is expected to eliminate any potential problems caused by endogenous env transcripts that might be present in murine cells. Cos cells are expected to permit high expression of the SV40 promoter-containing expression plasmids (Gluzman, 1981). The cells are transfected by the DEAE dextran method according to Stratagene protocols. Approximately 48 hrs after env gene transfection, $2x10^5$ cells are plated onto a six-well culture dish and infected with 10^2-10^3 ΨCRE/BAG virions in the presence of 8ug polybrene/ml according to the method of Heard and Danos (1991). When the cells have grown to confluence, they are fixed in 0.5 % gluteraldelhyde in PBS and stained with a histochemical solution containing 1mg/ml X-gal (Sanes et al, 1986). The number of β-gal foci are scored relative to cells which were transfected with the pSG5 vector alone. A decreased number of β-gal foci are expected to be scored relative to cells which were transfected with the pSG5 vector

alone. A decreased number of β-gal foci determines that the transfected env construct is likely to be able to bind the ERR and block virion interaction.

4. Analysis of modified-env biosynthesis in transfected cells.

To determine whether recombinant *env* gene products are produced by the transfected cells, protein analysis is performed. Cells are metabolically labeled with a mixture of ^{35}S methionine and ^{35}S cysteine (ICN), since the *env* protein is relatively rich in cysteine residues in the regions of interest. After labelling for 30-60 minutes, cells are pelleted and both the pellets and supernatants are processed for immunoprecipitation with polyclonal goat anti-Rauscher gp70 antibody (NIH repository) or non-murine sera followed by *S. aureaus* A cells. Process immunoprecipitates are analyzed on SDS-polyacrylamide gels. The absence of immunoprecipitated protein is expected to signify that the *env* protein is degraded or unable to be recognized by the anti-gp70 antibody. In this event, the presence of env RNA transcripts is determined by extracting total RNA from the transfected cells followed by northern blot analysis using an AKv env sequence probe.

Fragments which are able to be detected by immunoprecipitation and which block Ψ CRE/BAG virion infection are utilized in further examples and methods of the present invention.

5.1 Mutation of modified env fragments.

The DNA sequences of several ecotropic and non-ecotropic retrovirus gp70 genes have been determined.

Comparison of these sequences will suggest regions which are important for receptor interaction. For example, Figure 19, shows a comparison of 3 ecotropic and non-ecotropic gp70 amino acid sequences, within the N-terminal region, thought to be involved in receptor specificity. The three ecotropic retroviruses show strong sequence homology. There are considerable differences between the ecotropic and non-ecotropic sequences, the most salient being a gap of approximately 30 amino acids located at positions 68 to 97 (Battini et al, 1992). While this region may contain an ecotropic binding site, it is also likely to confer different conformations upon the different subgroups of retroviruses.

When limited regions of the N-terminal *env* protein are found to block viral-receptor interactions, then it is possible to systematically modify amino acids in order to identify critical residues. In vitro mutagenesis is then accomplished utilizing mutagenic oligonucleotides and the pSelected (pAlter) system of Promega as previously described for the modification of ERR (Yoshimoto et al, 1993) are used.

If it is not possible to limit the location of the receptor binding domain such that it is feasible to create point mutations, the creation of deleted molecules or chimeric molecules between limited gp70 regions of the different viral subgroups are provided.

a) Creation of deletion mutants criteria and protocol.

1. Digestion with an enzyme having sites within the region of interest and relatively few elsewhere in the recombinant plasmid.

2. If the reading frame is altered, use exonuclease III to progressively remove nucleotides.

3. Use S1 nuclease to create blunt ends (13). Ligate and transform the deleted molecules.

4. Determine the sequence of the deleted clones to find those which maintain the correct reading frame.

For example (Fig. 20), a 163bp SmaI fragment is present (404bp, 567bp) within the variable region of the Akv env N-terminal sequence (Lenz et al, 1992). This fragment includes the 30 amino acids which are deleted from the amphotropic sequence (Figures 19 and 20). Simple removal of this sequence will create an incorrect reading frame since one SmaI site (404bp) lacks 1 nucleotide of an amino acid triplet.

b) Creation of Insertions.

The insertion of a specific sequence is performed by using the polymerase chain reaction (PCR) to amplify the sequence of interest according to standard protocols (Sambrook, *supra*; Ausubel, *supra*). The PCR primers are designed to maintain the proper reading frame of the env protein.

For example, the insertion of the 30 amino acid region of the ecotropic virus into the amphotropic virus sequence is accomplished by the following steps.

1. Digestion of the amphotropic env sequence with RsaI at position 325 creates a blunt end site and separates the 1st nucleotide (T) from the 2nd and 3rd (A,C) of the amino acid codon for tyrosine.

2. In order to insert the ecotropic sequence, a primer is synthesized which contains an extra AC at the 5' end in order to retain the tyrosine residue. A 3' primer is synthesized with an extra T at its 5' end which adds a tyrosine residue and retains the reading frame. The position of the proposed primers on the Akv sequence are shown in Table III.

TABLE III

5' primer: 5' AC CCG GGG CCC CCC TGC 3' (SEQ ID NO:23)

3' primer: 5' A GGG AGT ATA ATG AAG 3' (SEQ ID NO:24)

Following PCR, the product is run on a 2% agarose gel, the 90bp fragment is excised, purified and ligated to the amphotropic env sequence at the Rsal site. Recombinant clones are sequenced to determine those having the correct orientation of the PCR fragment.

7. Identification of viral envelope sequences binding C1q.

It has been suggested that gp70 molecules have a domain resembling the C1q recognition site on the Fc fragment of immunoglobulins. If this correct, and if this region is in an unnecessary part of the molecule for virus binding and entry, its removal and replacement is expected to alleviate any potential effects of serum oncornavirus lytic activity (SOLA) on the direct, <u>in vivo</u> use of retroviral vectors for gene therapy. to identify amino acid residues critical for C1q binding, the MuLVE-gp70 is modified by in-frame deletions when the domain resembling the C1q recognition site on the Fc

fragment of immunoglobulins, and by oligonucleotide directed mutagenesis. Once the C1p recognition is identified, we will attempt to construct a modified MuLVE-gp70 env gene, is constructed which will have this region substituted by non-C1q binding sequence.

To assay for SOLA activity in serum, and virus sensitivity, sucrose banded and purified, cloned Radiation leukemia virus is diluted with PBS to a standardized concentration, so that at least 100,000 cpm are detectable in our standard reverse transcriptase assay (Brown et al, 1990). Human serum at various dilutions are added to the virus preparation and incubated for 30 minutes under normal laboratory lighting conditions. Control samples are treated with 0.5% Triton X-100 (Sigma). All samples are then analyzed for RT activity as previously described (Meruelo et al, 1988).

All references cited herein, including journal articles or abstracts, published or corresponding U.S. or foreign patent applications, issued U.S. or foreign patents, or any other references, are entirely incorporated by reference herein, including all data, tables, figures, and text presented in the cited references. Additionally, the contents of the references cited within the references cited herein are also entirely incorporated by reference.

Reference to known method steps, conventional methods steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the generic concept of the present invention. Therefore, such adaptations and modifications are intended to be comprehended within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein.

REFERENCES

Aaronson, S., et al. Endogenous C-type of BALB/c cells: frequencies of spontaneous and chemical induction, J. Virol. 13:181-185, 1974.

Adam, M.A., et al. J. Virol. 62:3802-3806, 1988.

Albritton, L.M., et al. A putative murine ecotropic retrovirus receptor gene encodes a multiple membrane-spanning protein and confers susceptibility to virus infection. Cell, 57:659-666, 1989.

Anderson, C. Gene therapy researcher under fire over controversial cancer trials. Nature, 360:399-400, 1992.

Anderson, K.P. et al. Endogenous origin of defective retroviruslike particles from a recombinant chinese hamster ovary cell line. Virology, 181:305-311, 1991.

Anderson, W.F. Human gene therapy. Science, 256:808-813, 1992.

Armentano, D., et al. Effect of internal viral sequences on the utility of retroviral vectors. J. Virol., 62:1647-1650, 1987.

Battini, J.-L., et al. Receptor choice determinants in the envelope glycoproteins of amphotropic, xenotropic, and polytropic murine leukemia viruses. J. Virol., 66:1468-1475, 1992.

Bender, M.A., et al. Evidence that the packaging signal of Moloney murine leukemia virus extends into the gag region. J. Virol., 61:1639-1649, 1987.

Bestwick, R.K., et al. Overcoming interference to retroviral superinfection results in amplified expression and transmission of clones genes. Proc. Natl. Acad. Sci. U.S.A., 85:5404-5408, 1988.

Bird, R.E., et al. Single-chain antigen-binding proteins. Science, 242:423-426, 1988.

Bodine, D.M., et al. Combination of interleukins 3 and 6 preserves stem cell function in culture and enhances retrovirus-mediated gene transfer into hematopoietic stem cells. Proc. Natl. Acad. Sci. U.S.A., 86:8897-8901, 1989.

Bodine, D.M., et al. Proc. Natl. Acad. Sci., ?7:3738-3742, 1990.

Brinkmann, U., et al. B3(Fv)-PE38KDEL, a single-chain immunotoxin that causes complete regression of a human carcinoma in mice. Proc. Natl. Acad. Sci. U.S.A., 88:8616-8620, 1991.

Brown, D.G., et al. Increased H-2D^d expression following infection by a molecularly cloned ecotropic MuLV. Immunogenetics, 31:94-103, 1990.

Chang, S.M.W., et al. Construction of a defective retrovirus containing the human hypoxanthine phosphoribosyltransferase cDNA and its expression in cultured cells and mouse bone marrow. Mol. Cell. Bio., 7:854-863, 1987.

Chaudhary, V.K., et al. Nature, 339:394, 1989.

Cooper, N.R., et al. Lysis of RNA tumor viruses by human serum: direct antibody-independent triggering of the classical complement pathway. J. Exp. Med., 144:970-985, 1976.

Culver, K.W., et al. In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. Science, 256:1550-1152, 1992.

Dalglelsh, A.G., et al. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature, 312:763-767, 1984.

Danos, O., et al. Safe and efficient generation of recombinant retroviruses with amphotropic and ecotropic host ranges, Proc. Natl. Acad. Sci. USA., 85:6460-6464, 1988.

Davies, D.R., et al. Ann. Rev. Immunol., 1:87, 1983.

Devereux, J., et al. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res., 12:387-395, 1984.

Dunn, W.A., et al. J. Cell Biol., 102:24, 1986.

Fraenkel, A.E., et al. J. Biol. Response Mod., 4:273, 1985.

Friedmann, T. Progress toward human gene therapy. Science, 244:1275-1281, 1989.

Gallagher, R.E., et al. Oncornavirus lytic activity in the serum of gibbon apes. J. Natl. Cancer Inst., 60:677-6?2, 1978.

Ghetie, M.A., et al. Cancer Res., 48:261, 1988.

Gluzman, Y. SV40-transformed simian cells support the replication of early SV40 mutants. Cell, 23:175-182, 1981.

Hartley, J.W., et al. Naturally occurring murine leukemia viruses in wild mice: Characterization of a new "amphotropic" class. J. Virol., 19:19-25, 1976.

Harwood, P.J., et al. Eur. J. Cancer Clin. Oncol., 21:1515, 1985.

Heard, J.M., et al. An amino-terminal fragment of the Friend Murine leukemia virus envelope glycoprotein binds the ecotropic receptor. J. Virol., 65:4026-4032, 1991.

Hellstrom, K.E., et al. FASEB J., 3:1715, 1989.

Hendler, F.J., et al. J. Clin. Invest., 74:647, 1984.

Henikoff, S. Unidirectional digestion with exonuclease III in DNA sequence analysis. Method Enz., 155:156, 1987.

Hertler, A.A., et al. J. Biol. Response Mod., 7:97, 1988.

Hesdorffer, C., et al. Somatic gene therapy. Hematology/Oncology Clinics of North America, 5:423-432, 1991.

Hock, R.A., et al. Retrovirus-mediated transfer and expression of drug-resistant genes in human hematopoietic progenitor cells. Nature, 32?:275-277, 1986.

Huang et al. J. Virol., 50:417-424, 198?4.

Huber, B.E., et al. Retroviral-mediated gene therapy for the treatment of hepatocellular carcinoma: An innovative approach for cancer therapy. Proc. Natl. Acad. Sci. U.S.A., 83:8039-8043, 1991.

Huston, J.S., et al. Protein engineering of antibody binding sites: Recovery of specific activity in an anti-digoxin single-chain Fv analogue produced in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A., 85:5879-5883, 1988.

Jones, N.R., et al. Cancer, 66:152, 1990.

Kawano, T.J., et al. Nature, 332:83, 1988.

Kim, J.W., et al. Transport of cationic amino acids by the mouse retrovirus receptor. Nature, 352:725-729, 1991.

Koch, W., et al. Nucleotide sequence of the env gene of a Friend murine leukemia virus. J. Virol., 45:1-9, 1983.

Kohn, D.B., et al. Genetic Therapy for Genetic Diseases. Cancer Invest., 7:179-192, 1989.

Kurth, R., et al. Recognition of simian sarcoma virus antigen by human sera. In *Modern trends in human leukemia* (R. Neth et al., eds.), vol. 3, pp. 385-394, Springer, Heidelberg, 1979.

Landau, N.R., et al. Packaging system for rapid production of murine leukemia virus vectors with variable tropism. J. Virol., 66:5110-5113, 1992.

Lander, R.C., U.S. Patent 4,704,692; 1987.

Lasky, L.A., et al. Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science, 233:209-212, 1986.

Lau, J.L.T., et al. J. Urol., 139:170, 1988.

Lenz, J., et al. Nucleotide sequence of the AKv env gene. J. Virol., 42:519-529, 1982.

Lieber, M.M., et al. Mammalian cells in culture frequently release type C viruses. Science, 182:56-58, 1973.

MacLeod, C.L., et al. Activated T cells express a novel gene on chromosome 8 that is closely related to the murine ecotropic retroviral receptor. Mol. Cell. Biol., 10:3663-3674, 1990.

Manlatis, T., et al. J. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, New York, New York, 1982.

Mann, R., et al. Construction of a retrovirus packaging mutant and its use to produce helper-free defective retrovirus. Cell, 33:153-159, 1983.

Markowitz, D., et al. A safe packaging in line for gene transfer: Separating viral genes on two different plasmids. J. Virol., 62:1120-1124, 1988.

Markowitz, D., et al. Construction and use of a safe and efficient amphotropic packaging cell line. Virol., 167:400-406, 1988a.

Meruelo, D., et al. Therapeutic agents with dramatic antiretroviral activity and little toxicity at effective doses: Aromatic polycyclic diones hypericin and pseudohypericin. Proc. Natl. Acad. Sci., 85:5230-5234, 1988.

Miller, A.D., et al. Re-design of retrovirus packaging cell lines to avoid recombination leading to helper virus production. Mol. Cell Biol., 6:2895-2902, 1986.

Miller, A.D., et al. Factors involved in production of helper virus-free retrovirus vectors. Somat. Cell. Mol. Genet., 12:175-183, 1986.

Muenchau, D., et al. Short communications: analysis of retroviral packaging lines for generation of replication-competent virus. Virology, 176:262-265, 1990.

Muraro, R., et al. Cancer Res., 45:5769, 1985.

Murphy, J.R. Cancer Treat. Res., 37:123, 1988.

Murray, M.J., et al. Cell, 25:355-361, 1981.

Neville, D.J. Crit. Rev. Therap. Drug Carr??ier Syst., 2:329, 1986.

Ogata, M., et al. Proc. Natl. Acad. Sci. U.S.A., 86:4215, 1989.

Ogata, M., et al. J. Immunol., 141:4224, 1988.

O'Hara, B., et al. Characterization of a human gene conferring sensitivity to infection by gibbon ape leukemia virus. Cell Growth Diff., 1:119-127, 1990.

Ott, D., et al. Basis for receptor specificity of non-ecotropic murine leukemia virus surface glycoprotein $gp70^{SU}$. J. Virol., 66:4632-4638, 1992.

Pastan, I., et al. Recombinant Toxins for Cancer Treatment. Science, 254:1173-177, 1991.

Pastan, I., et al. Characterization of monoclonal antibodies B1 and B3 that react with mucinous adenocarcinomas. Cancer Res., 51:3781-3787, 1991.

Pastan, I., et al. J. Biol. Chem., 264:15157, 1989.

Pastan, I., et al. Cell, 47:641, 1986.

Poljak, R.S., et al. Proc. Natl. Acad. Sci. U.S.A., 70:3305, 1973.

Porter, R.R. Science, 180:713, 1973.

Price, J., et al. Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. Proc. Natl. Acad. Sci., 84:15?-160, 1987.

Rasheed, S., et al. Am?photropic host range of naturally occurring wild mouse leukemia virus. J. Virol., 19:13-18, 1976.

Rein, A., et al. Different recombinant murine Leukemia viruses use different cell surface receptors. Virology, 136:144-152, 1984.

Sambrook, J., et al. In Molecular Cloning: a Laboratory Manual, CSH Press, 1989.

Sanes, J.R., et al. Use of recombinant retrovirus to study post-implantation cell lineage in mouse embryos. EMBO J., 5:?3133-3142, 1986.

Sattentau, Q.J., et al. The human and simian immunodeficiency viruses HIV-1, HIV-2 and SIV interact with similar epitopes on their cell surface receptor, the CD4 molecule. AIDS, 2:101-105, 1988.

Scadden, D.T., et al. Human cells infected with retrovirus vectors acquire an endogenous murine provirus. J. Virol., 64:424-427, 1990.

Schiffer, et al. Biochemistry, 12:4620, 1973.

Shih, C.-C., et al. Highly preferred targets for retrovirus integration. Cell, 53:531-537, 1988.

Shinnick, T.M., et al. Nucleotide sequence of Moloney murine leukemia virus. Nature, 293:543-548, 1981.

Simonsen, C.C., et al. Isolation and expression of an altered mouse dihydrofolate reductase cDNA. Proc. Natl. Acad. Sci. USA, 80:2495-2499, 1983.

Skerra, A., et al. Assembly of a functional immunoglobulin Fv fragment in *Eschrichia coli*. Science, 240:1038-1041, 1988.

Sommerfelt, M.A., et al. Receptor interference groups of 20 retroviruses plating on human cells. Virology, 176:58-69, 1990.

Sommerfelt, M.A., et al. Human T cell leukemia viruses use a receptor determined by human chromosome 17. Science, 242:1557-1559, 1988.

Sommerfelt, M.A., et al. Localization of the receptor gene for type D simian retroviruses on human chromosome 19. J. Virol., 64:6214-6220, 1990.

Stenback, W.A., et al. Virus particles in hamster tumors as revealed by electron miscroscopy. Proc. Soc. Exp. Biol. Med., 122:1219-1223, 1966.

Stoye, J.P., et al. The four classes of endogenous murine leukemia virus: Structural relationships and potential recombination. J. Virol., 61:2859-2669, 1987.

Thompson, L. Science, 258:744-746, 1992.

Thompson, L. Monkey Tests Spark Safety review. Science, 257:1854, 1992.

Urlab, G., et al. Isolation of Chinese hamster cell mutants deficient in dihydrofolate reductase activity. Proc. Natl. Acad. Sci. U.S.A., 77:4216-4220, 1980.

Varkl, N., et al. Cancer Res., 44:681, 1984.

Velu, T.J., et al. Science, 238:1408, 1987.

Verma, I.M., 1990. Gene therapy. Sci. Am., 262:68-84.

Vile, R.G., et al. Virus receptors as permeases. Nature, 352:666-667, 1991.

Vitetta, E.S., et al. Science, 238:1098-177, 1987.

Wahl, R.L., et al. J. Nucl. Med., 24:316, 1983.

Waldemann. Cell. Immunol., 99:53-731, 1986.

Walz, G., et al. Proc. Natl. Acad. Sci. U.S.A., 86:9485, 1989.

Wang, H., et al. Cell-surface receptor for ecotropic murine retroviruses is a basic amino-acid transporter. Nature, 352:729-731, 1991.

Weiss, R., et al. RNA tumor viruses: molecular biology of tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1984.

Welsh, R.M., et al. Human serum lyses RNA tumor viruses. Nature, 257:612-614, 1975.

Welsh, R.M., et al. Inactivation and lysis of oncornaviruses by human serum. Virology, 74:432-440, 1976.

Wigler, M., et al. Biochemical transfer of single-copy eucaryotic genes using total cellular DNA as donor. Cell, 14:725-731, 1978.

Williams, D.P., et al. Protein Eng., 1:49?, 1987.

Willingham, M.C., et al. Proc. Natl. Acad. Sci. U.S.A., 84:2474, 1987.

Yee, J.-K., et al. Cold Spring Harbor Symp. Quant., Biol., 51:1021, 1986.

Yoshimoto, T., et al. Molecular cloning and characterization of a novel human gene homologous to the murine ecotropic retroviral receptor. Virology, 185:10-17, 1991.

Yoshimoto, T., et al. Identification of Amino acid residues critical for infection with ecotropic murine leukemia retroviruses. J. Virol., in press, 1993.

Yu, S.-F., et al. Self-inactivating retroviral vectors designed for transfer of whole genes into mammalian cells. Proc. Natl. Acad. Sci. U.S.A., 83:3194-3198, 1986.

TABLE 2

Abolishment of ability of ERR to function as a receptor for MuLV-E by mutation.

ERR Oligonucleotide use for mutagenesis Mutant	AA change (ERR) (H13)	Infectivity
Extracellular domain 3		
1 AAGGCTCCGTTAAAAAC (SEQ ID No: 19)	I V	+++
2 TACAGGAGAAATCTTCCTCCGTGAGCTG** (SEQ ID No: 20)	KN ED	+++
3 GAGAAAAATTTCGGCAACTGTAACAACAAC (SEQ ID No: 21)	S- GN	+++
4 AAAAATTTCTCCCGTCTCTGTAACAACAAC (SEQ ID No: 22)	– RL	+++
5 AATTTCTCCTGTTTCAACAACGACAC (SEQ ID No: 23)	N L	+++
6 TCACCGTATTTCCCTTCTGTGTCGTTGTT** (SEQ ID No: 24)	NV EG	+++
7 ACAAACGTGAAACCCGGTGTGGGAGGGTTTAT (SEQ ID No: 25)	YGE PGV	-
7A ACAAACGTGAAACCCGGTGAGGGAGG (SEQ ID No: 26)	Y P	±
7B ATACGGTGTGGGAGGGT (SEQ ID No: 27)	E V	+++
Extracellular domain 4		
8 TCTGCCTGGACAACAACAGCCCGCTGC (SEQ ID No: 28)	ID NN	+++
9 GCCCGCTGCCTGACGCCTTCAAGCAC (SEQ ID No: 29)	G D	+++
10 GCCTTCAAGCACGTGGGCTGGGAAGGAGCTAAGTACGC (SEQ ID No: 30)	QGWEE VGWEG	+++
11 GCCTTCAAGCACGTGGGCTGGGAAGGAGCTAAGTACGC (SEQ ID No: 31)	E G	+++

This is one of the representative result of three different experiments.

*Letters above each oligonucleotide sequence are those in the original ERR sequence. - means the absence of corresponding nucleotide sequence in ERR sequence according to the alignment (Fig.

** These two are antisense oligonucleotides and the others are sense oligonucleotides.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: MERUELO, DANIEL
YOSHIMOTO, TAKAYUKI

(ii) TITLE OF INVENTION: Human Retrovirus Receptor and DNA Coding Therefor

(iii) NUMBER OF SEQUENCES: 31

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Browdy and Neimark
(B) STREET: 419 Seventh Street, N.W.
(C) CITY: Washington
(D) STATE: DC
(E) COUNTRY: USA
(F) ZIP: 20004

(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 #1.24

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Townsend, Guy Kevin
(B) REGISTRATION NUMBER: 34,033
(C) REFERENCE/DOCKET NUMBER: MERUELO=1B

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 202 628-5197

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1102 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1102

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CCG GGC GCC ACC TTC GAC GAG CTG ATA GGC AGA CCC ATC GGG GAG TTC
Pro Gly Ala Thr Phe Asp Glu Leu Ile Gly Arg Pro Ile Gly Glu Phe      48
 1               5                  10                  15

TCA CGG ACA CAC ATG ACT CTG AAC GCC CCC GGC GTG CTG GCT GAA AAC      96
Ser Arg Thr His Met Thr Leu Asn Ala Pro Gly Val Leu Ala Glu Asn
            20                  25                  30

CCC GAC ATA TTC GCA GTG ATC ATA ATT CTC ATC TTG ACA GGA CTT TTA     144
Pro Asp Ile Phe Ala Val Ile Ile Ile Leu Ile Leu Thr Gly Leu Leu
```

```
         35                  40                  45

ACT CTT GGT GTG AAA GAG TCG GCC ATG GTC AAC AAA ATA TTC ACT TGT    192
Thr Leu Gly Val Lys Glu Ser Ala Met Val Asn Lys Ile Phe Thr Cys
     50                  55                  60

ATT AAC GTC CTG GTC CTG GGC TTC ATA ATG GTG TCA GGA TTT GTG AAA    240
Ile Asn Val Leu Val Leu Gly Phe Ile Met Val Ser Gly Phe Val Lys
 65                  70                  75                  80

GGA TCG GTT AAA AAC TGG CAG CTC ACG GAG GAG GAT TTT GGG AAC ACA    288
Gly Ser Val Lys Asn Trp Gln Leu Thr Glu Glu Asp Phe Gly Asn Thr
                 85                  90                  95

TCA GGC CGT CTC TGT TTG AAC AAT GAC ACA AAA GAA GGG AAG CCC GGT    336
Ser Gly Arg Leu Cys Leu Asn Asn Asp Thr Lys Glu Gly Lys Pro Gly
            100                 105                 110

GTT GGT GGA TTC ATG CCC TTC GGG TTC TCT GGT GTC CTG TCG GGG GCA    384
Val Gly Gly Phe Met Pro Phe Gly Phe Ser Gly Val Leu Ser Gly Ala
        115                 120                 125

GCG ACT TGC TTC TAT GCC TTC GTG GGC TTT GAC TGC ATC GCC ACC ACA    432
Ala Thr Cys Phe Tyr Ala Phe Val Gly Phe Asp Cys Ile Ala Thr Thr
    130                 135                 140

GGT GAA GAG GTG AAG AAC CCA CAG AAG GCC ATC CCC GTG GGG ATC GTG    480
Gly Glu Glu Val Lys Asn Pro Gln Lys Ala Ile Pro Val Gly Ile Val
145                 150                 155                 160

GCG TCC CTC TTG ATC TGC TTC ATC GCC TAC TTT GGG GTG TCG GCT GCC    528
Ala Ser Leu Leu Ile Cys Phe Ile Ala Tyr Phe Gly Val Ser Ala Ala
                165                 170                 175

CTC ACG CTC ATG ATG CCC TAC TTC TGC CTG GAC AAT AAC AGC CCC CTG    576
Leu Thr Leu Met Met Pro Tyr Phe Cys Leu Asp Asn Asn Ser Pro Leu
            180                 185                 190

CCC GAC GCC TTT AAG CAC GTG GGC TGG GAA GGT GCC AAG TAC GCA GTG    624
Pro Asp Ala Phe Lys His Val Gly Trp Glu Gly Ala Lys Tyr Ala Val
        195                 200                 205


GCC GTG GGC TCC CTC TGC GCT CTT TCC GCC AGT CTT CTA GGT TCC ATG    672
Ala Val Gly Ser Leu Cys Ala Leu Ser Ala Ser Leu Leu Gly Ser Met
    210                 215                 220

TTT CCC ATG CCT CGG GTT ATC TAT GCC ATG GCT GAG GAT GGA CTG CTA    720
Phe Pro Met Pro Arg Val Ile Tyr Ala Met Ala Glu Asp Gly Leu Leu
225                 230                 235                 240

TTT AAA TTC TTA GCC AAC GTC AAT GAT AGG ACC AAA ACA CCA ATA ATC    768
Phe Lys Phe Leu Ala Asn Val Asn Asp Arg Thr Lys Thr Pro Ile Ile
                245                 250                 255

GCC ACA TTA GCC TCG GGT GCC GTT GCT GCT GTG ATG GCC TTC CTC TTT    816
Ala Thr Leu Ala Ser Gly Ala Val Ala Ala Val Met Ala Phe Leu Phe
            260                 265                 270

GAC CTG AAG GAC TTG GTG GAC CTC ATG TCC ATT GGC ACT CTC CTG GCT    864
Asp Leu Lys Asp Leu Val Asp Leu Met Ser Ile Gly Thr Leu Leu Ala
        275                 280                 285

TAC TCG TTG GTG GCT GCC TGT GTG TTG GTC TTA CGG TAC CAG CCA GAG    912
Tyr Ser Leu Val Ala Ala Cys Val Leu Val Leu Arg Tyr Gln Pro Glu
    290                 295                 300
```

```
CAG CCT AAC CTG GTA TAC CAG ATG GCC AGT ACT TCC GAC GAG TTA GAT    960
Gln Pro Asn Leu Val Tyr Gln Met Ala Ser Thr Ser Asp Glu Leu Asp
305                 310                 315                 320

CCA GCA GAC CAA AAT GAA TTG GCA AGC ACC AAT GAT TCC CAG CTG GGG   1008
Pro Ala Asp Gln Asn Glu Leu Ala Ser Thr Asn Asp Ser Gln Leu Gly
                325                 330                 335

TTT TTA CCA GAG GCA GAG ATG TTC TCT TTG AAA ACC ATA CTC TCA CCC   1056
Phe Leu Pro Glu Ala Glu Met Phe Ser Leu Lys Thr Ile Leu Ser Pro
            340                 345                 350

AAA AAC ATG GAG CCT TCC AAA ATC TCT GGG CTA ATT GTG AAC CCG G     1102
Lys Asn Met Glu Pro Ser Lys Ile Ser Gly Leu Ile Val Asn Pro
        355                 360                 365
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 367 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Pro Gly Ala Thr Phe Asp Glu Leu Ile Gly Arg Pro Ile Gly Glu Phe
 1               5                  10                  15

Ser Arg Thr His Met Thr Leu Asn Ala Pro Gly Val Leu Ala Glu Asn
            20                  25                  30

Pro Asp Ile Phe Ala Val Ile Ile Ile Leu Ile Leu Thr Gly Leu Leu
        35                  40                  45

Thr Leu Gly Val Lys Glu Ser Ala Met Val Asn Lys Ile Phe Thr Cys
    50                  55                  60

Ile Asn Val Leu Val Leu Gly Phe Ile Met Val Ser Gly Phe Val Lys
65                  70                  75                  80

Gly Ser Val Lys Asn Trp Gln Leu Thr Glu Glu Asp Phe Gly Asn Thr
                85                  90                  95

Ser Gly Arg Leu Cys Leu Asn Asn Asp Thr Lys Glu Gly Lys Pro Gly
            100                 105                 110

Val Gly Gly Phe Met Pro Phe Gly Phe Ser Gly Val Leu Ser Gly Ala
        115                 120                 125

Ala Thr Cys Phe Tyr Ala Phe Val Gly Phe Asp Cys Ile Ala Thr Thr
    130                 135                 140

Gly Glu Glu Val Lys Asn Pro Gln Lys Ala Ile Pro Val Gly Ile Val
145                 150                 155                 160

Ala Ser Leu Leu Ile Cys Phe Ile Ala Tyr Phe Gly Val Ser Ala Ala
                165                 170                 175

Leu Thr Leu Met Met Pro Tyr Phe Cys Leu Asp Asn Asn Ser Pro Leu
            180                 185                 190

Pro Asp Ala Phe Lys His Val Gly Trp Glu Gly Ala Lys Tyr Ala Val
        195                 200                 205

Ala Val Gly Ser Leu Cys Ala Leu Ser Ala Ser Leu Leu Gly Ser Met
```

```
    210                 215                 220

Phe Pro Met Pro Arg Val Ile Tyr Ala Met Ala Glu Asp Gly Leu Leu
225                 230                 235                 240

Phe Lys Phe Leu Ala Asn Val Asn Asp Arg Thr Lys Thr Pro Ile Ile
                245                 250                 255

Ala Thr Leu Ala Ser Gly Ala Val Ala Ala Val Met Ala Phe Leu Phe
            260                 265                 270

Asp Leu Lys Asp Leu Val Asp Leu Met Ser Ile Gly Thr Leu Leu Ala
        275                 280                 285

Tyr Ser Leu Val Ala Ala Cys Val Leu Val Leu Arg Tyr Gln Pro Glu
    290                 295                 300

Gln Pro Asn Leu Val Tyr Gln Met Ala Ser Thr Ser Asp Glu Leu Asp
305                 310                 315                 320

Pro Ala Asp Gln Asn Glu Leu Ala Ser Thr Asn Asp Ser Gln Leu Gly
                325                 330                 335

Phe Leu Pro Glu Ala Glu Met Phe Ser Leu Lys Thr Ile Leu Ser Pro
            340                 345                 350

Lys Asn Met Glu Pro Ser Lys Ile Ser Gly Leu Ile Val Asn Pro
        355                 360                 365
```

4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2425 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 199..2064

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

```
GATTCCGCCC GCGTGCGCCA TCCCCTCAGC TAGCAGGTGT GAGAGGCTTT CTACCCGCGG   60

TCTCCACACA GCTCAACATC TTGCCGCCTC CTCCGAGCCT GAAGCTACCG TGGACTCTGC  120

TGTGGCGTCT TGGCCCCCAG GTGCGGATCC TCCCCAGTGA GAAGTCCCAC GAGTCTTACA  180

GCAGATTCGC TCAGCACA ATG GGC TGC AAA AAC CTG CTC GGT CTG GGC CAG     231
                   Met Gly Cys Lys Asn Leu Leu Gly Leu Gly Gln
                       1               5                  10

CAG ATG CTG CGC CGG AAG GTG GTG GAC TGC AGC CGG GAG GAG AGC CGG    279
Gln Met Leu Arg Arg Lys Val Val Asp Cys Ser Arg Glu Glu Ser Arg
            15                  20                  25

CTG TCC CGC TGC CTC AAC ACC TAT GAC CTG GTA GCT CTT GGG GTG GGC    327
Leu Ser Arg Cys Leu Asn Thr Tyr Asp Leu Val Ala Leu Gly Val Gly
        30                  35                  40
```

```
AGC ACC TTG GGC GCT GGT GTC TAT GTC CTA GCC GGT GCC GTG GCC CGT   375
Ser Thr Leu Gly Ala Gly Val Tyr Val Leu Ala Gly Ala Val Ala Arg
     45                  50                  55

GAA AAT GCT GGC CCT GCC ATC GTC ATC TCC TTC TTG ATT GCT GCT CTC   423
Glu Asn Ala Gly Pro Ala Ile Val Ile Ser Phe Leu Ile Ala Ala Leu
 60                  65                  70                  75

GCC TCC GTG CTG GCC GGC CTG TGC TAC GGC GAG TTT GGT GCC CGT GTC   471
Ala Ser Val Leu Ala Gly Leu Cys Tyr Gly Glu Phe Gly Ala Arg Val
                 80                  85                  90

CCC AAG ACG GGC TCA GCC TAC CTC TAC AGC TAC GTG ACG GTG GGG GAG   519
Pro Lys Thr Gly Ser Ala Tyr Leu Tyr Ser Tyr Val Thr Val Gly Glu
            95                 100                 105

CTT TGG GCC TTC ATC ACT GGC TGG AAC CTG ATT CTC TCC TAC ATC ATC   567
Leu Trp Ala Phe Ile Thr Gly Trp Asn Leu Ile Leu Ser Tyr Ile Ile
        110                 115                 120

GGT ACT TCA AGC GTG GCA AGA GCC TGG AGT GCG ACT TTT GAC GAG CTG   615
Gly Thr Ser Ser Val Ala Arg Ala Trp Ser Ala Thr Phe Asp Glu Leu
    125                 130                 135

ATA GGC AAG CCC ATC GGA GAG TTC TCA CGT CAG CAC ATG GCC CTG AAT   663
Ile Gly Lys Pro Ile Gly Glu Phe Ser Arg Gln His Met Ala Leu Asn
140                 145                 150                 155

GCT CCT GGG GTG CTG GCC CAA ACC CCG GAC ATA TTT GCT GTG ATT ATA   711
Ala Pro Gly Val Leu Ala Gln Thr Pro Asp Ile Phe Ala Val Ile Ile
                160                 165                 170

ATT ATC ATC TTA ACA GGA CTG TTA ACT CTT GGC GTG AAG GAG TCA GCC   759
Ile Ile Ile Leu Thr Gly Leu Leu Thr Leu Gly Val Lys Glu Ser Ala
            175                 180                 185

ATG GTC AAC AAA ATT TTC ACC TGT ATC AAT GTC CTG GTC TTG TGC TTC   807
Met Val Asn Lys Ile Phe Thr Cys Ile Asn Val Leu Val Leu Cys Phe
        190                 195                 200

ATC GTG GTG TCC GGG TTC GTG AAA GGC TCC ATT AAA AAC TGG CAG CTC   855
Ile Val Val Ser Gly Phe Val Lys Gly Ser Ile Lys Asn Trp Gln Leu
    205                 210                 215

ACG GAG AAA AAT TTC TCC TGT AAC AAC AAC GAC ACA AAC GTG AAA TAC   903
Thr Glu Lys Asn Phe Ser Cys Asn Asn Asn Asp Thr Asn Val Lys Tyr
220                 225                 230                 235

GGT GAG GGA GGG TTT ATG CCC TTT GGA TTC TCT GGT GTC CTG TCA GGG   951
Gly Glu Gly Gly Phe Met Pro Phe Gly Phe Ser Gly Val Leu Ser Gly
                240                 245                 250

GCA GCG ACC TGC TTT TAT GCC TTC GTG GGC TTT GAC TGC ATC GCC ACC   999
Ala Ala Thr Cys Phe Tyr Ala Phe Val Gly Phe Asp Cys Ile Ala Thr
            255                 260                 265

ACA GGG GAA GAA GTC AAG AAC CCC CAG AAG GCC ATT CCT GTG GGC ATC  1047
Thr Gly Glu Glu Val Lys Asn Pro Gln Lys Ala Ile Pro Val Gly Ile
        270                 275                 280

GTG GCG TCC CTC CTC ATT TGC TTC ATA GCG TAC TTT GGC GTG TCC GCC  1095
Val Ala Ser Leu Leu Ile Cys Phe Ile Ala Tyr Phe Gly Val Ser Ala
    285                 290                 295

GCT CTC ACG CTC ATG ATG CCT TAC TTC TGC CTG GAC ATC GAC AGC CCG  1143
Ala Leu Thr Leu Met Met Pro Tyr Phe Cys Leu Asp Ile Asp Ser Pro
300                 305                 310                 315
```

```
CTG CCT GGT GCC TTC AAG CAC CAG GGC TGG GAA GAA GCT AAG TAC GCA  1191
Leu Pro Gly Ala Phe Lys His Gln Gly Trp Glu Glu Ala Lys Tyr Ala
            320                 325                 330

GTG GCC ATT GGC TCT CTC TGC GCA CTT TCC ACC AGT CTC CTA GGC TCC  1239
Val Ala Ile Gly Ser Leu Cys Ala Leu Ser Thr Ser Leu Leu Gly Ser
        335                 340                 345

ATG TTT CCC ATG CCC CGA GTT ATC TAT GCC ATG GCT GAA GAT GGA CTA  1287
Met Phe Pro Met Pro Arg Val Ile Tyr Ala Met Ala Glu Asp Gly Leu
    350                 355                 360

CTG TTT AAA TTT TTG GCC AAA ATC AAC AAT AGG ACC AAA ACA CCC GTA  1335
Leu Phe Lys Phe Leu Ala Lys Ile Asn Asn Arg Thr Lys Thr Pro Val
365                 370                 375

ATC GCC ACT GTG ACC TCA GGC GCC ATT GCT GCT GTG ATG GCC TTC CTC  1383
Ile Ala Thr Val Thr Ser Gly Ala Ile Ala Ala Val Met Ala Phe Leu
380                 385                 390                 395

TTT GAA CTG AAG GAC CTG GTG GAC CTC ATG TCC ATT GGC ACT CTC CTG  1431
Phe Glu Leu Lys Asp Leu Val Asp Leu Met Ser Ile Gly Thr Leu Leu
            400                 405                 410

GCT TAC TCT TTG GTG GCT GCC TGT GTT TTG GTC TTA CGG TAC CAG CCA  1479
Ala Tyr Ser Leu Val Ala Ala Cys Val Leu Val Leu Arg Tyr Gln Pro
        415                 420                 425

GAA CAA CCT AAT CTG GTA TAC CAG ATG GCC AGA ACC ACC GAG GAG CTA  1527
Glu Gln Pro Asn Leu Val Tyr Gln Met Ala Arg Thr Thr Glu Glu Leu
    430                 435                 440

GAT CGA GTA GAT CAG AAT GAG CTG GTC AGT GCC AGT GAA TCA CAG ACA  1575
Asp Arg Val Asp Gln Asn Glu Leu Val Ser Ala Ser Glu Ser Gln Thr
445                 450                 455

GGC TTT TTA CCG GTA GCC GAG AAG TTT TCT CTG AAA TCC ATC CTC TCA  1623
Gly Phe Leu Pro Val Ala Glu Lys Phe Ser Leu Lys Ser Ile Leu Ser
460                 465                 470                 475

CCC AAG AAC GTG GAG CCC TCC AAA TTC TCA GGG CTA ATT GTG AAC ATT  1671
Pro Lys Asn Val Glu Pro Ser Lys Phe Ser Gly Leu Ile Val Asn Ile
            480                 485                 490

TCA GCC GGC CTC CTA GCC GCT CTT ATC ATC ACC GTG TGC ATT GTG GCC  1719
Ser Ala Gly Leu Leu Ala Ala Leu Ile Ile Thr Val Cys Ile Val Ala
        495                 500                 505

GTG CTT GGA AGA GAG GCC CTG GCC GAA GGG ACA CTG TGG GCA GTC TTT  1767
Val Leu Gly Arg Glu Ala Leu Ala Glu Gly Thr Leu Trp Ala Val Phe
    510                 515                 520

GTA ATG ACA GGG TCA GTC CTC CTC TGC ATG CTG GTG ACA GGC ATC ATC  1815
Val Met Thr Gly Ser Val Leu Leu Cys Met Leu Val Thr Gly Ile Ile
525                 530                 535

TGG AGA CAG CCT GAG AGC AAG ACC AAG CTC TCA TTT AAG GTA CCC TTT  1863
Trp Arg Gln Pro Glu Ser Lys Thr Lys Leu Ser Phe Lys Val Pro Phe
540                 545                 550                 555

GTC CCC GTA CTT CCT GTC TTG AGC ATC TTC GTG AAC ATC TAT CTC ATG  1911
Val Pro Val Leu Pro Val Leu Ser Ile Phe Val Asn Ile Tyr Leu Met
            560                 565                 570

ATG CAG CTG GAC CAG GGC ACG TGG GTC CGG TTT GCA GTG TGG ATG CTG  1959
Met Gln Leu Asp Gln Gly Thr Trp Val Arg Phe Ala Val Trp Met Leu
        575                 580                 585
```

```
ATA GGT TTC ACC ATC TAT TTC GGT TAT GGG ATC TGG CAC AGT GAG GAA  2007
Ile Gly Phe Thr Ile Tyr Phe Gly Tyr Gly Ile Trp His Ser Glu Glu
        590                 595                 600

GCG TCC CTG GCT GCT GGC CAG GCA AAG ACT CCT GAC AGC AAC TTG GAC  2055
Ala Ser Leu Ala Ala Gly Gln Ala Lys Thr Pro Asp Ser Asn Leu Asp
    605                 610                 615

CAG TGC AAA TGACGTGCAG CCCCACCCAC CAGGGTGACA GCGGTTGACG          2104
Gln Cys Lys
620

GGTGCCCGTA GAAGCCTGGG ACCCTCACAA TCTCTCCACT CATGCCTCAG GATCAGCTCA 2164

CACCCCCAAT GTCACCAAAG CTGGTTTGCT GCCAGCTCGT GAGATCCTGG TCATTTCTGG 2224
ACAGTCCCTT GGTTTACTCA TCTCCCTCTG AACAAAGAAA GCAGCCCTTC TCCTTGCCGG 2284

CCGGCCGGGC GCTTCGCTGC TGCGGCCCCA GCAGAAGGGA GGCCCCCTTC TCCTCTCACT 2344
TGGGAAGCAG GCCTCCCTCC CTCCCTGGGA CCACCCTGGC ATCGCCCATG TGCACACTCC 2404
AGATGGCTAG TGAGCCTCTC C                                          2425
```

(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 622 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Gly Cys Lys Asn Leu Leu Gly Leu Gly Gln Gln Met Leu Arg Arg
1               5                  10                  15

Lys Val Val Asp Cys Ser Arg Glu Glu Ser Arg Leu Ser Arg Cys Leu
            20                  25                  30

Asn Thr Tyr Asp Leu Val Ala Leu Gly Val Gly Ser Thr Leu Gly Ala
        35                  40                  45

Gly Val Tyr Val Leu Ala Gly Ala Val Ala Arg Glu Asn Ala Gly Pro
    50                  55                  60

Ala Ile Val Ile Ser Phe Leu Ile Ala Ala Leu Ala Ser Val Leu Ala
65                  70                  75                  80

Gly Leu Cys Tyr Gly Glu Phe Gly Ala Arg Val Pro Lys Thr Gly Ser
                85                  90                  95

Ala Tyr Leu Tyr Ser Tyr Val Thr Val Gly Glu Leu Trp Ala Phe Ile
            100                 105                 110

Thr Gly Trp Asn Leu Ile Leu Ser Tyr Ile Ile Gly Thr Ser Ser Val
        115                 120                 125

Ala Arg Ala Trp Ser Ala Thr Phe Asp Glu Leu Ile Gly Lys Pro Ile
    130                 135                 140

Gly Glu Phe Ser Arg Gln His Met Ala Leu Asn Ala Pro Gly Val Leu
145                 150                 155                 160

Ala Gln Thr Pro Asp Ile Phe Ala Val Ile Ile Ile Ile Ile Leu Thr
                165                 170                 175
```

Gly Leu Leu Thr Leu Gly Val Lys Glu Ser Ala Met Val Asn Lys Ile
180 185 190

Phe Thr Cys Ile Asn Val Leu Val Leu Cys Phe Ile Val Val Ser Gly
195 200 205

Phe Val Lys Gly Ser Ile Lys Asn Trp Gln Leu Thr Glu Lys Asn Phe
210 215 220

Ser Cys Asn Asn Asn Asp Thr Asn Val Lys Tyr Gly Glu Gly Gly Phe
225 230 235 240

Met Pro Phe Gly Phe Ser Gly Val Leu Ser Gly Ala Ala Thr Cys Phe
245 250 255

Tyr Ala Phe Val Gly Phe Asp Cys Ile Ala Thr Thr Gly Glu Glu Val
260 265 270

Lys Asn Pro Gln Lys Ala Ile Pro Val Gly Ile Val Ala Ser Leu Leu
275 280 285

Ile Cys Phe Ile Ala Tyr Phe Gly Val Ser Ala Ala Leu Thr Leu Met
290 295 300

Met Pro Tyr Phe Cys Leu Asp Ile Asp Ser Pro Leu Pro Gly Ala Phe
305 310 315 320

Lys His Gln Gly Trp Glu Glu Ala Lys Tyr Ala Val Ala Ile Gly Ser
325 330 335

Leu Cys Ala Leu Ser Thr Ser Leu Leu Gly Ser Met Phe Pro Met Pro
340 345 350

Arg Val Ile Tyr Ala Met Ala Glu Asp Gly Leu Leu Phe Lys Phe Leu
355 360 365

Ala Lys Ile Asn Asn Arg Thr Lys Thr Pro Val Ile Ala Thr Val Thr
370 375 380

Ser Gly Ala Ile Ala Ala Val Met Ala Phe Leu Phe Glu Leu Lys Asp
385 390 395 400

Leu Val Asp Leu Met Ser Ile Gly Thr Leu Leu Ala Tyr Ser Leu Val
405 410 415

Ala Ala Cys Val Leu Val Leu Arg Tyr Gln Pro Glu Gln Pro Asn Leu
420 425 430

Val Tyr Gln Met Ala Arg Thr Thr Glu Glu Leu Asp Arg Val Asp Gln
435 440 445

Asn Glu Leu Val Ser Ala Ser Glu Ser Gln Thr Gly Phe Leu Pro Val
450 455 460

Ala Glu Lys Phe Ser Leu Lys Ser Ile Leu Ser Pro Lys Asn Val Glu
465 470 475 480

Pro Ser Lys Phe Ser Gly Leu Ile Val Asn Ile Ser Ala Gly Leu Leu
485 490 495

Ala Ala Leu Ile Ile Thr Val Cys Ile Val Ala Val Leu Gly Arg Glu
500 505 510

Ala Leu Ala Glu Gly Thr Leu Trp Ala Val Phe Val Met Thr Gly Ser
515 520 525

```
Val Leu Leu Cys Met Leu Val Thr Gly Ile Ile Trp Arg Gln Pro Glu
    530                 535                 540

Ser Lys Thr Lys Leu Ser Phe Lys Val Pro Phe Val Pro Val Leu Pro
545                 550                 555                 560

Val Leu Ser Ile Phe Val Asn Ile Tyr Leu Met Met Gln Leu Asp Gln
                565                 570                 575

Gly Thr Trp Val Arg Phe Ala Val Trp Met Leu Ile Gly Phe Thr Ile
            580                 585                 590


Tyr Phe Gly Tyr Gly Ile Trp His Ser Glu Glu Ala Ser Leu Ala Ala
        595                 600                 605

Gly Gln Ala Lys Thr Pro Asp Ser Asn Leu Asp Gln Cys Lys
    610                 615                 620
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2397 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:410..1768

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

```
GGGTGTCTTT CCTCATCGCT GCCCTGGCCT CGGTTATGGC CGGCCTTTGC TATGCTGAAT   60
TTGGGGCCCG AGTACCCAAG ACTGGATCTG CGTATCTATA CACTTACGTC ACGGTCGGAG  120
AGCTGTGGGC CTTCATCACT GGCTGGAATC TCATCCTGTC ATATGTCATA GGTACGTCCA  180
GTGTCGCAAG AGCATGGAGT GGCACCTTTG ACGAACTTCT TAATAAACAG ATTGGCCAGT  240
TTTTCAAAAC GTACTTCAAA ATGAATTACA CTGGTCTGGC AGAGTATCCA GACTTCTTTG  300
CCGTGTGCCT TGTATTACTC CTGGCAGGTC TTTTATCTTT TGGAGTAAAA GAGTCTGCTT  360
GGGTGAATAA ATTTTTACAG CTATTAATAT CCTGGTCCTT CTCTTTGTC ATG GTG      415
                                                      Met Val
                                                        1

GCT GGG TTT GTG AAA GGA AAT GTG GCT AAC TGG AAG ATC AGT GAA GAG    463
Ala Gly Phe Val Lys Gly Asn Val Ala Asn Trp Lys Ile Ser Glu Glu
          5                  10                  15

TTT CTC AAA AAT ATA TCA GCA AGT GCT AGA GAA CCA CCT TCT GAG AAC    511
Phe Leu Lys Asn Ile Ser Ala Ser Ala Arg Glu Pro Pro Ser Glu Asn
      20                  25                  30

GGA ACA AGC ATC TAC GGG GCT GGC GGC TTT ATG CCC TAT GGC TTT ACA    559
Gly Thr Ser Ile Tyr Gly Ala Gly Gly Phe Met Pro Tyr Gly Phe Thr
  35                  40                  45                  50

GGG ACG TTG GCT GGT GCT GCA ACG TGC TTT TAT GCC TTT GTG GGC TTT    607
Gly Thr Leu Ala Gly Ala Ala Thr Cys Phe Tyr Ala Phe Val Gly Phe
                  55                  60                  65

GAC TGC ATT GCA ACA ACC GGT GAA GAG GTT CGG AAT CCA CAA AAG GCG    655
Asp Cys Ile Ala Thr Thr Gly Glu Glu Val Arg Asn Pro Gln Lys Ala
```

```
                70                  75                  80

ATC CCC ATC GGA ATA GTG ACG TCC TTA CTT GTC TGC TTT ATG GCT TAC    703
Ile Pro Ile Gly Ile Val Thr Ser Leu Leu Val Cys Phe Met Ala Tyr
         85                  90                  95

TTT GGG GTT TCT GCA GCT TTA ACG CTT ATG ATG CCT TAC TAC CTC CTG    751
Phe Gly Val Ser Ala Ala Leu Thr Leu Met Met Pro Tyr Tyr Leu Leu
    100                 105                 110

GAT GAG AAA AGT CCA CTC CCA GTC GCG TTT GAG TAT GTC AGA TGG GGC    799
Asp Glu Lys Ser Pro Leu Pro Val Ala Phe Glu Tyr Val Arg Trp Gly
115                 120                 125                 130

CCC GCC AAA TAC GTT GTC GCA GCA GGC TCC CTC TGC GCC TTA TCA ACA    847
Pro Ala Lys Tyr Val Val Ala Ala Gly Ser Leu Cys Ala Leu Ser Thr
                135                 140                 145

AGT CTT CTT GGA TCC ATT TTC CCA ATG CCT CGT GTA ATC TAT GCT ATG    895
Ser Leu Leu Gly Ser Ile Phe Pro Met Pro Arg Val Ile Tyr Ala Met
            150                 155                 160

GCG GAG GAT GGG TTG CTT TTC AAA TGT CTA GCT CAA ATC AAT TCC AAA    943
Ala Glu Asp Gly Leu Leu Phe Lys Cys Leu Ala Gln Ile Asn Ser Lys
        165                 170                 175

ACG AAG ACA CCA GTA ATT GCT ACT TTG TCA TCG GGT GCA GTG GCA GCT    991
Thr Lys Thr Pro Val Ile Ala Thr Leu Ser Ser Gly Ala Val Ala Ala
    180                 185                 190

GTG ATG GCC TTT CTT TTT GAC CTG AAG GCC CTC GTG GAC ATG ATG TCT    039
Val Met Ala Phe Leu Phe Asp Leu Lys Ala Leu Val Asp Met Met Ser
195                 200                 205                 210

ATT GGC ACC CTC ATG GCC TAC TCT CTG GTG GCA GCC TGT GTG CTT ATT    087
Ile Gly Thr Leu Met Ala Tyr Ser Leu Val Ala Ala Cys Val Leu Ile
                215                 220                 225

CTC AGG TAC CAA CCT GGC TTG TGT TAC GAG CAG CCC AAA TAC ACC CCT   1135
Leu Arg Tyr Gln Pro Gly Leu Cys Tyr Glu Gln Pro Lys Tyr Thr Pro
            230                 235                 240

GAG AAA GAA ACT CTG GAA TCA TGT ACC AAT GCG ACT TTG AAG AGC GAG   1183
Glu Lys Glu Thr Leu Glu Ser Cys Thr Asn Ala Thr Leu Lys Ser Glu
        245                 250                 255

TCC CAG GTC ACC ATG CTG CAA GGA CAG GGT TTC AGC CTA CGA ACC CTC   1231
Ser Gln Val Thr Met Leu Gln Gly Gln Gly Phe Ser Leu Arg Thr Leu
    260                 265                 270

TTC AGC CCC TCT GCC CTG CCC ACA CGA CAG TCG ECT TCC CTT GTG AGC   1279
Phe Ser Pro Ser Ala Leu Pro Thr Arg Gln Ser Ala Ser Leu Val Ser
275                 280                 285                 290

TTT CTG GTG GGA TTC CTG GCT TTC CTC ATC CTG GGC TTG AGT ATT CTA   1327
Phe Leu Val Gly Phe Leu Ala Phe Leu Ile Leu Gly Leu Ser Ile Leu
                295                 300                 305

ACC ACG TAT GGC GTC CAG GCC ATT GCC AGA CTG GAA GCC TGG AGC CTG   1375
Thr Thr Tyr Gly Val Gln Ala Ile Ala Arg Leu Glu Ala Trp Ser Leu
            310                 315                 320

GCT CTT CTC GCC CTG TTC CTT GTC CTC TGC GCT GCC GTC ATT CTG ACC   1423
Ala Leu Leu Ala Leu Phe Leu Val Leu Cys Ala Ala Val Ile Leu Thr
        325                 330                 335

ATT TGG AGG CAG CCA CAG AAT CAG CAA AAA GTA GCC TTC ATG GTC CCG   1471
Ile Trp Arg Gln Pro Gln Asn Gln Gln Lys Val Ala Phe Met Val Pro
```

```
    340                 345                 350

TTC TTA CCG TTT CTG CCG GCC TTC AGC ATC CTG GTC AAC ATT TAC TTG   1519.
Phe Leu Pro Phe Leu Pro Ala Phe Ser Ile Leu Val Asn Ile Tyr Leu
355                 360                 365                 370

ATG GTC CAG TTA AGT GCG GAC ACT TGG ATC AGA TTC AGC ATC TGG ATG   1567
Met Val Gln Leu Ser Ala Asp Thr Trp Ile Arg Phe Ser Ile Trp Met
                375                 380                 385

GCG CTT GGC TTT CTG ATC TAT TTC GCC TAT GGC ATT AGA CAC AGC TTG   1615
Ala Leu Gly Phe Leu Ile Tyr Phe Ala Tyr Gly Ile Arg His Ser Leu
            390                 395                 400

GAG GGT AAC CCC AGG GAC GAA GAA GAC GAT GAG GAT GCC TTT TCA GAA   1663
Glu Gly Asn Pro Arg Asp Glu Glu Asp Asp Glu Asp Ala Phe Ser Glu
        405                 410                 415

AAC ATC AAT GTA GCA ACA GAA GAA AAG TCC GTC ATG CAA GCA AAT GAC   1711
Asn Ile Asn Val Ala Thr Glu Glu Lys Ser Val Met Gln Ala Asn Asp
    420                 425                 430

CAT CAC CAA AGA AAC CTC AGC TTA CCT TTC ATA CTT CAT GAA AAG ACA   1759
His His Gln Arg Asn Leu Ser Leu Pro Phe Ile Leu His Glu Lys Thr
435                 440                 445                 450

AGT GAA TGT TGATGCTGGC CCTCGGTCTT ACCACGCATA CCTTAACAAT           1808
Ser Glu Cys

GAGTACACTG TGGCCGGATG CCACCATCGT GCTGGGCTGT CGTGGGTCTG CTGTGGACAT 1868

GGCTTGCCTA ACTTGTACTT CCTCCTCCAG ACAGCTTCTC TTCAGATGGT GGATTCTGTG 1928

TCTGAGGAGA CTGCCTGAGA GCACTCCTCA GCTATATGTA TCCCCAAAAC AGTATGTCCG 1988

TGTGCGTACA TGTATGTCTG CGATGTGAGT GTTCAATGTT GTCCGTTATT AGTCTGTGAC 2048

ATAATTCCAG CATGGTAATT GGTGGCATAT ACTGCACACA CTAGTAAACA GTATATTGCT 2108

GAATAGAGAT GTATTCTGTA TATGTCCTAG GTGGCTGGGG AAATAGTGGT GGTTTCTTTA 2168

TTAGGTATAT GACCATCAGT TTGGACATAC TGAAATGCCA TCCCCTGTCA GGATGTTTAA 2228

CAGTGGTCAT GGGTGGGGAA GGGATAAGGA ATGGGCATTG TCTATAAATT GTAATGCATA 2288

TATCCTTCTC CTACTTGCTA AGACAGCTTT CTTAAACGGC CAGGGAGAGT GTTTCTTTCC 2348

TCTGTATGAC AAGATGAAGA GGTAGTCTGT GGCTGGAGAT GGCCAATCC             2397
```

(7) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 453 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Val Ala Gly Phe Val Lys Gly Asn Val Ala Asn Trp Lys Ile Ser
1               5                  10                  15
```

Glu Glu Phe Leu Lys Asn Ile Ser Ala Ser Ala Arg Glu Pro Pro Ser
20 25 30

Glu Asn Gly Thr Ser Ile Tyr Gly Ala Gly Gly Phe Met Pro Tyr Gly
35 40 45

Phe Thr Gly Thr Leu Ala Gly Ala Ala Thr Cys Phe Tyr Ala Phe Val
50 55 60

Gly Phe Asp Cys Ile Ala Thr Thr Gly Glu Glu Val Arg Asn Pro Gln
65 70 75 80

Lys Ala Ile Pro Ile Gly Ile Val Thr Ser Leu Leu Val Cys Phe Met
85 90 95

Ala Tyr Phe Gly Val Ser Ala Ala Leu Thr Leu Met Met Pro Tyr Tyr
100 105 110

Leu Leu Asp Glu Lys Ser Pro Leu Pro Val Ala Phe Glu Tyr Val Arg
115 120 125

Trp Gly Pro Ala Lys Tyr Val Val Ala Ala Gly Ser Leu Cys Ala Leu
130 135 140

Ser Thr Ser Leu Leu Gly Ser Ile Phe Pro Met Pro Arg Val Ile Tyr
145 150 155 160

Ala Met Ala Glu Asp Gly Leu Leu Phe Lys Cys Leu Ala Gln Ile Asn
165 170 175

Ser Lys Thr Lys Thr Pro Val Ile Ala Thr Leu Ser Ser Gly Ala Val
180 185 190

Ala Ala Val Met Ala Phe Leu Phe Asp Leu Lys Ala Leu Val Asp Met
195 200 205

Met Ser Ile Gly Thr Leu Met Ala Tyr Ser Leu Val Ala Ala Cys Val
210 215 220

Leu Ile Leu Arg Tyr Gln Pro Gly Leu Cys Tyr Glu Gln Pro Lys Tyr
225 230 235 240

Thr Pro Glu Lys Glu Thr Leu Glu Ser Cys Thr Asn Ala Thr Leu Lys
245 250 255

Ser Glu Ser Gln Val Thr Met Leu Gln Gly Gln Gly Phe Ser Leu Arg
260 265 270

Thr Leu Phe Ser Pro Ser Ala Leu Pro Thr Arg Gln Ser Ala Ser Leu
275 280 285

Val Ser Phe Leu Val Gly Phe Leu Ala Phe Leu Ile Leu Gly Leu Ser
290 295 300

Ile Leu Thr Thr Tyr Gly Val Gln Ala Ile Ala Arg Leu Glu Ala Trp
305 310 315 320

Ser Leu Ala Leu Leu Ala Leu Phe Leu Val Leu Cys Ala Ala Val Ile
325 330 335

Leu Thr Ile Trp Arg Gln Pro Gln Asn Gln Gln Lys Val Ala Phe Met
340 345 350

Val Pro Phe Leu Pro Phe Leu Pro Ala Phe Ser Ile Leu Val Asn Ile
355 360 365

Tyr Leu Met Val Gln Leu Ser Ala Asp Thr Trp Ile Arg Phe Ser Ile
370 375 380

```
Trp Met Ala Leu Gly Phe Leu Ile Tyr Phe Ala Tyr Gly Ile Arg His
385                 390                 395                 400

Ser Leu Glu Gly Asn Pro Arg Asp Glu Glu Asp Asp Glu Asp Ala Phe
                405                 410                 415

Ser Glu Asn Ile Asn Val Ala Thr Glu Glu Lys Ser Val Met Gln Ala
            420                 425                 430

Asn Asp His His Gln Arg Asn Leu Ser Leu Pro Phe Ile Leu His Glu
        435                 440                 445

Lys Thr Ser Glu Cys
    450
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2157 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 148..2034

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

```
CGATCCTGCC GGAGCCCCGC CGCCGCCGGC TTGGATTCTG AAACCTTCCT TGTATCCCTC   60

CTGAGACATC TTTGCTGCAA GATCGAGGCT GTCCTCTGGT GAGAAGGTGG TGAGGCTTCC  120

CGTCATATTC CAGCTCTGAA CAGCAAC ATG GGG TGC AAA GTC CTG CTC AAC ATT  174
                                 Met Gly Cys Val Leu Leu Asn Ile Ile
                                   5

GGG CAG CAG ATG CTG CGG CGG AAG GTG GTG GAC TGT AGC CGG GAG GAG    222
Gly Gln Gln Met Leu Arg Arg Lys Val Val Asp Cys Ser Arg Glu Glu
 10                  15                  20                  25

ACG CGG CTG TCT CGC TGC CTG AAC ACT TTT GAT CTG GTG GCC CTC GGG    270
Thr Arg Leu Ser Arg Cys Leu Asn Thr Phe Asp Leu Val Ala Leu Gly
                30                  35                  40

GTG GGC AGC ACA CTG GGT GCT GGT GTC TAC GTC CTG GCT GGA GCT GTG    318
Val Gly Ser Thr Leu Gly Ala Gly Val Tyr Val Leu Ala Gly Ala Val
            45                  50                  55

GCC CGT GAG AAT GCA GGC CCT GCC ATT GTC ATC TCC TTC CTG ATC GCT    366
Ala Arg Glu Asn Ala Gly Pro Ala Ile Val Ile Ser Phe Leu Ile Ala
        60                  65                  70

GCG CTG GCC TCA GTG CTG GCT GGC CTG TGC TAT GGC GAG TTT GGT GCT    414
Ala Leu Ala Ser Val Leu Ala Gly Leu Cys Tyr Gly Glu Phe Gly Ala
    75                  80                  85

CGG GTC CCC AAG ACG GGC TCA GCT TAC CTC TAC AGC TAT GTC ACC GTT    462
Arg Val Pro Lys Thr Gly Ser Ala Tyr Leu Tyr Ser Tyr Val Thr Val
 90                  95                  100                 105
```

```
GGA GAG CTC TGG GCC TTC ATC ACC GGC TGG AAC TTA ATC CTC TCC TAC    507
Gly Glu Leu Trp Ala Phe Ile Thr Gly Trp Asn Leu Ile Leu Ser Tyr
                110                 115                 120

ATC ATC GGT ACT TCA AGC GTA GCG AGG GCC TGG AGC GCC ACC TTC GAC    558
Ile Ile Gly Thr Ser Ser Val Ala Arg Ala Trp Ser Ala Thr Phe Asp
            125                 130                 135

GAG CTG ATA GGC AGA CCC ATC GGG GAG TTC TCA CGG ACA CAC ATG ACT    606
Glu Leu Ile Gly Arg Pro Ile Gly Glu Phe Ser Arg Thr His Met Thr
        140                 145                 150


CTG AAC GCC CCC GGC GTG CTG GCT GAA AAC CCC GAC ATA TTC GCA GTG    654
Leu Asn Ala Pro Gly Val Leu Ala Glu Asn Pro Asp Ile Phe Ala Val
    155                 160                 165

ATC ATA ATT CTC ATC TTG ACA GGA CTT TTA ACT CTT GGT GTG AAA GAG    702
Ile Ile Ile Leu Ile Leu Thr Gly Leu Leu Thr Leu Gly Val Lys Glu
170                 175                 180                 185
TCG GCC ATG GTC AAC AAA ATA TTC ACT TGT ATT AAC GTC CTG GTC CTG    750
Ser Ala Met Val Asn Lys Ile Phe Thr Cys Ile Asn Val Leu Val Leu
                190                 195                 200
GGC TTC ATA ATG GTG TCA GGA TTT GTG AAA GGA TCG GTT AAA AAC TGG    798
Gly Phe Ile Met Val Ser Gly Phe Val Lys Gly Ser Val Lys Asn Trp
            205                 210                 215
CAG CTC ACG GAG GAG GAT TTT GGG AAC ACA TCA GGC CGT CTC TGT TTG    846
Gln Leu Thr Glu Glu Asp Phe Gly Asn Thr Ser Gly Arg Leu Cys Leu
      220                 225                 230

AAC AAT GAC ACA AAA GAA GGG AAG CCC GGT GTT GGT GGA TTC ATG CCC    894
Asn Asn Asp Thr Lys Glu Gly Lys Pro Gly Val Gly Gly Phe Met Pro
    235                 240                 245
TTC GGG TTC TCT GGT GTC CTG TCG GGG GCA GCG ACT TGC TTC TAT GCC    942
Phe Gly Phe Ser Gly Val Leu Ser Gly Ala Ala Thr Cys Phe Tyr Ala
250                 255                 260                 265
TTC GTG GGC TTT GAC TGC ATC GCC ACC ACA GGT GAA GAG GTG AAG AAC    990
Phe Val Gly Phe Asp Cys Ile Ala Thr Thr Gly Glu Glu Val Lys Asn
                270                 275                 280
CCA CAG AAG GCC ATC CCC GTG GGG ATC GTG GCG TCC CTC TTG ATC TGC   1038
Pro Gln Lys Ala Ile Pro Val Gly Ile Val Ala Ser Leu Leu Ile Cys
            285                 290                 295
TTC ATC GCC TAC TTT GGG GTG TCG GCT GCC CTC ACG CTC ATG ATG CCC   1086
Phe Ile Ala Tyr Phe Gly Val Ser Ala Ala Leu Thr Leu Met Met Pro
        300                 305                 310

TAC TTC TGC CTG GAC AAT AAC AGC CCC CTG CCC GAC GCC TTT AAG CAC   1134
Tyr Phe Cys Leu Asp Asn Asn Ser Pro Leu Pro Asp Ala Phe Lys His
    315                 320                 325
GTG GGC TGG GAA GGT GCC AAG TAC GCA GTG GCC GTG GGC TCC CTC TGC   1182
Val Gly Trp Glu Gly Ala Lys Tyr Ala Val Ala Val Gly Ser Leu Cys
330                 335                 340                 345
GCT CTT TCC GCC AGT CTT CTA GGT TCC ATG TTT CCC ATG CCT CGG GTT   1230
Ala Leu Ser Ala Ser Leu Leu Gly Ser Met Phe Pro Met Pro Arg Val
                350                 355                 360
ATC TAT GCC ATG GCT GAG GAT GGA CTG CTA TTT AAA TTC TTA GCC AAC   1278
Ile Tyr Ala Met Ala Glu Asp Gly Leu Leu Phe Lys Phe Leu Ala Asn
            365                 370                 375


GTC AAT GAT AGG ACC AAA ACA CCA ATA ATC GCC ACA TTA GCC TCG GGT   1326
Val Asn Asp Arg Thr Lys Thr Pro Ile Ile Ala Thr Leu Ala Ser Gly
        380                 385                 390

GCC GTT GCT GCT GTG ATG GCC TTC CTC TTT GAC CTG AAG GAC TTG GTG   1374
Ala Val Ala Ala Val Met Ala Phe Leu Phe Asp Leu Lys Asp Leu Val
    395                 400                 405
```

```
GAC CTC ATG TCC ATT GGC ACT CTC CTG GCT TAC TCG TTG GTG GCT GCC   1422
Asp Leu Met Ser Ile Gly Thr Leu Leu Ala Tyr Ser Leu Val Ala Ala
410                 415                 420                 425

TGT GTG TTG GTC TTA CGG TAC CAG CCA GAG CAG CCT AAC CTG GTA TAC   1470
Cys Val Leu Val Leu Arg Tyr Gln Pro Glu Gln Pro Asn Leu Val Tyr
                430                 435                 440

CAG ATG GCC AGT ACT TCC GAC GAG TTA GAT CCA GCA GAC CAA AAT GAA   1518
Gln Met Ala Ser Thr Ser Asp Glu Leu Asp Pro Ala Asp Gln Asn Glu
            445                 450                 455

TTG GCA AGC ACC AAT GAT TCC CAG CTG GGG TTT TTA CCA GAG GCA GAG   1566
Leu Ala Ser Thr Asn Asp Ser Gln Leu Gly Phe Leu Pro Glu Ala Glu
        460                 465                 470

ATG TTC TCT TTG AAA ACC ATA CTC TCA CCC AAA AAC ATG GAG CCT TCC   1614
Met Phe Ser Leu Lys Thr Ile Leu Ser Pro Lys Asn Met Glu Pro Ser
    475                 480                 485

AAA ATC TCT GGG CTA ATT GTG AAC ATT TCA ACC AGC CTT ATA GCT GTT   1662
Lys Ile Ser Gly Leu Ile Val Asn Ile Ser Thr Ser Leu Ile Ala Val
490                 495                 500                 505

CTC ATC ATC ACC TTC TGC ATT GTG ACC GTG CTT GGA AGG GAG GCT CTC   1710
Leu Ile Ile Thr Phe Cys Ile Val Thr Val Leu Gly Arg Glu Ala Leu
                510                 515                 520

ACC AAA GGG GCG CTG TGG GCA GTC TTT CTG CTC GCA GGG TCT GCC CTC   1758
Thr Lys Gly Ala Leu Trp Ala Val Phe Leu Leu Ala Gly Ser Ala Leu
            525                 530                 535

CTC TGT GCC GTG GTC ACG GGC GTC ATC TGG AGG CAG CCC GAG AGC AAG   1806
Leu Cys Ala Val Val Thr Gly Val Ile Trp Arg Gln Pro Glu Ser Lys
        540                 545                 550

ACC AAG CTC TCA TTT AAG GTT CCC TTC CTG CCA GTG CTC CCC ATC CTG   1854
Thr Lys Leu Ser Phe Lys Val Pro Phe Leu Pro Val Leu Pro Ile Leu
    555                 560                 565

AGC ATC TTC GTG AAC GTC TAT CTC ATG ATG CAG CTG GAC CAG GGC ACC   1902
Ser Ile Phe Val Asn Val Tyr Leu Met Met Gln Leu Asp Gln Gly Thr
570                 575                 580                 585

TGG GTC CGG TTT GCT GTG TGG ATG CTG ATA GGC TTC ATC ATC TAC TTT   1950
Trp Val Arg Phe Ala Val Trp Met Leu Ile Gly Phe Ile Ile Tyr Phe
                590                 595                 600

GGC TAT GGC CTG TGG CAC AGC GAG GAG GCG TCC CTG GAT GCC GAC CAA   1998
Gly Tyr Gly Leu Trp His Ser Glu Glu Ala Ser Leu Asp Ala Asp Gln
            605                 610                 615

GCA AGG ACT CCT GAC GGC AAC TTG GAC CAG TGC AAG TGACGCACAG        2044
Ala Arg Thr Pro Asp Gly Asn Leu Asp Gln Cys Lys
        620                 625

CCCCGCCCCC CGGAGGTGGC AGCAGCCCCG AGGGACGCCC CCAGAGGACC GGGAGGCACC 2104

CCACCCTCCC CACCAGTGCA ACAGAAACCA CCTGCGTCCA CACCCTCACT GCA        2157
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 629 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Gly Cys Val Leu Leu Asn Ile Ile Gly Gln Gln Met Leu Arg Arg
                5                  10                  15
Lys Val Val Asp Cys Ser Arg Glu Glu Thr Arg Leu Ser Arg Cys Leu
            20                  25                  30

Asn Thr Phe Asp Leu Val Ala Leu Gly Val Gly Ser Thr Leu Gly Ala
        35                  40                  45

Gly Val Tyr Val Leu Ala Gly Ala Val Ala Arg Glu Asn Ala Gly Pro
    50                  55                  60

Ala Ile Val Ile Ser Phe Leu Ile Ala Ala Leu Ala Ser Val Leu Ala
65                  70                  75                  80

Gly Leu Cys Tyr Gly Glu Phe Gly Ala Arg Val Pro Lys Thr Gly Ser
                85                  90                  95

Ala Tyr Leu Tyr Ser Tyr Val Thr Val Gly Glu Leu Trp Ala Phe Ile
            100                 105                 110

Thr Gly Trp Asn Leu Ile Leu Ser Tyr Ile Ile Gly Thr Ser Ser Val
        115                 120                 125

Ala Arg Ala Trp Ser Ala Thr Phe Asp Glu Leu Ile Gly Arg Pro Ile
    130                 135                 140

Gly Glu Phe Ser Arg Thr His Met Thr Leu Asn Ala Pro Gly Val Leu
145                 150                 155                 160

Ala Glu Asn Pro Asp Ile Phe Ala Val Ile Ile Ile Leu Ile Leu Thr
                165                 170                 175

Gly Leu Leu Thr Leu Gly Val Lys Glu Ser Ala Met Val Asn Lys Ile
            180                 185                 190

Phe Thr Cys Ile Asn Val Leu Val Leu Gly Phe Ile Met Val Ser Gly
        195                 200                 205

Phe Val Lys Gly Ser Val Lys Asn Trp Gln Leu Thr Glu Glu Asp Phe
    210                 215                 220

Gly Asn Thr Ser Gly Arg Leu Cys Leu Asn Asn Asp Thr Lys Glu Gly
225                 230                 235                 240

Lys Pro Gly Val Gly Gly Phe Met Pro Phe Gly Phe Ser Gly Val Leu
                245                 250                 255

Ser Gly Ala Ala Thr Cys Phe Tyr Ala Phe Val Gly Phe Asp Cys Ile
            260                 265                 270

Ala Thr Thr Gly Glu Glu Val Lys Asn Pro Gln Lys Ala Ile Pro Val
        275                 280                 285

Gly Ile Val Ala Ser Leu Leu Ile Cys Phe Ile Ala Tyr Phe Gly Val
    290                 295                 300

Ser Ala Ala Leu Thr Leu Met Met Pro Tyr Phe Cys Leu Asp Asn Asn
305                 310                 315                 320

Ser Pro Leu Pro Asp Ala Phe Lys His Val Gly Trp Glu Gly Ala Lys
                325                 330                 335
```

```
Tyr Ala Val Ala Val Gly Ser Leu Cys Ala Leu Ser Ala Ser Leu Leu
            340                 345                 350

Gly Ser Met Phe Pro Met Pro Arg Val Ile Tyr Ala Met Ala Glu Asp
        355                 360                 365

Gly Leu Leu Phe Lys Phe Leu Ala Asn Val Asn Asp Arg Thr Lys Thr
    370                 375                 380

Pro Ile Ile Ala Thr Leu Ala Ser Gly Ala Val Ala Ala Val Met Ala
385                 390                 395                 400

Phe Leu Phe Asp Leu Lys Asp Leu Val Asp Leu Met Ser Ile Gly Thr
                405                 410                 415

Leu Leu Ala Tyr Ser Leu Val Ala Ala Cys Val Leu Val Leu Arg Tyr
            420                 425                 430

Gln Pro Glu Gln Pro Asn Leu Val Tyr Gln Met Ala Ser Thr Ser Asp
        435                 440                 445

Glu Leu Asp Pro Ala Asp Gln Asn Glu Leu Ala Ser Thr Asn Asp Ser
    450                 455                 460

Gln Leu Gly Phe Leu Pro Glu Ala Glu Met Phe Ser Leu Lys Thr Ile
465                 470                 475                 480

Leu Ser Pro Lys Asn Met Glu Pro Ser Lys Ile Ser Gly Leu Ile Val
                485                 490                 495

Asn Ile Ser Thr Ser Leu Ile Ala Val Leu Ile Ile Thr Phe Cys Ile
            500                 505                 510

Val Thr Val Leu Gly Arg Glu Ala Leu Thr Lys Gly Ala Leu Trp Ala
        515                 520                 525

Val Phe Leu Leu Ala Gly Ser Ala Leu Leu Cys Ala Val Val Thr Gly
    530                 535                 540

Val Ile Trp Arg Gln Pro Glu Ser Lys Thr Lys Leu Ser Phe Lys Val
545                 550                 555                 560

Pro Phe Leu Pro Val Leu Pro Ile Leu Ser Ile Phe Val Asn Val Tyr
                565                 570                 575

Leu Met Met Gln Leu Asp Gln Gly Thr Trp Val Arg Phe Ala Val Trp
            580                 585                 590

Met Leu Ile Gly Phe Ile Ile Tyr Phe Gly Tyr Gly Leu Trp His Ser
        595                 600                 605

Glu Glu Ala Ser Leu Asp Ala Asp Gln Ala Arg Thr Pro Asp Gly Asn
    610                 615                 620
```

Leu Asp Gln Cys Lys
625

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
AAAGAAGGGA AGTACGGTGT TGGTGG 26

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
ACACAAAAGA AGTGAAGTAC GGTGTTGGTG G
31

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
AATGACACAA AAAACGTGAA GTACGGTGTT GGTGG
35

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
AAAGAAGGGA AGTACGGTGA GGGTGGATTC ATG
33

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
TGAAGTACGG TGTTGGTGGA TTCATG 26

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
ACACAAAAGA AGTGAAGTAC GGTGA
25

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
AATGACACAA AAAACGTGAA GTACGGTGA
29

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:
AACAATGACA CAAACGTGAA GTACGGTGAG GGTGGATTCA TG
42

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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

(2) INFORMATION FOR SEQ ID NO:18:

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

(ii) MOLECULE TYPE: DNA

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

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:
AAGGCTCCGT TAAAAAC 17

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:
TACAGGAGAA ATCTTCCTCC GTGAGCTG 28

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:
GAGAAAAATT TCGGCAACTG TAACAACAAC 30

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:
AAAAATTTCT CCCGTCTCTG TAACAACAAC 30

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

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

AATTTCTCCT GTTTCAACAA CGACAC 26

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:
TCACCGTATT TCCCTTCTGT GTCGTTGTT 29

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:
ACAAACGTGA AACCCGGTGT GGGAGGGTTT AT 32

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
ACAAACGTGA AACCCGGTGA GGGAGG 26

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:
ATACGGTGTG GGAGGGT 17

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
TCTGCCTGGA CAACAACAGC CCGCTGC 27

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
GCCCGCTGCC TGACGCCTTC AAGCAC 26

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
GCCTTCAAGC ACGTGGGCTG GGAAGGAGCT AAGTACGC 28

(2) INFORMATION FOR SEQ ID NO:31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: DNA
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
GCCTTCAAGC ACGTGGGCTG GGAAGGAGCT AAGTACGC 28

WHAT IS CLAIMED IS

1. A chimeric receptor polypeptide, consisting essentially of a polypeptide having an amino acid sequence of a receptor for a first species specific virus, said receptor sequence modified to contain at least one chimeric viral receptor binding site which binds a viral *env* binding domain of a second species specific virus,
wherein

(a) said first species is different from said second species; and

(b) said chimeric receptor binding site comprises at least a first amino acid residue and a second amino acid residue which confer to said polypeptide binding activity for said second virus *env* binding domain.

2. A chimeric receptor polypeptide according to claim 1, wherein said virus is selected from a retrovirus, an adenovirus, or an adenovirus associated virus.

3. A chimeric receptor polypeptide according to claim 2, wherein said retrovirus is a leukemia virus.

4. A chimeric receptor polypeptide according to claim 3, wherein said leukemia virus is selected from a murine leukemia virus, a hamster leukemia virus and a human leukemia virus.

5. A chimeric receptor polypeptide according to claim 1, wherein said first species is selected from the group consisting of human, mouse, rat, hamster, rabbit, guinea pig, baboon, and monkey.

6. A chimeric receptor polypeptide according to claim 5, wherein said first species is human.

7. A chimeric receptor polypeptide according to claim 6, wherein said second species is non-human.

8. A chimeric receptor polypeptide according to claim 7, wherein said second species is selected from the group consisting of mouse, rat, hamster, baboon, rabbit, guinea pig, and monkey.

9. A chimeric receptor polypeptide according to claim 1, wherein said second species is selected from the group consisting of human, mouse, rat, hamster, baboon, rabbit, guinea pig, and monkey.

10. A chimeric receptor polypeptide according to claim 1, wherein said at least first and second residue replaces an amino acid corresponding to amino acids 210-250 of SEQ ID NO:8.

11. A chimeric receptor polypeptide according to claim 10, wherein

(i) said first residue is tyrosine which replaces an amino acid in said mutant amino acid sequence corresponding to Pro242 of H13 (SEQ ID NO:8); and

(ii) said second residue of said second species specific viral receptor replaces an amino acid selected from the group consisting of Val244, Glu239, Gly240, Gly 225, of SEQ ID NO:8.

12. A chimeric receptor polypeptide according to claim 11, wherein

(i) said second residue is selected from valine, replacing an amino acid corresponding to Gly240 of H13 (SEQ ID NO:8), and glutamine, replacing an amino acid corresponding to Val244 of H13 (SEQ ID NO:8).

13. A chimeric receptor polypeptide according to claim 1, wherein said mutant amino acid sequence further comprises at least one transmembrane domain peptide having an amino acid sequence corresponding to a transmembrane domain of H13 (SEQ ID NO:8).

14. A chimeric receptor polypeptide according to claim 1, wherein said chimeric receptor polypeptide, when expressed in a host cell, is capable of binding said viral binding domain extracellularly.

15. A chimeric receptor polypeptide according to claim 1, wherein the sequence of said polypeptide has at least 80% homology to the corresponding amino acid sequence 210-250 of H13 (SEQ ID NO:8).

16. A chimeric receptor polypeptide according to claim 15, wherein the sequence of said polypeptide has at least 95% homology to the corresponding amino acid sequence 210-250 of SEQ ID NO:8.

17. A method for rendering a eukaryotic cell or tissue susceptible to binding by a viral binding domain of a second species specific virus, comprising

(a) transforming, *in vitro, in vivo or in situ*, said eukaryotic cell or tissue with an expressible mutant nucleic acid encoding a chimeric receptor polypeptide according to claim 1 to produce a chimeric receptor cell or tissue; and

(b) providing conditions such that said at least one chimeric viral receptor binding site of said chimeric receptor polypeptide is expressed in said chimeric receptor cell and is capable of binding an extracellular viral binding domain of said second species specific virus.

18. A method according to claim 17, wherein said *in vivo* transforming is carried out by one selected from

(i) injection of said mutant nucleic acid into said chimeric receptor tissue or cell;

(ii) retroviral infection using a recombinant retrovirus comprising said mutant nucleic acid under control of a tissue specific regulatory sequence specific for said chimeric receptor tissue or cell;

(iii) liposome delivery of said mutant nucleic acid to said chimeric receptor tissue or cell;

(iv) contacting said cell or tissue specific antibody conjugated to said mutant nucleic acid to said chimeric receptor tissue or cell; or

(v) contacting said cell or tissue specific antibody conjugated to said mutant nucleic acid to said tissue or cell.

19. A method according to claim 17, wherein said *in situ* or *in vitro* transforming is carried out by one selected from

(i) injection of said mutant nucleic acid into said chimeric receptor cell or tissue;

(ii) retroviral infection using a recombinant retrovirus comprising said mutant nucleic acid under control of a tissue specific regulatory sequence specific for said chimeric receptor tissue or cell;

(iii) liposome delivery of said mutant nucleic acid;

(iv) transfection of said tissue or cell comprising said nucleic acid; or

(v) contacting said cell or tissue specific antibody conjugated to said mutant nucleic acid to said tissue or cell.

20. A chimeric receptor cell or tissue produced by a method according to claim 17, wherein said eukaryotic

cell or tissue is selected from the group consisting of mammalian, insect, bird and yeast.

21. A chimeric receptor cell according to claim 20, wherein said mammalian cell or tissue is of human, primate, hamster, rabbit, rodent, cow, pig, sheep, horse, goat, dog or cat origin.

22. A method for transferring at least one therapeutic agent or diagnostic agent to a chimeric receptor cell or tissue, comprising:

(a) providing a chimeric receptor cell or tissue according to claim 20;

(b) contacting said chimeric receptor cell or tissue, *in vitro*, *in vivo* or *in situ*, with a delivery vector comprising an *env* binding domain of a non-human virus and said at least one therapeutic or diagnostic agent, such that said delivery vector binds said chimeric modified cell or tissue and said therapeutic or diagnostic agent has a therapeutic or diagnostic effect on said chimeric receptor cell or tissue.

23. A method according to claim 22, wherein said therapeutic agent is a therapeutic nucleic acid and wherein said therapeutic effect on said chimeric receptor cell is selected from the group consisting of

(i) inhibiting transcription of a DNA sequence;

(ii) inhibiting translation of an RNA sequence;

(iii) inhibiting reverse transcription of an RNA or DNA sequence;

(iv) inhibiting a post-translational modification of a protein;

(v) inducing transcription of a DNA sequence;

(vi) inducing translation of an RNA sequence;

(vii) inducing reverse transcription of an RNA or DNA sequence;

(viii) inducing a post-translational modification of a protein;

(ix) transcription of said nucleic acid as an RNA;

(x) translation of said nucleic acid as a protein; and

(xi) incorporating said nucleic acid into a chromosome of said chimeric receptor cell.

24. A method according to claim 23, wherein said delivery vector is a recombinant, non-human specific virus, such that binding of said non-human specific virus to said chimeric receptor cell or tissue results both in infection of said modified receptor cell and in said therapeutic effect of said therapeutic nucleic acid in said chimeric receptor cell.

25. A method according to claim 22, wherein said delivery vector comprises

(i) said *env* binding domain; bound by a linker to

(ii) said therapeutic or diagnostic agent,

such that said contacting results in said therapeutic or diagnostic effect.

26. A method according to claim 22, wherein said delivery vector further comprises a liposome, said liposome containing said *env* binding domain and said therapeutic or diagnostic agent, such that said *env* binding domain is capable of binding said chimeric receptor binding site of said chimeric receptor cell.

27. A method according to claim 23, wherein said contacting results in said modified receptor cell or tissue expressing a therapeutically effective amount of the expression product of said therapeutic nucleic acid.

28. A method according to claim 23, wherein said therapeutic nucleic acid encodes a normal form of protein which acts to correct a pathology associated with an abnormal form of the said protein.

29. A method according to claim 23, wherein said therapeutic nucleic acid encodes a toxin which acts to selectively kill the chimeric receptor cell or tissue.

30. A method according to claim 29, wherein said chimeric cell is a pathologic cell.

31. A method according to claim 30, wherein said therapeutic nucleic acid further encodes a growth factor selected from epidermal growth factor, interleukin-2, interleukin-4, interleukin-6, tissue growth factor-α, insulin growth factor-1 or fibroblast growth factor.

32. A method according to claim 30, wherein said toxin is a recombinant toxin or a toxin fragment comprising at least one functional cytotoxic domain of toxin selected from at least one of ricin, *Pseudomonas* exotoxin, diphtheria toxin and thymidine kinase.

33. A method according to claim 23, wherein said therapeutic nucleic acid encodes an antisense nucleotide which acts to block expression of an abnormal protein in said chimeric receptor cell or tissue.

34. A method according to claim 23, wherein said therapeutic nucleic acid encodes a single chain ribosome inhibitory protein which acts to block expression of an abnormal protein in said chimeric receptor cell or tissue.

35. A method according to claim 23, wherein said therapeutic nucleic acid encodes a cytokine.

36. A method according to claim 23, wherein said therapeutic nucleic acid encodes a growth factor.

37. A method according to claim 22, wherein said virus is a recombinant leukemia retrovirus.

38. A method according to claim 22, wherein said therapeutic agent is a toxin which acts to kill the chimeric receptor cell or tissue as a pathologic cell or tissue.

39. A method according to claim 38, wherein said pathologic cell is a neoplastic cell.

40. A method according to claim 38, wherein said toxin is a recombinant toxin or a toxin fragment comprising at least one functional cytotoxic domain of toxin selected from at least one of ricin, *Pseudomonas* exotoxin and diphtheria toxin.

41. A method according to claim 22, wherein said diagnostic agent is a detectable label which can be detected *in vivo*, *in situ*, or *in vitro*.

42. A method according to claim 41, wherein said detectable label is a radiolabel, an enzymatic label or a fluorescent label.

43. A recombinant nucleic acid comprising a nucleotide sequence encoding a chimeric receptor polypeptide according to claim 1.

44. A host cell comprising the nucleic acid of claim 43.

45. A host cell according to claim 44, wherein said host cell is selected from a mammalian cell, a yeast cell, a bird cell or an insect cell.

46. A host cell according to claim 44, wherein, when said nucleic acid is expressed as said receptor polypeptide in said host cell, a receptor binding molecule comprising said *env* binding domain binds to said receptor polypeptide.

47. A host cell according to claim 45, wherein said mammalian cell is selected from a human cell, a primate cell or a rodent cell.

48. A chimeric receptor antibody, anti-idiotype antibody or fragment thereof, which binds an epitope specific for a chimeric receptor polypeptide according to claim 1, a fragment thereof or an anti-idiotype to said antibody.

49. A chimeric receptor antibody, anti-idiotype antibody or fragment thereof of claim 48 which is monoclonal.

50. A chimeric receptor antibody, fragment or anti-idiotype antibody of claim 48 which is detectably labeled with a label which can be detected *in vivo*, *in situ*, or *in vitro*.

51. An antibody according to claim 50, wherein said detectable label is a radiolabel, an enzymatic label or a fluorescent label.

52. A method for producing a chimeric receptor polypeptide according to claim 1, comprising:

(a) culturing a recombinant host comprising a nucleic acid encoding a chimeric receptor polypeptide according to claim 1 in expressible form;

(b) culturing the recombinant host, such that said chimeric receptor polypeptide is expressed in recoverable amounts; and

(c) recovering said chimeric receptor polypeptide from said host or culture.

53. The method of claim 52 further comprising:

(d) purifying said chimeric receptor polypeptide.

54. A method for detecting an antibody specific for an epitope of a chimeric receptor polypeptide according to claim 1 in a sample, comprising:

(a) contacting said sample with a chimeric receptor polypeptide according to claim 1, or a fragment thereof, attached to a solid support, such that said antibody associates with the polypeptide; and

(b) detecting said antibody in the sample which is associated with said chimeric receptor polypeptide attached to said support.

55. A method for recovering an antibody specific for an epitope of a chimeric receptor polypeptide according to claim 1 in a sample comprising:

(a) contacting said sample with a chimeric receptor polypeptide according to claim 1, or a fragment thereof, attached to a solid support, such that said antibody associates with the chimeric receptor polypeptide; and

(b) recovering said antibody in the sample which is associated with said chimeric receptor polypeptide attached to said support.

56. A transgenic non-human mammal substantially all of whose germ cells and somatic cells contain a recombinant nucleic acid comprising a nucleotide sequence encoding a chimeric receptor polypeptide according to claim 1.

57. The transgenic mammal of claim 56, wherein said recombinant nucleic acid has been introduced into said mammal or an ancestor of said mammal at an embryonic stage.

58. An isolated or recombinant H13 polypeptide, consisting of a polypeptide having an amino acid sequence substantially corresponding to the amino acid sequence of an H13 polypeptide of SEQ ID NO:8.

59. A pharmaceutical composition useful for preventing or treating a retrovirus infection, comprising at least one chimeric receptor polypeptide according to any of claim 1, and a pharmaceutically acceptable carrier.

60. A method for preventing or treating a retrovirus infection in a subject, comprising administering a retrovirus inhibiting effecting amount of a composition according to claim 59.

61. A method for inhibiting the infectivity of a retrovirus, comprising contacting said retrovirus with an amount of a chimeric receptor polypeptide according to claim 1, sufficient to prevent said virus from infecting said cell.

62. The method of claim 61, wherein said retrovirus is a human immunodeficiency virus.

63. The method of claim 61, wherein said contacting is *in vivo*, *in vitro* or *in situ*.

64. A method for detecting a human retrovirus, a retroviral protein or a peptide derived therefrom, in a sample wherein said retrovirus, retroviral protein or retroviral peptide is capable of binding a detectably labeled chimeric receptor polypeptide, comprising:

(a) contacting said sample with a chimeric receptor polypeptide according to claim 1 which has been detectably labeled, such that said resulting labeled chimeric receptor polypeptide associates with said retrovirus, retroviral protein or retroviral peptide to provide a detectably labeled retrovirus, retroviral protein or retroviral peptide; and

(b) detecting said labeled retrovirus, retroviral protein or retroviral peptide in said sample which is bound to said labeled chimeric receptor polypeptide.

65. A method for detecting a human retrovirus, a retroviral protein or a peptide derived therefrom, in a sample wherein said retrovirus, retroviral protein or retroviral peptide is capable of binding a detectably labeled H13 polypeptide, comprising:

(a) contacting said sample with an chimeric receptor polypeptide according to claim 58 which has been detectably labeled, such that said resulting labeled receptor polypeptide associates with said retrovirus, retroviral protein or retroviral peptide to provide a detectably labeled retrovirus, retroviral protein or retroviral peptide; and

(b) detecting said labeled retrovirus, retroviral protein or retroviral peptide in said sample which is bound to said labeled human receptor polypeptide.

Nucleotide and Deduced Amino Acid Sequences of H13 cDNA

```
   1 CGATCCTGCCGGAGCCCCGCCGCCGCCGGCTTGGATTCTGAAACCTTCCTTGTATCCCTCCTGAGACATCTTTGCTGCAAGATCGAGGCT
  91 GTCCTCTGGTGAGAAGGTGGTGAGGCTTCCCGTCATATTCCAGCTCTGAACAGCAACATGGGGTGCAAAGTCCTGCTCAACATTGGGCAG
                                                          M  G  C  K  V  L  L  N  I  G  Q   11
 181 CAGATGCTGCGGCGGAAGGTGGTGGACTGTAGCCGGGAGGAGACGCGGCTGTCTCGCTGCCTGAACACTTTTGATCTGGTGGCCCTCGGG
       Q  M  L  R  K  K  V  V  D  C  S  R  E  E  T  R  L  S  R  C  L  N  T  F  D  L  V  A  L  G   41
 271 GTGGGCAGCACACTGGGTGCTGGTGTCTACGTCCTGGCTGGAGCTGTGGCCCGTGAGAATGCAGGCCCTGCCATTGTCATCTCCTTCCTG
       V  G  S  T  L  G  A  G  V  Y  V  L  A  G  A  V  A  R  E  N  A  G  P  A  I  V  I  S  F  L   71
 361 ATCGCTGCGCTGGCCTCAGTGCTGGCTGGCCTGTGCTATGGGGAGTTTGGTGCTCGGGTCCCCAAGACGGGCTCAGCTTACCTCTACAGC
       I  A  A  L  A  S  V  L  A  G  L  C  Y  G  E  F  G  A  R  V  P  K  T  G  S  A  Y  L  Y  S  101
 451 TATGTCACCGTTGGAGAGCTCTGGGCCTTCATCACCGGCTGGAACTTAATCCTCTCCTACATCATCGGTACTTCAAGCGTAGCGAGGGCC
       Y  V  T  V  G  E  L  W  A  F  I  T  G  W  N  L  I  L  S  Y  I  I  G  T  S  S  V  A  R  A  131
 541 TGGAGCGCCACCTTCGACGAGCTGATAGGCAGACCCATCGGGGAGTTCTCACGGACACACATGACTCTGAACGCCCCCGGCGTGCTGGCT
       W  S  A  T  F  D  E  L  I  G  R  P  I  G  E  F  S  R  T  H  M  T  L  N  A  P  G  V  L  A  161
 631 GAAAACCCCGACATATTCGCAGTGATCATAATTCTCATCTTGACAGGACTTTTAACTCTTGGTGTGAAAGAGTCGGCCATGGTCAACAAA
       E  N  P  D  I  F  A  V  I  I  I  L  I  L  T  G  L  L  T  L  G  V  K  E  S  A  M  V  N  K  191
 721 ATATTCACTTGTATTAACGTCCTGGTCCTGGGCTTCATAATGGTGTCAGGATTTGTGAAAGGATCGGTTAAAAACTGGCAGCTCACGGAG
       I  F  T  C  I  N  V  L  V  L  G  F  I  M  V  S  G  F  V  K  G  S  V  K  N  W  Q  L  T  E  221
 811 GAGGATTTTGGGAACACATCAGGCCGTCTCTGTTTGAACAATGACACAAAAGAAGGGAAGCCCGGTGTTGGTGGATTCATGCCCTTCGGG
       E  D  F  G  N  T  S  G  R  L  C  L  N  N  D  T  K  E  G  K  P  G  V  G  G  F  M  P  F  G  251
 901 TTCTCTGGTGTCCTGTCGGGGGCAGCGACTTGCTTCTATGCCTTCGTGGGCTTTGACTGCATCGCCACCACAGGTGAAGAGGTGAAGAAC
       F  S  G  V  L  S  G  A  A  T  C  F  Y  A  F  V  G  F  D  C  I  A  T  T  G  E  E  V  K  N  291
 991 CCACAGAAGGCCATCCCCGTGGGGATCGTGGCGTCCCTCTTGATCTGCTTCATCGCCTACTTTGGGGTGTCGGCTGCCCTCACGCTCATG
       P  Q  K  A  I  P  V  G  I  V  A  S  L  L  I  C  F  I  A  Y  F  G  V  S  A  A  L  T  L  M  331
1081 ATGCCCTACTTCTGCCTGGACAATAACAGCCCCCTGCCCGACGCCTTTAAGCACGTGGGCTGGGAAGGTGCCAAGTACGCAGTGGCCGTG
       M  P  Y  F  C  L  D  N  N  S  P  L  P  D  A  F  K  H  V  G  W  E  G  A  K  Y  A  V  A  V  341
1171 GGCTCCCTCTGCGCTCTTTCCGCCAGTCTTCTAGGTTCCATGTTTCCCATGCCTCGGGTTATCTATGCCATGGCTGAGGATGGACTGCTA
       G  S  L  C  A  L  S  A  S  L  L  G  S  M  F  P  M  P  R  V  I  Y  A  M  A  E  D  G  L  L  371
1261 TTTAAATTCTTAGCCAACGTCAATGATAGGACCAAAACACCAATAATCGCCACATTAGCCTCGGGTGCCGTTGCTGCTGTGATGGCCTTC
       F  K  F  L  A  N  V  N  D  R  T  K  T  P  I  I  A  T  L  A  S  G  A  V  A  A  V  M  A  F  401
1351 CTCTTTGACCTGAAGGACTTGGTGGACCTCATGTCCATTGGCACTCTCCTGGCTTACTCGTTGGTGGCTGCCTGTGTGTTGGTCTTACGG
       L  F  D  L  K  D  L  V  D  L  M  S  I  G  T  L  L  A  Y  S  L  V  A  A  C  V  L  V  L  R  431
1441 TACCAGCCAGAGCAGCCTAACCTGGTATACCAGATGGCCAGTACTTCCGACGAGTTAGATCCAGCAGACCAAAATGAATTGGCAAGCACC
       Y  Q  P  E  Q  P  N  L  V  Y  Q  M  A  S  T  S  D  E  L  D  P  A  D  Q  N  E  L  A  S  T  461
1531 AATGATTCCCAGCTGGGGTTTTTACCAGAGGCAGAGATGTTCTCTTTGAAAACCATACTCTCACCCAAAAACATGGAGCCTTCCAAAATC
       N  D  S  Q  L  G  F  L  P  E  A  E  M  F  S  L  K  T  I  L  S  P  K  N  M  E  P  S  K  I  491
1621 TCTGGGCTAATTGTGAACATTTCAACCAGCCTTATAGCTGTTCTCATCATCACCTTCTGCATTGTGACCGTGCTTGGAAGGGAGGCTCTC
       S  G  L  I  V  N  I  S  T  S  L  I  A  V  L  I  I  T  F  C  I  V  T  V  L  G  R  E  A  L  521
1711 ACCAAAGGGGCGCTGTGGGCAGTCTTTCTGCTCGCAGGGTCTGCCCTCCTCTGTGCCGTGGTCACGGGCGTCATCTGGAGGCAGCCCGAG
       T  K  G  A  L  W  A  V  F  L  L  A  G  S  A  L  L  C  A  V  V  T  G  V  I  W  R  Q  P  E  551
1801 AGCAAGACCAAGCTCTCATTTAAGGTTCCCTTCCTGCCAGTGCTCCCCATCCTGAGCATCTTCGTGAACGTCTATCTCATGATGCAGCTG
       S  K  T  K  L  S  F  K  V  P  F  L  P  V  L  P  I  L  S  I  F  V  N  V  Y  L  M  M  Q  L  581
1891 GACCAGGGCACCTGGGTCCGGTTTGCTGTGTGGATGCTGATAGGCTTCATCATCTACTTTGGCTATGGCCTGTGGCACAGCGAGGAGGCG
       D  Q  G  T  W  V  R  F  A  V  W  M  L  I  G  F  I  I  Y  F  G  Y  G  L  W  H  S  E  E  A  611
1981 TCCCTGGATGCCGACCAAGCAAGGACTCCTGACGGCAACTTGGACCAGTGCAAGTGACGCACAGCCCCGCCCCCCCGGAGGTGGCAGCAGC
       S  L  D  A  D  Q  A  R  T  P  D  G  N  L  D  Q  C  K  *                                  629
2071 CCCGAGGGACGCCCCCAGAGGACCGGGAGGCACCCCACCCTCCCCACCAGTGCAACAGAAACCACCTGCGTCCACACCCTCACTGCA
```

Fig. 1

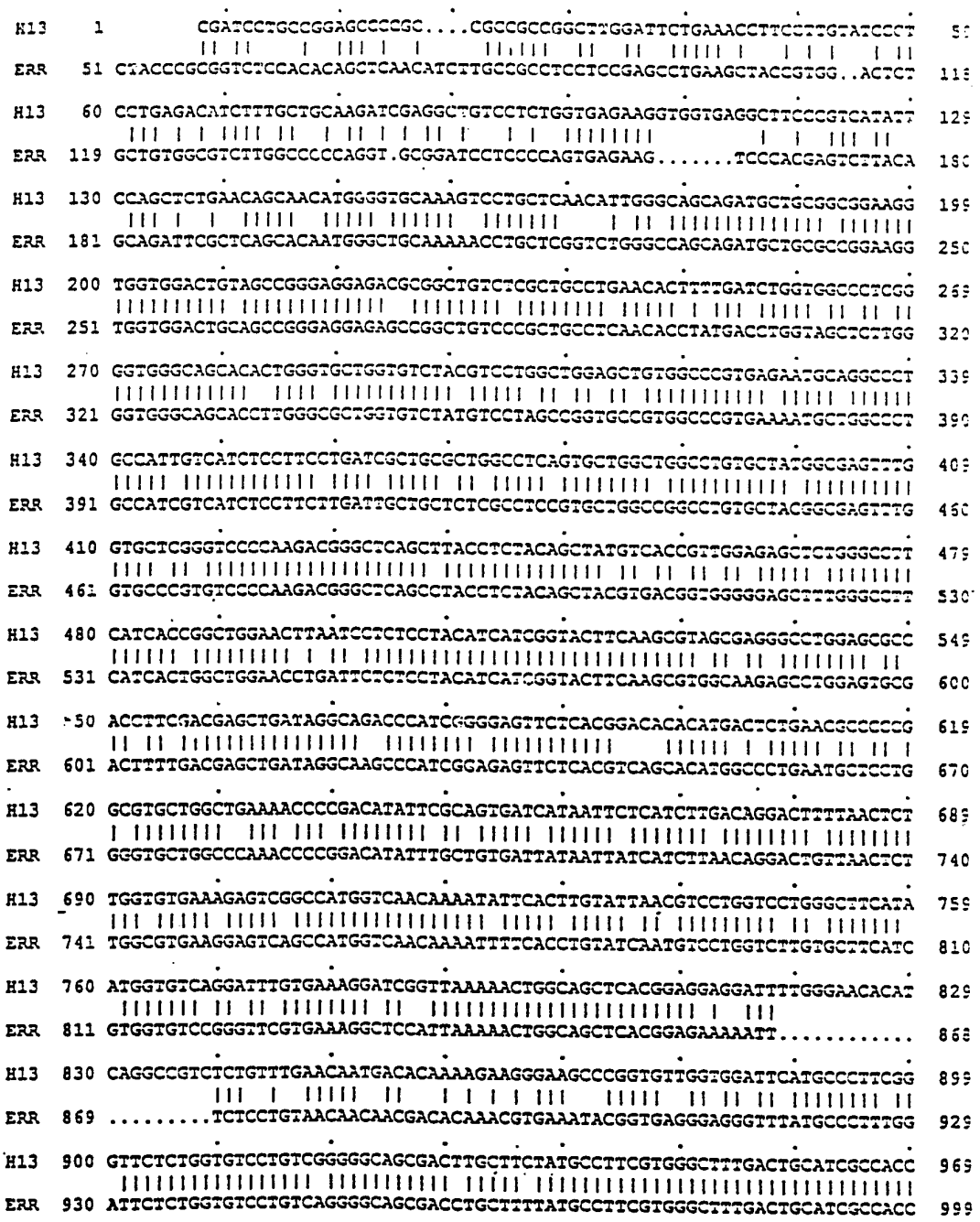

H13 1 CGATCCTGCCGGAGCCCCGC....CGCCGCCGGCTTGGATTCTGAAACCTTCCTTGTATCCCT 5
ERR 51 CTACCCGCGGTCTCCACACAGCTCAACATCTTGCCGCCTCCTCCGAGCCTGAAGCTACCGTGG..ACTCT 11
H13 60 CCTGAGACATCTTTGCTGCAAGATCGAGGCTGTCCTCTGGTGAGAAGGTGGTGAGGCTTCCCGTCATATT 12
ERR 119 GCTGTGGCGTCTTGGCCCCCAGGT.GCGGATCCTCCCCAGTGAGAAG.....TCCCACGAGTCTTACA 18
H13 130 CCAGCTCTGAACAGCAACATGGGGTGCAAAGTCCTGCTCAACATTGGGCAGCAGATGCTGCGGCGGAAGG 19
ERR 181 GCAGATTCGCTCAGCACAATGGGCTGCAAAAACCTGCTCGGTCTGGGCCAGCAGATGCTGCGCCGGAAGG 25
H13 200 TGGTGGACTGTAGCCGGGAGGAGACGCGGCTGTCTCGCTGCCTGAACACTTTTGATCTGGTGGCCCTCGG 26
ERR 251 TGGTGGACTGCAGCCGGGAGGAGAGCCGGCTGTCCCGCTGCCTCAACACCTATGACCTGGTAGCTCTTGG 32
H13 270 GGTGGGCAGCACACTGGGTGCTGGTGTCTACGTCCTGGCTGGAGCTGTGGCCCGTGAGAATGCAGGCCCT 33
ERR 321 GGTGGGCAGCACCTTGGGCGCTGGTGTCTATGTCCTAGCCGGTGCCGTGGCCCGTGAAAATGCTGGCCCT 39
H13 340 GCCATTGTCATCTCCTTCCTGATCGCTGCGCTGGCCTCAGTGCTGGCTGGCCTGTGCTATGGCGAGTTTG 40
ERR 391 GCCATCGTCATCTCCTTCTTGATTGCTGCTCTCGCCTCCGTGCTGGCCGGCCTGTGCTACGGCGAGTTTG 46
H13 410 GTGCTCGGGTCCCCAAGACGGGCTCAGCTTACCTCTACAGCTATGTCACCGTTGGAGAGCCTCTGGGCCTT 47
ERR 461 GTGCCCGTGTCCCCAAGACGGGCTCAGCCTACCTCTACAGCTACGTGACGGTGGGGGAGCTTTGGGCCTT 53
H13 480 CATCACCGGCTGGAACTTAATCCTCTCCTACATCATCGGTACTTCAAGCGTAGCGAGGGCCTGGAGCGCC 54
ERR 531 CATCACTGGCTGGAACCTGATTCTCTCCTACATCATCGGTACTTCAAGCGTGGCAAGAGCCTGGAGTGCG 60
H13 550 ACCTTCGACGAGCTGATAGGCAGACCCATCGGGGAGTTCTCACGGACACACATGACTCTGAACGCCCCG 61
ERR 601 ACTTTTGACGAGCTGATAGGCAAGCCCATCGGAGAGTTCTCACGTCAGCACATGGCCCTGAATGCTCCTG 67
H13 620 GCGTGCTGGCTGAAAACCCCGGACATATTCGCAGTGATCATAATTCTCATCTTGACAGGACTTTTAACTCT 68
ERR 671 GGGTGCTGGCCCAAACCCCGGACATATTTGCTGTGATTATAATTATCATCTTAACAGGACTGTTAACTCT 74
H13 690 TGGTGTGAAAGAGTCGGCCATGGTCAACAAAATATTCACTTGTATTAACGTCCTGGTCCTGGGCTTCATA 75
ERR 741 TGGCGTGAAGGAGTCAGCCATGGTCAACAAAATTTTCACCTGTATCAATGTCCTGGTCTTGTGCTTCATC 81
H13 760 ATGGTGTCAGGATTTGTGAAAGGATCGGTTAAAAACTGGCAGCTCACGGAGGAGGATTTTGGGAACACAT 82
ERR 811 GTGGTGTCCGGGTTCGTGAAAGGCTCCATTAAAAACTGGCAGCTCACGGAGAAAAATT..... 86
H13 830 CAGGCCGTCTCTGTTTGAACAATGACACAAAAGAAGGGAAGCCCGGTGTTGGTGGATTCATGCCCTTCGG 89
ERR 869 .....TCTCCTGTAACAACAACGACACAAACGTGAAATACGGTGAGGGAGGGTTTATGCCCTTTGG 92
H13 900 GTTCTCTGGTGTCCTGTCGGGGGCAGCGACTTGCTTCTATGCCTTCGTGGGCTTTGACTGCATCGCCACC 96
ERR 930 ATTCTCTGGTGTCCTGTCAGGGGCAGCGACCTGCTTTTATGCCTTCGTGGGCTTTGACTGCATCGCCACC 99

Fig. 2A

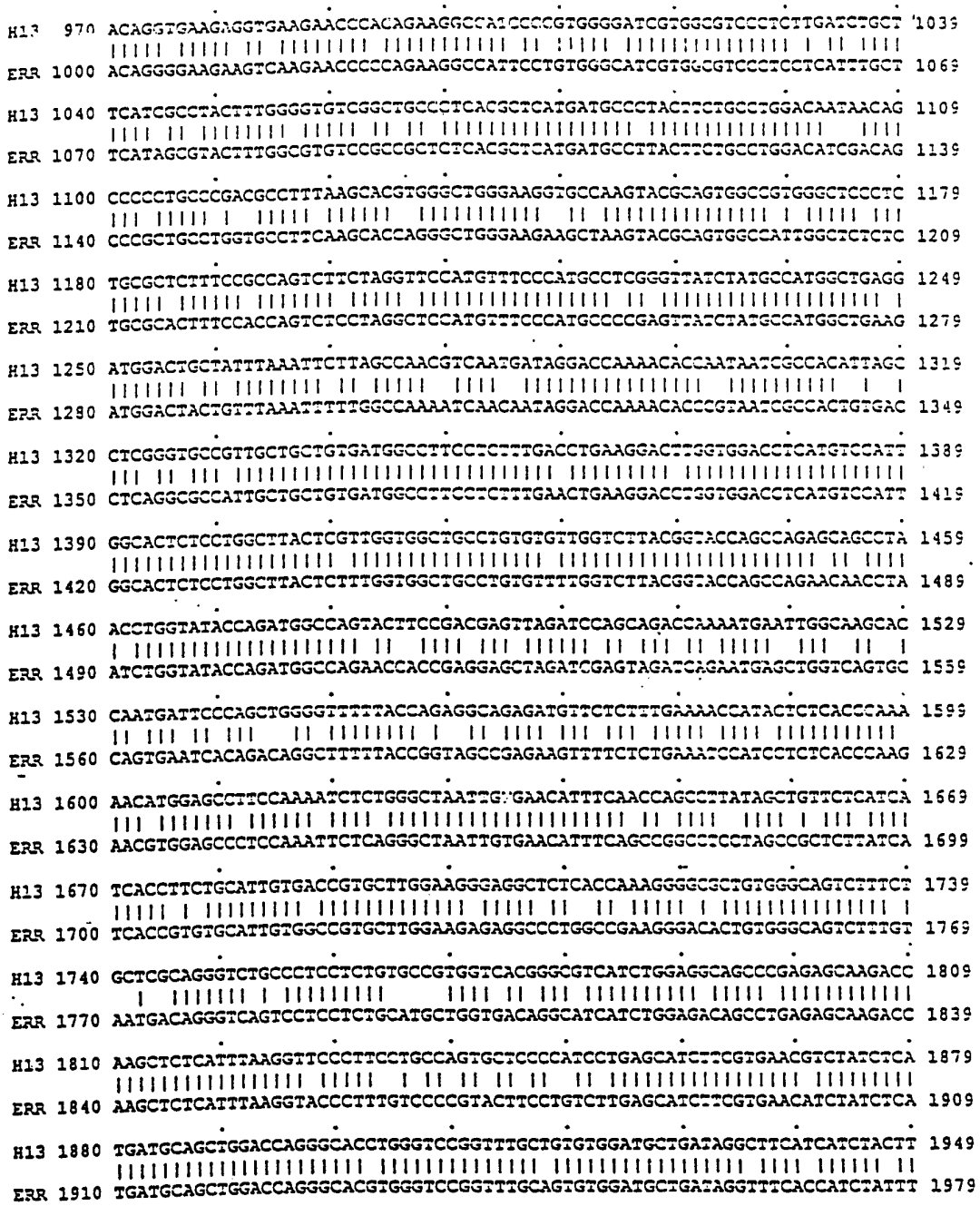


Fig 2B

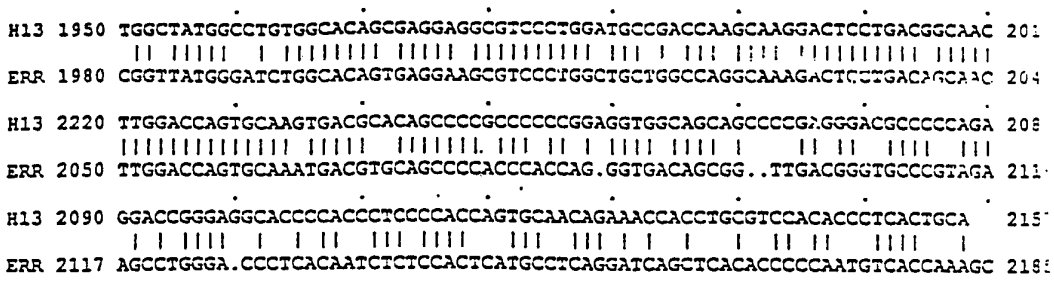

H13 1950 TGGCTATGGCCTGTGGCACAGCGAGGAGGCGTCCCTGGATGCCGACCAAGCAAGGACTCCTGACGGCAAC 201
ERR 1980 CGGTTATGGGATCTGGCACAGTGAGGAAGCGTCCCTGGCTGCTGGCCAGGCAAAGACTCCTGACAGCAAC 204
H13 2220 TTGGACCAGTGCAAGTGACGCACAGCCCCGCCCCCCGGAGGTGGCAGCAGCCCCGAGGGACGCCCCCAGA 208
ERR 2050 TTGGACCAGTGCAAATGACGTGCAGCCCCACCCACCAG.GGTGACAGCGG..TTGACGGGTGCCCGTAGA 211
H13 2090 GGACCGGGAGGCACCCCACCCTCCCCACCAGTGCAACAGAAACCACCTGCGTCCACACCCTCACTGCA 215
ERR 2117 AGCCTGGGA.CCCTCACAATCTCTCCACTCATGCCTCAGGATCAGCTCACACCCCCAATGTCACCAAAGC 218

Fig 2C

Alignment of H13, ERR and TEA Deduced Protein Sequences

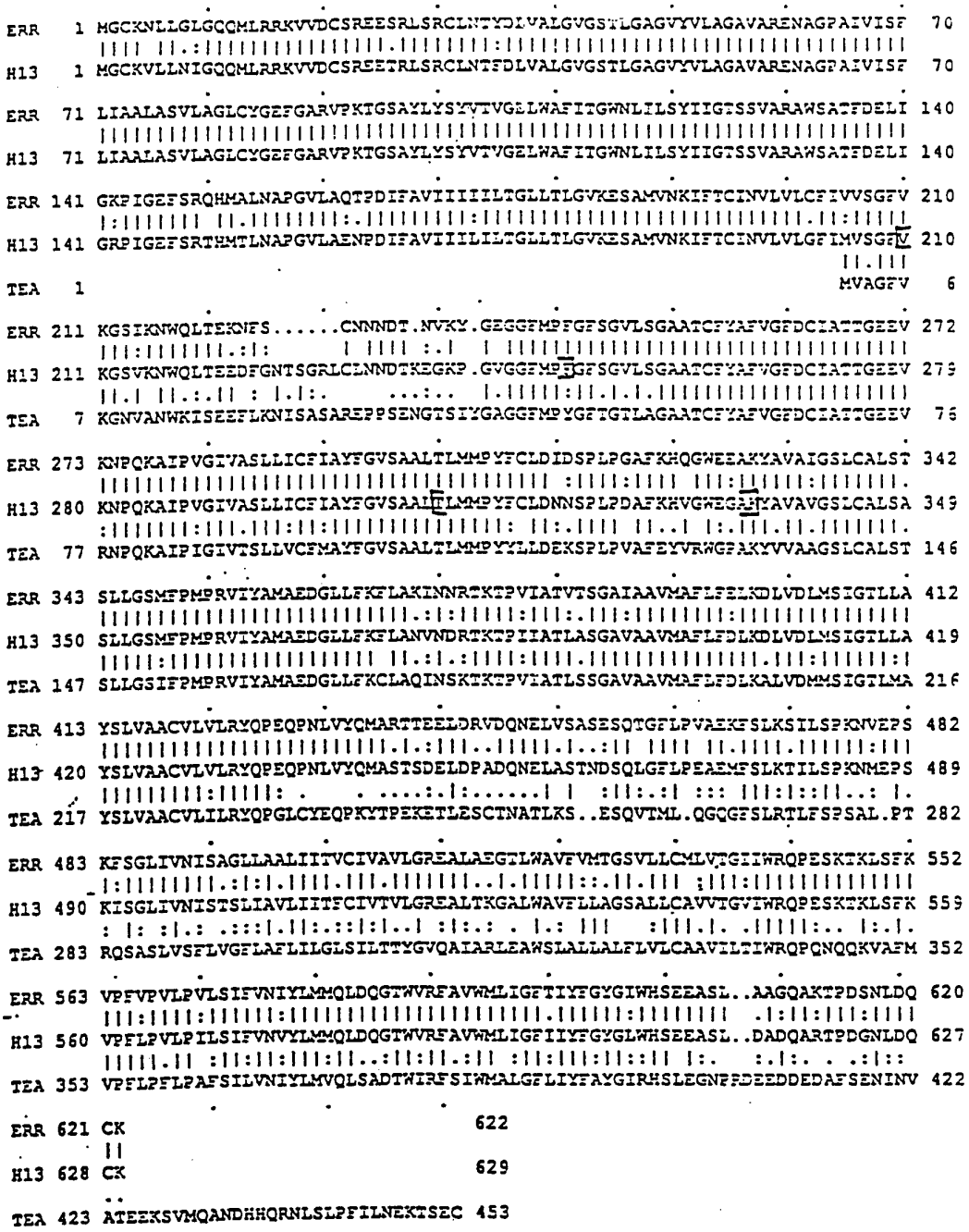


Fig. 3

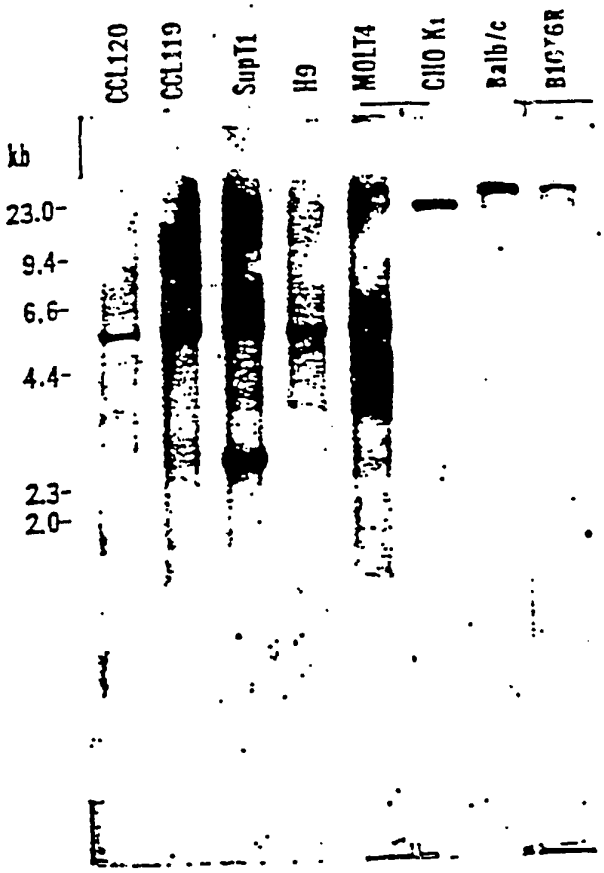

CCL120
CCL119
SupT1
H9
MOLT4
CHO K1
Balb/c
B16F6R
kb
23.0-
9.4-
6.6-
4.4-
2.3-
2.0-

Fig. 4

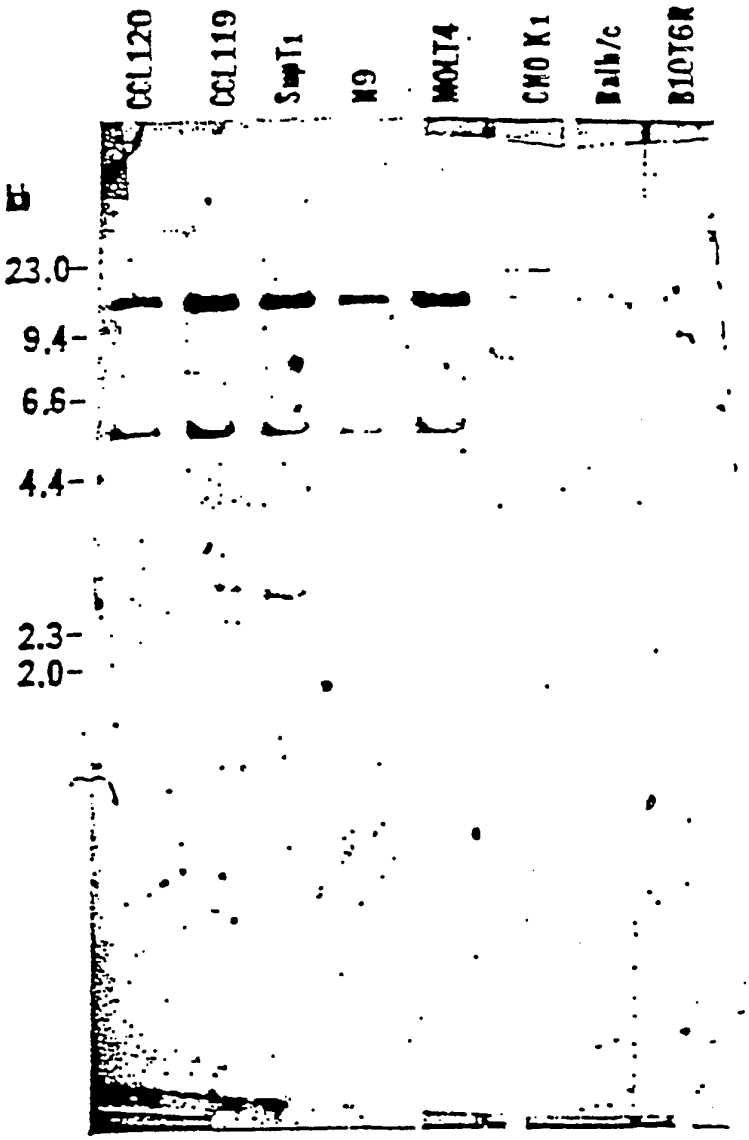

CCL120
CCL119
SupT1
M9
MOLT4
CHO K1
Balb/c
B1OT6R
kb
23.0
9.4
6.6
4.4
2.3
2.0

Fig. 5

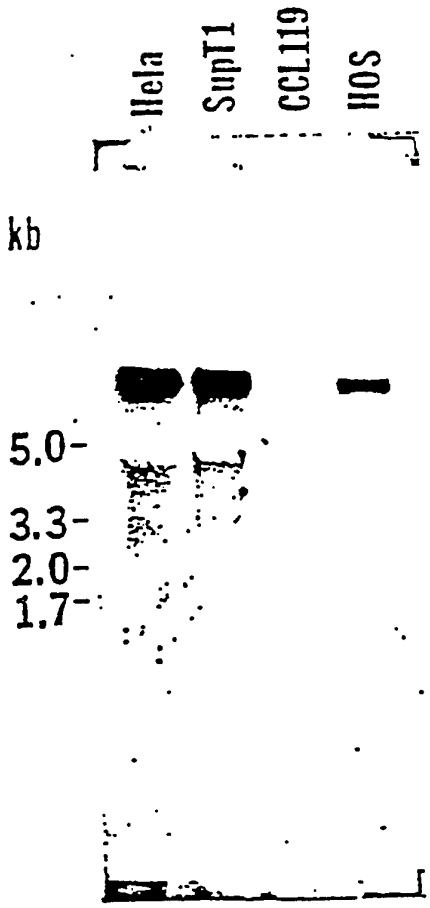
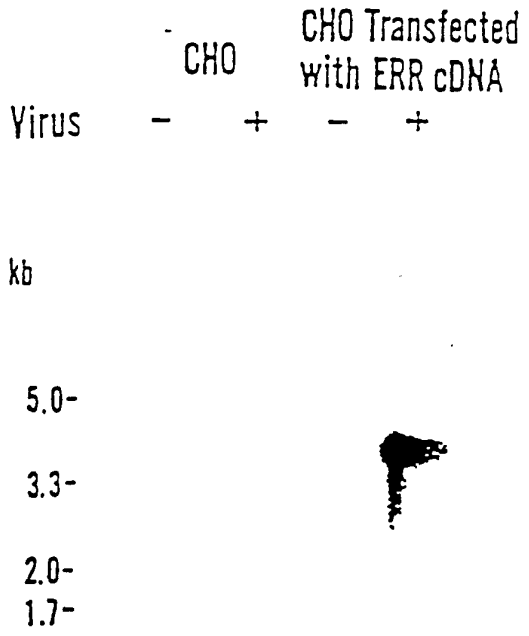


Fig. 6



Fig. 7



	Virus	RT Activity (cpm)
Medium		2146
CHO	-	1872
	+	2099
CHO Transfected with ERR cDNA	-	1792
	+	28172

Fig. 8

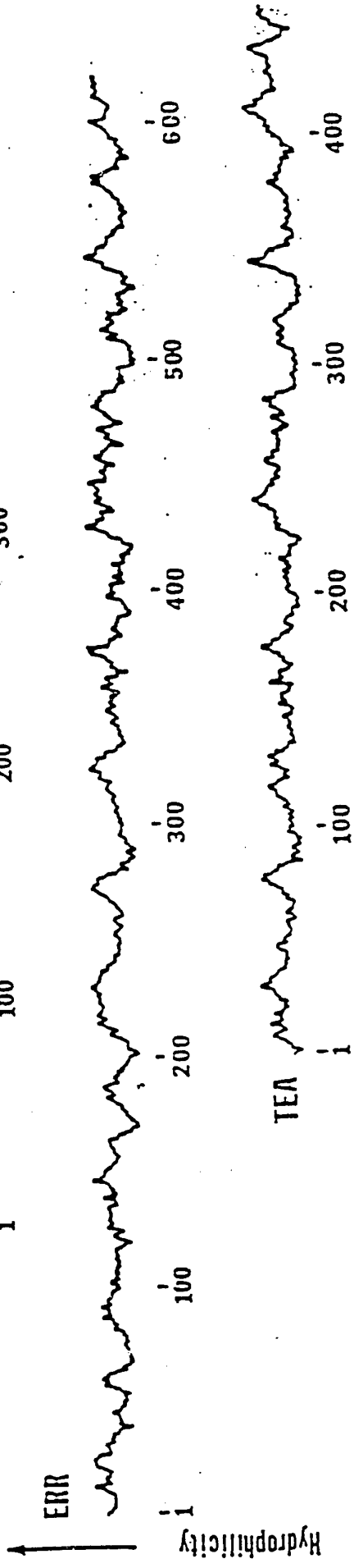

ERR
Hydrophilicity
1
100
200
300
400
500
600
II13
1
100
200
300
400
TEA
1
100
200
300
400
Amino Acid

Fig. 9

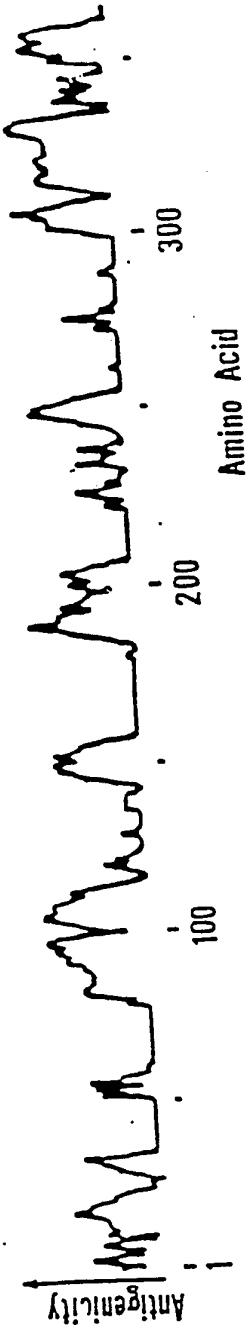


Fig. 10


pGEX-2T
pGEX-2T-H13
IPTG
Purified
IPTG
Purified
- +
- +
kDa
120-
105-
80-
46-
35-
27-
19-

Fig. 11

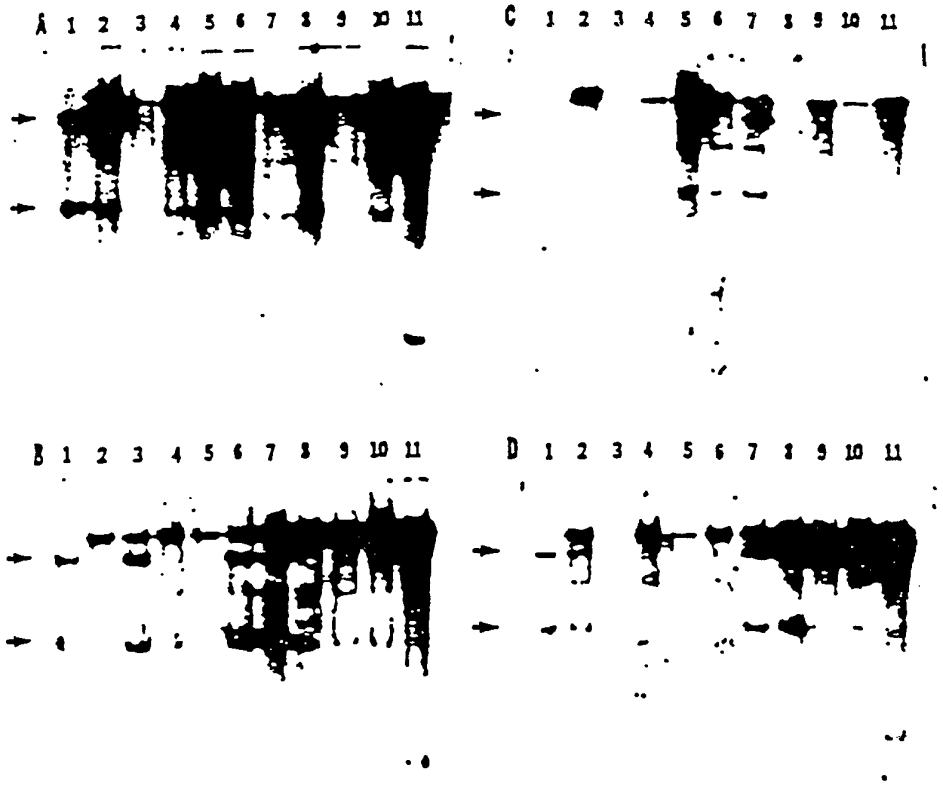

A 1 2 3 4 5 6 7 8 9 10 11
C 1 2 3 4 5 6 7 8 9 10 11
B 1 2 3 4 5 6 7 8 9 10 11
D 1 2 3 4 5 6 7 8 9 10 11

Fig. 12A

GENETIC MAPPING OF HL3 GENE TO HUMAN CHROMOSOME 13

Lane Number of Panel A

Chromosome	1	2	3	4	5	6	7	8	9	10	11
HL3	+	+						+			
1	+	60									
2	+		+								
3	+			+	15						
4	+					+		55			
5	+	+	+		+	+	D	+			+
6	+				+		+				
7	+							+			
8	+					+	+	+	+		+
9	+										
10	+				15						
11	+					15					
12	+										
13	+	+						+			
14	+	+					+				
15	+							+			
16	+										+
17	+								+		
18	+	+							+		
19	+	+			45			+			
20	+										
21	+	.			+			+			
22	+					+					
x	+	.				+	+				
y	+										

Lane Number of Panel C

Chromosome	1	2	3	4	5	6	7	8	9	10
HL3	+						+	+		
1	+	+								
2	+		+							
3	+			+		+				
4	+						55			
5	+	+	+	+	+	+	+	D	D	I
6	+							+	+	
7	+						+	+		
8	+						+			
9	+									
10	+				+	45				
11	+									
12	+			+				+	+	
13	+						+	+		
14	+	+		+				45		
15	+	+				10	+			
16	+					+			5	
17	+	+								
18	+									
19	+						+	+		
20	+			40				+		
21	+	+					+	+	+	
22	+			25						
x	+									
y	+			+				+	+	

Lane Number of Panel B

Chromosome	1	2	3	4	5	6	7	8	9	10	11
HL3	+		+			+	+				
1	+						+				
2	+										
3	+				+						
4	+										
5	+	+	+	+	+	D	D		+	+	
6	+										
7	+										
8	+										
9	+	+	+								
10	+									+	
11	+			+							
12	+			45	+						
13	+		+			+	+				
14	+			+	+	+					
15	+					+					
16	+										
17	+										
18	+	+						+			
19	+			+		+	+				
20	+				40				+		
21	+			+			+				
22	+			+	25		+				
x	+		+								
y	+				+						

Lane Number of Panel D

Chromosome	1	2	3	4	5	6	7	8	9	10
HL3	+						+	+		
1	+						60			
2	+									
3	+			15						
4	+									
5	+	+	+	+	+	+	+	D	Dq	
6	+			+						
7	+	-								
8	+	+								
9	+		+							
10	+			15						
11	+				+	+				
12	+					45				
13	+						+	+		
14	+					+	+	+		
15	+							+		
16	+	+								
17	+									
18	+						+			
19	+			45		+	+	+		
20	+									
21	+			+		+				
22	+					+				
x	+									
y	+									+

Numbers are the percent of the cell population containing the noted chromosome. Lane 1, human; lane 11, hamster; D, deletion at p15.1 -15.2; Dq, multiple deletion in 5q.

Fig, 12B

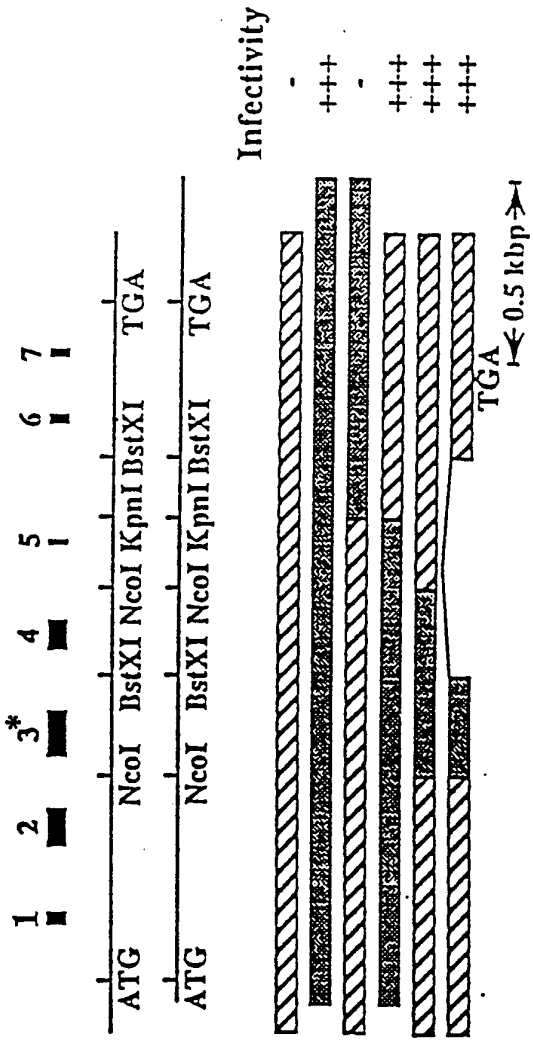

Chimeric Ecotropic Retroviral Receptor
Extracellular Domain
1 2 3* 4 5 6 7
H13
ATG NcoI BstXI NcoI KpnI BstXI TGA
ERR
ATG NcoI BstXI NcoI KpnI BstXI TGA
Infectivity
H13 -
ERR +++
Chimera I -
II +++
III +++
IV +++
TGA
0.5 kbp

Fig. 13

Comparison of Sequences of Extracellular Domain 3 between H13 and ERR

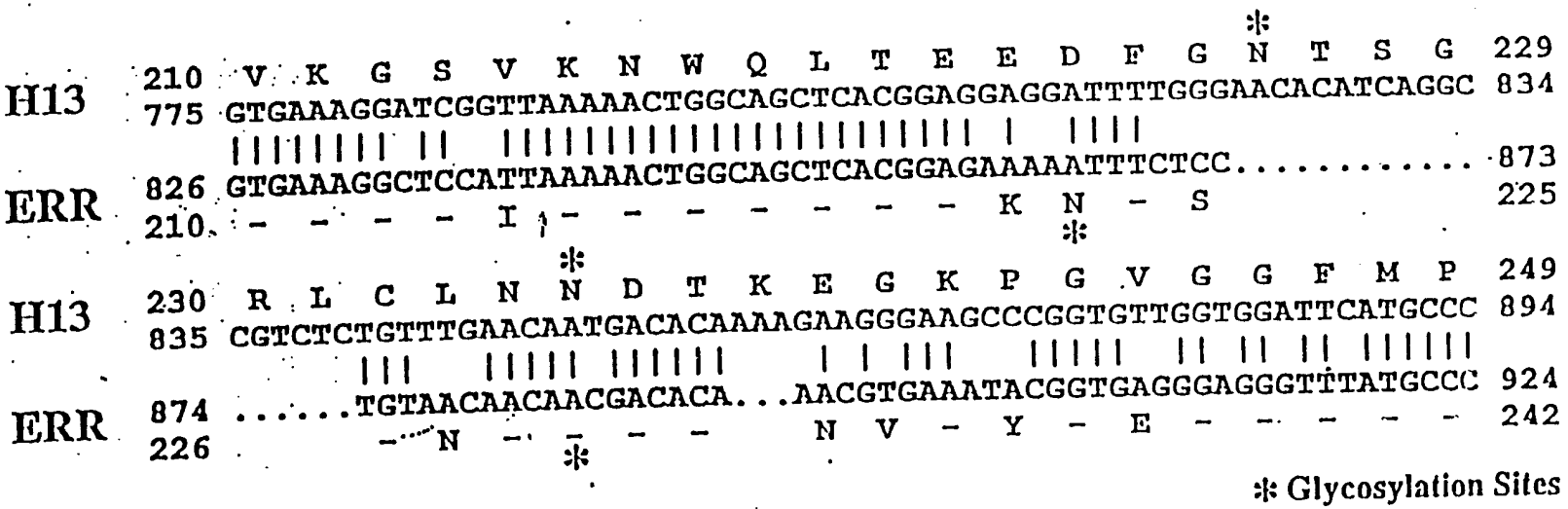


Fig. 14

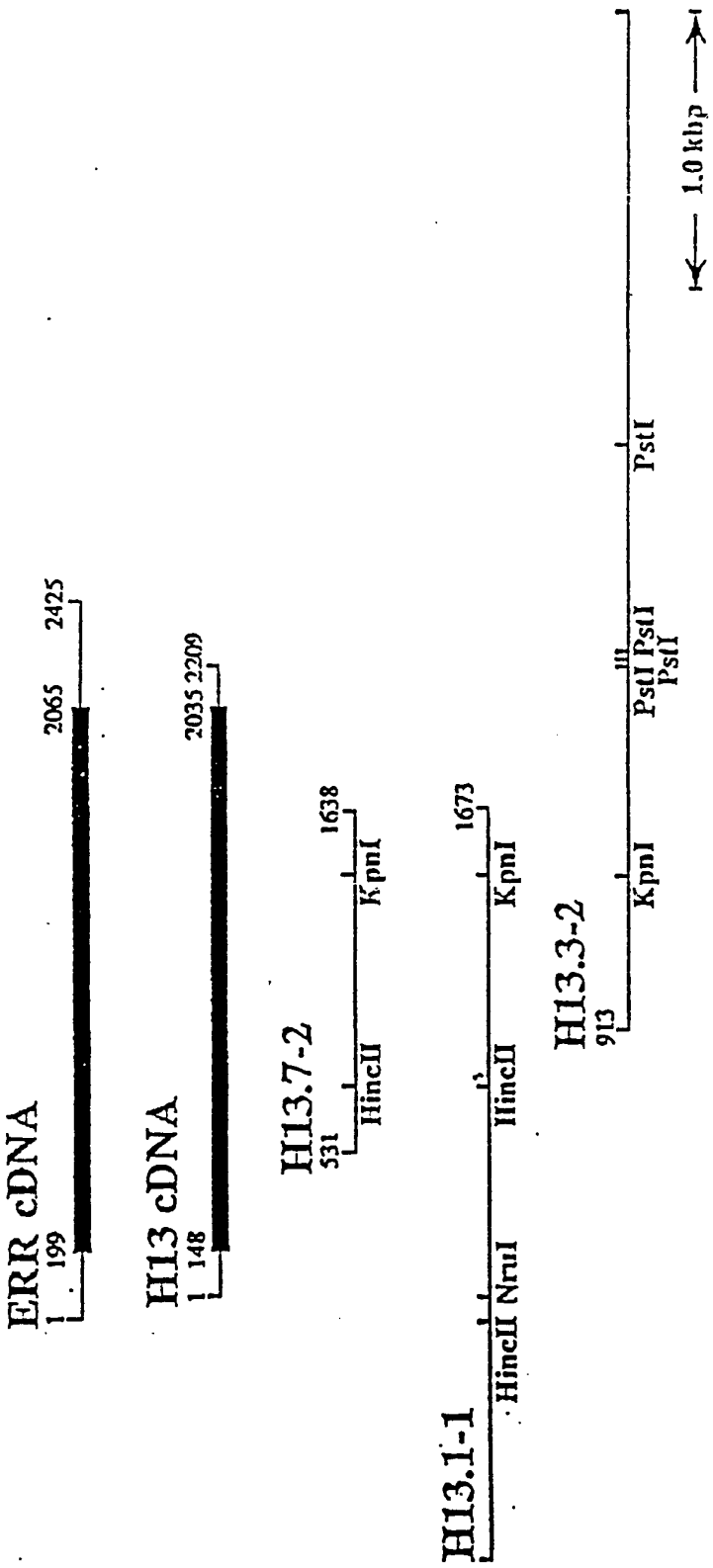

ERR cDNA
1
199
2065
2425
H13 cDNA
1
148
2035
2209
H13.7-2
531
HincII
1638
KpnI
H13.1-1
HincII
NruI
1673
KpnI
H13.3-2
913
KpnI
PstI
PstI
PstI
PstI
1.0 kbp

Fig. 15

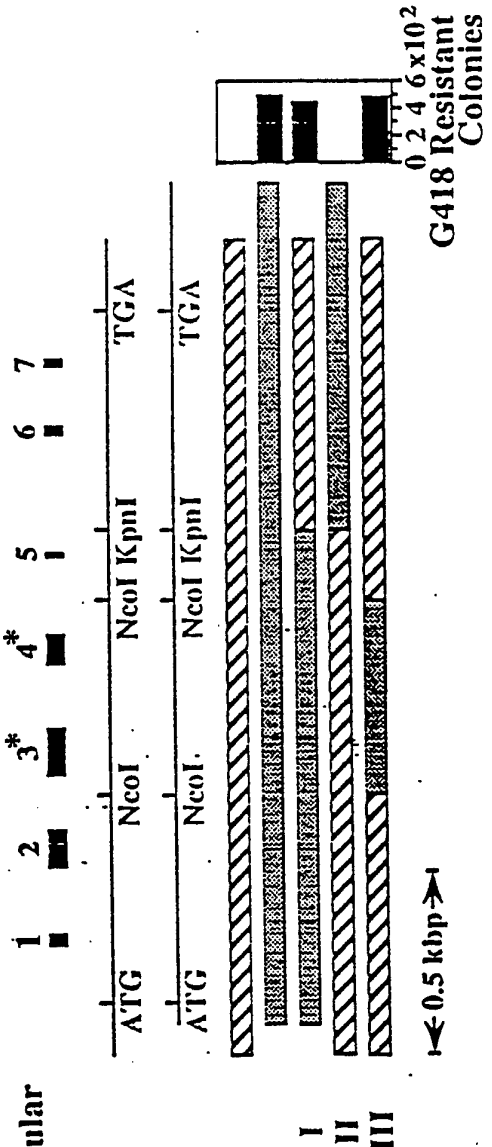

Chimeric Ecotropic Retroviral Receptor
Extracellular Doamin
1
2
3*
4*
5
6
7
H13
ERR
H13
ERR
Chimera I
II
III
ATG
NcoI
NcoI KpnI
TGA
ATG
NcoI
NcoI KpnI
TGA
0.5 kbp
0 2 4 6 x10²
G418 Resistant Colonies

Fig. 16

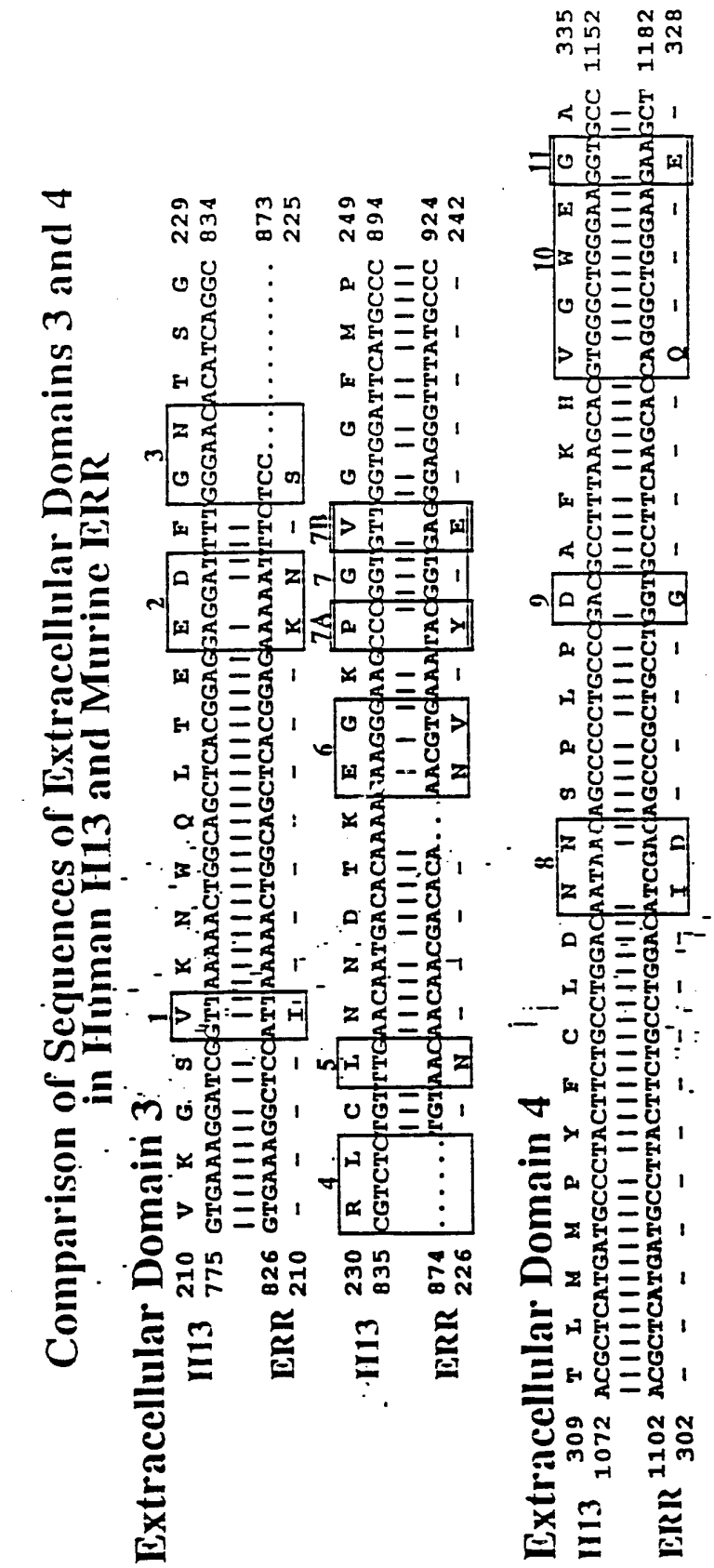

Comparison of Sequences of Extracellular Domains 3 and 4
in Human H13 and Murine ERR
Extracellular Domain 3
H13
ERR
Extracellular Domain 4
H13
ERR

Fig. 17

Acquirement of Ability of H13 to Function as A Receptor for MuLV-E by Mutation

	238						244
H13	K	E	G	K	P	G	V
ERR	–	N	V	K	Y	G	E
	232						237
H13 Mutant 1	.	.	.	.	Y	.	.
2	.	.	V	.	Y	.	.
3	.	N	V	.	Y	.	.
4	–	N	V	.	Y	.	.
5	.	.	.	.	Y	.	E
6	.	.	V	.	Y	.	E
7	.	N	V	.	Y	.	E
8	–	N	V	.	Y	.	E

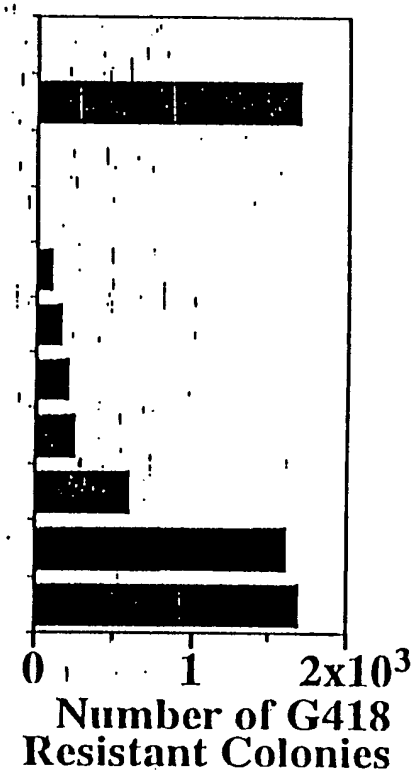


Fig18

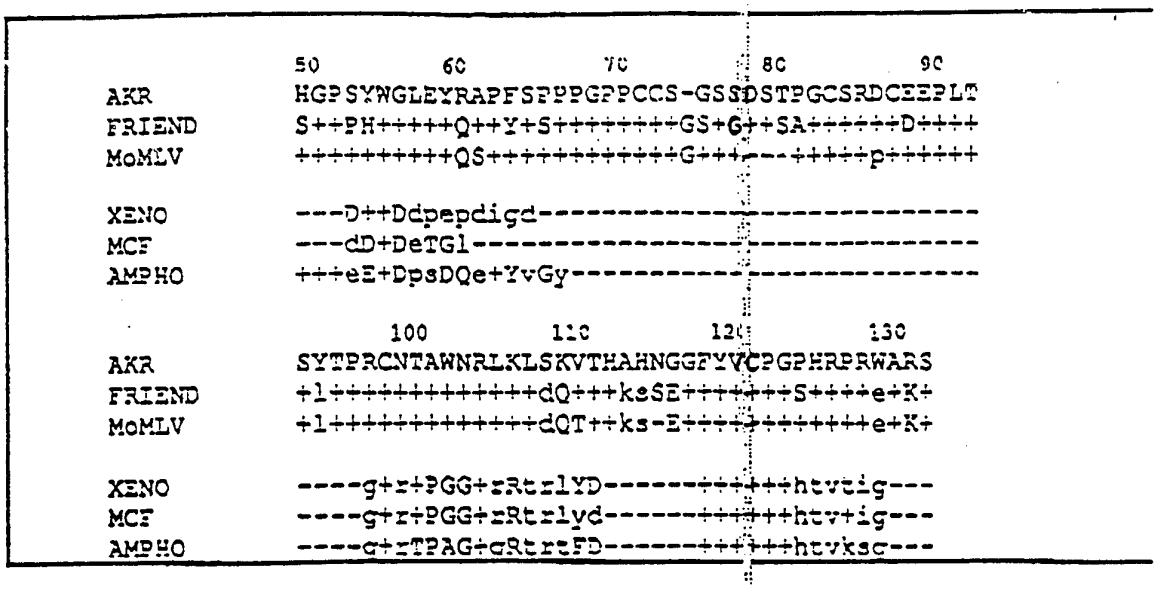

50 60 70 80 90
AKR HGPSYWGLEYRAPFSPPPGPPCCS-GSSDSTPGCSRDCEEPLT
FRIEND S++PH+++++Q++Y+S++++++++GS+G++SA++++++D++++
MoMLV ++++++++++QS++++++++++++G+++---+++++p++++++
XENO ---D++Ddpepdigd----------
MCF ---dD+DeTGl----------
AMPHO +++eE+DpsDQe+YvGy----------
100 110 120 130
AKR SYTPRCNTAWNRLKLSKVTHAHNGGFYYCPGPHRPRWARS
FRIEND +l++++++++++++dQ+++keSE++++++++S++++e+K+
MoMLV +l++++++++++++dQT++ks-E++++++++++++e+K+
XENO ----g+r+PGG+rRtrlYD----------htvtig---
MCF ----g+r+PGG+rRtrlyd----------htv+ig---
AMPHO ----g+rTPAG+gRtrtFD----------htvksc---

FIG. 19

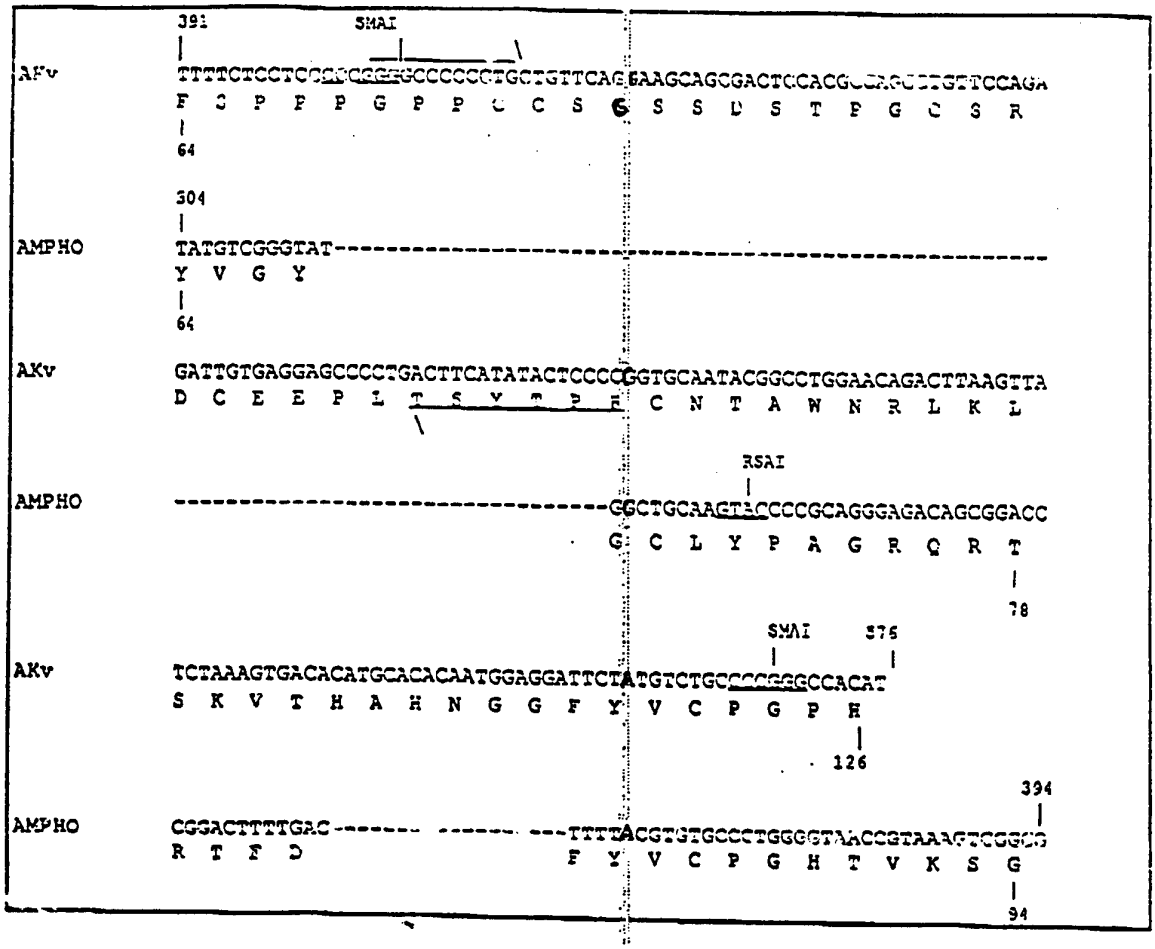

391
SMAI
AFv
F C P P P G P P C C S G S S S D S T P G C S R
64
304
AMPHO
TATGTCGGGTAT----------
Y V G Y
64
AKv
GATTGTGAGGAGCCCCTGACTTCATATACTCCCCGGTGCAATACGGCCTGGAACAGACTTAAGTTA
D C E E P L T S Y T P H C N T A W N R L K L
RSAI
AMPHO
----------GGCTGCAAGTACCCCGCAGGGAGACAGCGGACC
G C L Y P A G R Q R T
78
SMAI
376
AKv
TCTAAAGTGACACATGCACACAATGGAGGATTCTATGTCTGCCCCGGGCCACAT
S K V T H A H N G G F Y V C P G P H
126
394
AMPHO
CGGACTTTTGAC------- ------- ---TTTTACGTGTGCCCTGGGGTAACCGTAAAGTCGGCG
R T F D F Y V C P G H T V K S G
94

FIG. 20