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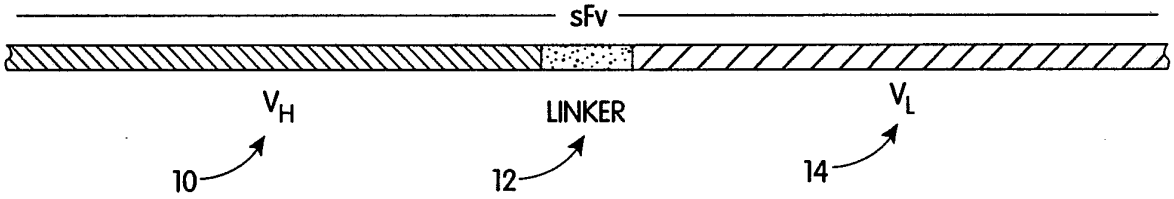

(57) Abstract

Disclosed is a single-chain Fv (sFv) polypeptide defining a binding site which exhibits the immunological binding properties of an immunoglobulin molecule which binds c-erbB-2 or a c-erbB-2-related tumor antigen, the sFv includes at least two polypeptide domains connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each of the polypeptide domains includes a set of complementarity determining regions (CDRs) interposed between a set of framework regions (FRs), the CDRs conferring immunological binding to the c-erbB-2 or c-erbB-2-related tumor antigen.

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BIOSYNTHETIC BINDING PROTEIN FOR CANCER MARKER

This invention relates in general to novel biosynthetic compositions of matter and, specifically, to biosynthetic antibody binding site (BABS) proteins, and conjugates thereof. Compositions of the invention are useful, for example, in drug and toxin targeting, imaging, immunological treatment of various cancers, and in specific binding assays, affinity purification schemes, and biocatalysis.

Background of the Invention

Carcinoma of the breast is the most common malignancy among women in North America, with 130,000 new cases in 1987. Approximately one in 11 women develop breast cancer in their lifetimes, causing this malignancy to be the second leading cause of cancer death among women in the United States, after lung cancer. Although the majority of women with breast cancer present with completely resectable disease, metastatic disease remains a formidable obstacle to cure. The use of adjuvant chemotherapy or hormonal therapy has definite positive impact on disease-free survival and overall survival in selected subsets of women with completely resected primary breast cancer, but a substantial proportion of women still relapse with metastatic disease (see, e.g., Fisher et al. (1986) J. Clin. Oncol. 4:929-941; "The Scottish trial", Lancet (1987) 2:171-175). In spite of the regularly induced objective responses induced by chemotherapy and hormonal therapy in appropriately selected patients, cure of metastatic breast cancer has not been achieved (see e.g., Aisner, et al. (187) J. Clin. Oncol.

5:1523-1533). To this end, many innovative treatment programs including the use of new agents, combinations of agents, high dose therapy (Henderson, ibid.) and increased dose intensity (Kernan et al. (1988) Clin. Invest. 259:3154-3157) have been assembled. Although improvements have been observed, routine achievement of complete remissions of metastatic disease, the first step toward cure, has not occurred. There remains a pressing need for new approaches to treatment.

The Fv fragment of an immunoglobulin molecule from IgM, and on rare occasions IgG or IgA, is produced by proteolytic cleavage and includes a non-covalent V_H-V_L heterodimer representing an intact antigen binding site. A single chain Fv (sFv) polypeptide is a covalently linked V_H-V_L heterodimer which is expressed from a gene fusion including V_H- and V_L-encoding genes connected by a peptide-encoding linker. See Huston et al., 1988, Proc. Nat. Aca. Sci. 85: 5879, hereby incorporated by reference.

U.S. Patent 4,753,894 discloses murine monoclonal antibodies which bind selectively to human breast cancer cells and, when conjugated to ricin A chain, exhibit a TCID 50% against at least one of MCF-7, CAMA-1, SKBR-3, or BT-20 cells of less than about 10 nM. The SKBR-3 cell line is recognized specifically by the monoclonal antibody 520C9. The antibody designated 520C9 is secreted by a murine hybridoma and is now known to recognize c-erbB-2 (Ring et al., 1991, Molecular Immunology 28:915).

Summary of the Invention

The invention features the synthesis of a class of novel proteins known as single chain Fv (sFv) polypeptides, which include biosynthetic single polypeptide chain binding sites (BABS) and define a binding site which exhibits the immunological binding properties of an immunoglobulin molecule which binds c-erbB-2 or a c-erbB-2-related tumor antigen.

The sFv includes at least two polypeptide domains connected by a polypeptide linker spanning the distance between the carboxy (C)- terminus of one domain and the amino (N)- terminus of the other domain, the amino acid sequence of each of the polypeptide domains including a set of complementarity determining regions (CDRs) interposed between a set of framework regions (FRs), the CDRs conferring immunological binding to c-erbB-2 or a c-erbB-2 related tumor antigen.

In its broadest aspects, this invention features single-chain Fv polypeptides including biosynthetic antibody binding sites, replicable expression vectors prepared by recombinant DNA techniques which include and are capable of expressing DNA sequences encoding these polypeptides, methods for the production of these polypeptides, methods of imaging a tumor expressing c-erbB-2 or a c-erbB-2-related tumor antigen, and methods of treating a tumor using targetable therapeutic agents by virtue of conjugates or fusions with these polypeptides.

As used herein, the term "immunological binding" or "immunologically reactive" refers to the non-covalent interactions of the type that occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific; "c-erbB-2" refers to a

protein antigen expressed on the surface of tumor cells, such as breast and ovarian tumor cells, which is an approximately 200,000 molecular weight acidic glycoprotein having an isoelectric point of about 5.3 and including the amino acid sequence set forth in SEQ ID NOS:1 and 2. A "c-erbB-2-related tumor antigen" is a protein located on the surface of tumor cells, such as breast and ovarian tumor cells, which is antigenically related to the c-erbB-2 antigen, i.e., bound by an immunoglobulin that is capable of binding the c-erbB-2 antigen, examples of such immunoglobulins being the 520C9, 741F8, and 454C11 antibodies; or which has an amino acid sequence that is at least 80% homologous, preferably 90% homologous, with the amino acid sequence of c-erbB-2. An example of a c-erbB-2 related antigen is the receptor for epidermal growth factor.

An sFv CDR that is "substantially homologous with" an immunoglobulin CDR retains at least 70%, preferably 80% or 90%, of the amino acid sequence of the immunoglobulin CDR, and also retains the immunological binding properties of the immunoglobulin.

The term "domain" refers to that sequence of a polypeptide that folds into a single globular region in its native conformation, and may exhibit discrete binding or functional properties. The term "CDR" or complementarity determining region, as used herein, refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site, or a synthetic polypeptide which mimics this function. CDRs typically are not wholly homologous to hypervariable regions of natural Fvs, but rather may also include specific amino acids or amino acid sequences which

flank the hypervariable region and have heretofore been considered framework not directly determinative of complementarity. The term "FR" or framework region, as used herein, refers to amino acid sequences which are naturally found between CDRs in immunoglobulins.

Single-chain Fv polypeptides produced in accordance with the invention include biosynthetically-produced novel sequences of amino acids defining polypeptides designed to bind with a preselected c-erbB-2 or related antigen material. The structure of these synthetic polypeptides is unlike that of naturally occurring antibodies, fragments thereof, or known synthetic polypeptides or "chimeric antibodies" in that the regions of the single-chain Fv responsible for specificity and affinity of binding (analogous to native antibody variable (V_H/V_L) regions) may themselves be chimeric, e.g., include amino acid sequences derived from or homologous with portions of at least two different antibody molecules from the same or different species. These analogous V_H and V_L regions are connected from the N-terminus of one to the C-terminus of the other by a peptide bonded biosynthetic linker peptide.

The invention thus provides a single-chain Fv polypeptide defining at least one complete binding site capable of binding c-erbB-2 or a c-erbB-2-related tumor antigen. One complete binding site includes a single contiguous chain of amino acids having two polypeptide domains, e.g., V_H and V_L, connected by a amino acid linker region. An sFv that includes more than one complete binding site capable of binding a c-erbB-2-related antigen, e.g., two binding sites, will be a single contiguous chain of amino acids having four polypeptide domains, each of which is covalently linked

by an amino acid linker region, e.g., V_{H1}-linker-V_{L1}-linker-V_{H2}-linkerV_{L2}. sFv's of the invention may include any number of complete binding sites (V_{Hn}-linker-V_{Ln})$_n$, where n > 1, and thus may be a single contiguous chain of amino acids having n antigen binding sites and n X 2 polypeptide domains.

In one preferred embodiment of the invention, the single-chain Fv polypeptide includes CDRs that are substantially homologous with at least a portion of the amino acid sequence of CDRs from a variable region of an immunoglobulin molecule from a first species, and includes FRs that are substantially homologous with at least a portion of the amino acid sequence of FRs from a variable region of an immunoglobulin molecule from a second species. Preferably, the first species is mouse and the second species is human.

The amino acid sequence of each of the polypeptide domains includes a set of CDRs interposed between a set of FRs. As used herein, a "set of CDRs" refers to 3 CDRs in each domain, and a "set of FRS" refers to 4 FRs in each domain. Because of structural considerations, an entire set of CDRs from an immunoglobulin may be used, but substitutions of particular residues may be desirable to improve biological activity, e.g., based on observations of conserved residues within the CDRs of immunoglobulin species which bind c-erbB-2 related antigens.

In another preferred aspect of the invention, the CDRs of the polypeptide chain have an amino acid sequence substantially homologous with the CDRs of the variable region of any one of the 520C9, 741F8, and 454C11 monoclonal antibodies. The CDRs of the 520C9 antibody are set forth in the Sequence Listing as amino acid residue numbers 31 through 35, 50 through 66, 99

through 104, 159 through 169, 185 through 191, and 224 through 232 in SEQ ID NOS: 3 and 4, and amino acid residue numbers 31 through 35, 50 through 66, 99 through 104, 157 through 167, 183 through 189, and 222 through 230 in SEQ ID NOS: 5, and 6.

In one embodiment, the sFv is a humanized hybrid molecule which includes CDRs from the mouse 520C9 antibody interposed between FRs derived from one or more human immunoglobulin molecules. This hybrid sFv thus contains binding regions which are highly specific for the c-erbB-2 antigen or c-erbB-2-related antigens held in proper immunochemical binding conformation by human FR amino acid sequences, and thus will be less likely to be recognized as foreign by the human body.

In another embodiment, the polypeptide linker region includes the amino acid sequence set forth in the Sequence Listing as amino acid residue numbers 123 through 137 in SEQ ID NOS:3 and 4, and as amino acid residues 1-16 in SEQ ID NOS:11 and 12. In other embodiments, the linker sequence has the amino acid sequence set forth in the Sequence Listing as amino acid residues 121-135 in SEQ ID NOS:5 and 6, or the amino acid sequence of residues 1-15 in SEQ ID NOS:13 and 14.

The single polypeptide chain described above also may include a remotely detectable moiety bound thereto to permit imaging or radioimmunotherapy of tumors bearing a c-erbB-2 or related tumor antigen. "Remotely detectable" moiety means that the moiety that is bound to the sFv may be detected by means external to and at a distance from the site of the moiety. Preferable remotely detectable moieties for imaging include radioactive atom such as ^{99m}Technetium (^{99m}Tc), a gamma emitter. Preferable nucleotides for high dose

radioimmunotherapy include radioactive atoms such as, (^{90}Yttrium (^{90}Yt), ^{131}Iodine (^{131}I) or ^{111}Indium (^{111}In).

In addition, the sFv may include a fusion protein derived from a gene fusion, such that the expressed sFv fusion protein includes an ancillary polypeptide that is peptide bonded to the binding site polypeptide. In some preferred aspects, the ancillary polypeptide segment also has a binding affinity for a c-erbB-2 or related antigen and may include a third and even a fourth polypeptide domain, each comprising an amino acid sequence defining CDRs interposed between FRs, and which together form a second single polypeptide chain biosynthetic binding site similar to the first described above.

In other aspects, the ancillary polypeptide sequence forms a toxin linked to the N or C terminus of the sFv, e.g., at least a toxic portion of Pseudomonas exotoxin, phytolaccin, ricin, ricin A chain, or diphtheria toxin, or other related proteins known as ricin A chain-like ribosomal inhibiting proteins, i.e., proteins capable of inhibiting protein synthesis at the level of the ribosome, such as pokeweed antiviral protein, gelonin, and barley ribosomal protein inhibitor. In still another aspect, the sFv may include at least a second ancillary polypeptide or moiety which will promote internalization of the sFv.

The invention also includes a method for producing sFv, which includes the steps of providing a replicable expression vector which includes and which expresses a DNA sequence encoding the single polypeptide chain; transfecting the expression vector into a host cell to produce a transformant; and culturing the transformant to produce the sFv polypeptide.

The invention also includes a method of imaging a tumor expressing a c-erbB-2 or related tumor antigen. This method includes the steps of providing an imaging agent including a single-chain Fv polypeptide as described above, and a remotely detectable moiety linked thereto; administering the imaging agent to an organism harboring the tumor in an amount of the imaging agent with a physiologically-compatible carrier sufficient to permit extracorporeal detection of the tumor; and detecting the location of the moiety in the subject after allowing the agent to bind to the tumor and unbound agent to have cleared sufficiently to permit visualization of the tumor image.

The invention also includes a method of treating cancer by inhibiting in vivo growth of a tumor expressing a c-erbB-2 or related antigen, the method including administering to a cancer patient a tumor inhibiting amount of a therapeutic agent which includes an sFv of the invention and at least a first moiety peptide bonded thereto, and which has the ability to limit the proliferation of a tumor cell.

Preferably, the first moiety includes a toxin or a toxic fragment thereof, e.g., ricin A; or includes a radioisotope sufficiently radioactive to inhibit proliferation of the tumor cell, e.g., ^{90}Yt, ^{111}In, or ^{131}I. The therapeutic agent may further include at least a second moiety that improves its effectiveness.

The clinical administration of the single-chain Fv or appropriate sFv fusion proteins of the invention, which display the activity of native, relatively small Fv of the corresponding immunoglobulin, affords a number of advantages over the use of larger fragments or entire antibody molecules. The single chain Fv and sFv fusion proteins of this invention offer fewer

cleavage sites to circulating proteolytic enzymes and thus offer greater stability. They reach their target tissue more rapidly, and are cleared more quickly from the body, which makes them ideal imaging agents for tumor detection and ideal radioimmunotherapeutic agents for tumor killing. They also have reduced non-specific binding and immunogenicity relative to murine immunoglobulins. In addition, their expression from single genes facilitates targeting applications by fusion to other toxin proteins or peptide sequences that allow specific coupling to other molecules or drugs. In addition, some sFv analogues or fusion proteins of the invention have the ability to promote the internalization of c-erbB-2 or related antigens expressed on the surface of tumor cells when they are bound together at the cell surface. These methods permit the selective killing of cells expressing such antigens with the single-chain-Fv-toxin fusion of appropriate design. sFv-toxin fusion proteins of the invention possess 15-200-fold greater tumor cell killing activity than conjugates which include a toxin that is chemically crosslinked to whole antibody or Fab.

Overexpression of c-erbB-2 or related receptors on malignant cells thus allows targeting of sFv species to the tumor cells, whether the tumor is well-localized or metastatic. In the above cases, the internalization of sFv-toxin fusion proteins permits specific destruction of tumor cells bearing the over expressed c-erbB-2 or related antigen. In other cases, depending on the infected cells, the nature of the malignancy, or other factors operating in a given individual, the same c-erbB-2 or related receptors may be poorly internalized or even represent a static tumor antigen

population. In this event, the single-chain Fv and its fusion proteins can also be used productively, but in a different mode than applicable to internalization of the toxin fusion. Where c-erbB-2 receptor/sFv or sFv fusion protein complexes are poorly internalized, toxins, such as ricin A chain, which operate cytoplasmically by inactivation of ribosomes, are not effective to kill cells. Nevertheless, single-chain unfused Fv is useful, e.g., for imaging or radioimmunotherapy, and bispecific single-chain Fv fusion proteins of various designs, i.e., that have two distinct binding sites on the same polypeptide chain, can be used to target via the two antigens for which the molecule is specific. For example, a bispecific single-chain antibody may have specificity for both the c-erbB-2 and CD3 antigens, the latter of which is present on cytotoxic lymphocytes (CTLs). This bispecific molecule could thus mediate antibody dependent cellular cytotoxicity (ADCC) that results in CTL-induced lysis of tumor cells. Similar results could be obtained using a bispecific single-chain Fv specific for c-erbB-2 and the Fcγ receptor type I or II. Other bispecific sFv formulations include domains with c-erbB-2 specificity paired with a growth factor domain specific for hormone or growth factor receptors, such as receptors for transferrin or epidermal growth factor (EGF).

Brief Description of the Drawings

The foregoing and other objects of this invention, the various features thereof, as well as the invention itself, may be more fully understood from the following description, when read together with the accompanying drawings.

FIG. 1A is a schematic drawing of a DNA construct encoding an sFv of the invention, which shows the V_H and V_L encoding domains and the linker region; FIG. 1B is a schematic drawing of the structure of Fv illustrating V_H and V_L domains, each of which comprises three complementarity determining regions (CDRs) and four framework regions (FRs) for monoclonal 520C9, a well known and characterized murine monoclonal antibody specific for c-erbB-2;

FIGS. 2A-2E are schematic representations of embodiments of the invention, each of which comprises a biosynthetic single-chain Fv polypeptide which recognizes a c-erbB-2-related antigen: FIG. 2A is an sFv having a pendant leader sequence, FIG. 2B is an sFv-toxin (or other ancillary protein) construct, and FIG. 2C is a bivalent or bispecific sFv construct; FIG. 2D is a bivalent sFv having a pendant protein attached to the carboxyl-terminal end; FIG. 2E is a bivalent sFv having pendant proteins attached to both amino- and carboxyl-terminal ends.

FIG. 3 is a diagrammatic representation of the construction of a plasmid encoding the 520C9 sFv-ricin A fused immunotoxin gene; and

FIG. 4 is a graphic representation of the results of a competition assay comparing the c-erbB-2 binding activity of the 520C9 monoclonal antibody (specific for c-erbB-2), an Fab fragment of that monoclonal antibody (filled dots), and different affinity purified

fractions of the single-chain-Fv binding site for c-erbB-2 constructed from the variable regions of the 520C9 monoclonal antibody (sFv whole sample (+), sFv bound and eluted from a column of immobilized extracellular domain of C-erbB-2 (squares) and sFv flow-through (unbound, *)).

Detailed Description of the Invention

Disclosed are single-chain Fv's and sFv fusion proteins having affinity for a c-erbB-2-related antigen expressed at high levels on breast and ovarian cancer cells and on other tumor cells as well, in certain other forms of cancer. The polypeptides are characterized by one or more sequences of amino acids constituting a region which behaves as a biosynthetic antibody binding site. As shown in FIG. 1, the sites comprise heavy chain variable region (V_H) 10, light chain variable region (V_L) 14 single chains wherein V_H 10 and V_L 14 are attached by polypeptide linker 12. The binding domains include CDRs 2, 4, 6 and 2', 4', 6' from immunoglobulin molecules able to bind a c-erbB-2-related tumor antigen linked to FRs 32, 34, 36, 38 and 32', 34', 36' 38' which may be derived from a separate immunoglobulin. As shown in FIGS. 2A, 2B, and 2C, the BABS single polypeptide chains (V_H 10, V_L 14 and linker 12) may also include remotely detectable moieties and/or other polypeptide sequences 16, 18, or 22, which function e.g., as an enzyme, toxin, binding site, or site of attachment to an immobilization matrix or radioactive atom. Also disclosed are methods for producing the proteins and methods of their use.

The single-chain Fv polypeptides of the invention are biosynthetic in the sense that they are synthesized and recloned in a cellular host made to express a protein encoded by a plasmid which includes genetic sequence based in part on synthetic DNA, that is, a recombinant DNA made from ligation of plural, chemically synthesized and recloned oligonucleotides, or by ligation of fragments of DNA derived from the genome of a hybridoma, mature B cell clone, or a cDNA library derived from such natural sources. The

proteins of the invention are properly characterized as "antibody binding sites" in that these synthetic single polypeptide chains are able to refold into a 3-dimensional conformation designed specifically to have affinity for a preselected c-erbB-2 or related tumor antigen. Single-chain Fv's may be produced as described in PCT application US88/01737, which corresponds to USSN 342,449, filed February 6, 1989, and claims priority from USSN 052,800, filed May 21, 1987, assigned to Creative BioMolecules, Inc., hereby incorporated by reference. The polypeptides of the invention are antibody-like in that their structure is patterned after regions of native antibodies known to be responsible for c-erbB-2-related antigen recognition.

More specifically, the structure of these biosynthetic antibody binding sites (BABS) in the region which imparts the binding properties to the protein, is analogous to the Fv region of a natural antibody to a c-erbB-2 or related antigen. It includes a series of regions consisting of amino acids defining at least three polypeptide segments which together form the tertiary molecular structure responsible for affinity and binding. The CDRs are held in appropriate conformation by polypeptide segments analogous to the framework regions of the Fv fragment of natural antibodies.

The CDR and FR polypeptide segments are designed empirically based on sequence analysis of the Fv region of preexisting antibodies, such as those described in U.S. Patent No. 4,753,894, herein incorporated by reference, or of the DNA encoding such antibody molecules.

One such antibody, 520C9, is a murine monoclonal antibody that is known to react with an antigen expressed by the human breast cancer cell line SK-Br-3 (U.S. Patent 4,753,894). The antigen is an approximately 200 kD acidic glycoprotein that has an isoelectric point of 5.3, and is present at about 5 million copies per cell. The association constant measured using radiolabelled antibody is approximately $4.6 \times 10^8 \ M^{-1}$.

In one embodiment, the amino acid sequences constituting the FRs of the single polypeptide chains are analogous to the FR sequences of a first preexisting antibody, for example, a human IgG. The amino acid sequences constituting the CDRs are analogous to the sequences from a second, different preexisting antibody, for example, the CDRs of a rodent or human IgG which recognizes c-erbB-2 or related antigens expressed on the surface of ovarian and breast tumor cells. Alternatively, the CDRs and FRs may be copied in their entirety from a single preexisting antibody from a cell line which may be unstable or, difficult to culture; e.g., an sFv-producing cell line that is based upon a murine, mouse/human, or human monoclonal antibody-secreting cell line.

Practice of the invention enables the design and biosynthesis of various reagents, all of which are characterized by a region having affinity for a preselected c-erbB-2 or related antigen. Other regions of the biosynthetic protein are designed with the particular planned utility of the protein in mind. Thus, if the reagent is designed for intravascular use in mammals, the FRs may include amino acid sequences that are similar or identical to at least a portion of the FR amino acids of antibodies native to that

mammalian species. On the other hand, the amino acid sequences that include the CDRs may be analogous to a portion of the amino acid sequences from the hypervariable region (and certain flanking amino acids) of an antibody having a known affinity and specificity for a c-erbB-2 or related antigen that is from, e.g., a mouse or rat, or a specific human antibody or immunoglobulin.

Other sections of native immunoglobulin protein structure, e.g., C_H and C_L, need not be present and normally are intentionally omitted from the biosynthetic proteins of this invention. However, the single polypeptide chains of the invention may include additional polypeptide regions defining a leader sequence or a second polypeptide chain that is bioactive, e.g., a cytokine, toxin, ligand, hormone, immunoglobulin domain(s), or enzyme, or a site onto which a toxin, drug, or a remotely detectable moiety, e.g., a radionuclide, can be attached.

One useful toxin is ricin, an enzyme from the castor bean that is highly toxic, or the portion of ricin that confers toxicity. At concentrations as low as 1 ng/ml ricin efficiently inhibits the growth of cells in culture. The ricin A chain has a molecular weight of about 30,000 and is glycosylated. The ricin B chain has a larger size (about 34,000 molecular weight) and is also glycosylated. The B chain contains two galactose binding sites, one in each of the two domains in the folded subunit. The crystallographic structure for ricin shows the backbone tracing of the A chain. There is a cleft, which is probably the active site, that runs diagonally across the molecule. Also present is a mixture of α-helix, ß-structure, and irregular structure in the molecule.

The A chain enzymatically inactivates the 60S ribosomal subunit of eucaryotic ribosomes. The B chain binds to galactose-based carbohydrate residues on the surfaces of cells. It appears to be necessary to bind the toxin to the cell surface, and also facilitates and participates in the mechanics of entry of the toxin into the cell. Because all cells have galactose-containing cell surface receptors, ricin inhibits all types of mammalian cells with nearly the same efficiency.

Ricin A chain and ricin B chain are encoded by a gene that specifies both the A and B chains. The polypeptide synthesized from the mRNA transcribed from the gene contains A chain sequences linked to B chain sequences by a 'J' (for joining) peptide. The J peptide fragment is removed by post-translational modification to release the A and B chains. However, A and B chains are still held together by the interchain disulfide bond. The preferred form of ricin is recombinant A chain as it is totally free of B chain and, when expressed in E. coli, is unglycosylated and thus cleared from the blood more slowly than the gycosylated form. The specific activity of the recombinant ricin A chain against ribosomes and that of native A chain isolated from castor bean ricin are equivalent. An amino acid sequence and corresponding nucleic acid sequence of ricin A chain is set forth in the Sequence Listing as SEQ ID NOS:7 and 8.

Recombinant ricin A chain, plant-derived ricin A chain, deglycosylated ricin A chain, or derivatives thereof, can be targeted to a cell expressing a c-erbB-2 or related antigen by the single-chain Fv polypeptide of the present invention. To do this, the sFv may be chemically crosslinked to ricin A chain or

an active analog thereof, or in a preferred embodiment a single-chain Fv-ricin A chain immunotoxin may be formed by fusing the single-chain Fv polypeptide to one or more ricin A chains through the corresponding gene fusion. By replacing the B chain of ricin with an antibody binding site to c-erbB-2 or related antigens, the A chain is guided to such antigens on the cell surface. In this way the selective killing of tumor cells expressing these antigens can be achieved. This selectivity has been demonstrated in many cases against cells grown in culture. It depends on the presence or absence of antigens on the surface of the cells to which the immunotoxin is directed.

The invention includes the use of humanized single-chain-Fv binding sites as part of imaging methods and tumor therapies. The proteins may be administered by intravenous or intramuscular injection. Effective dosages for the single-chain Fv constructs in antitumor therapies or in effective tumor imaging can be determined by routine experimentation, keeping in mind the objective of the treatment.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions. In all cases, the form must be sterile and must be fluid so as to be easily administered by syringe. It must be stable under the conditions of manufacture and storage, and must be preserved against the contaminating action of microorganisms. This may, for example, be achieved by filtration through a sterile 0.22 micron filter and/or lyophilization followed by sterilization with a gamma ray source.

Sterile injectable solutions are prepared by incorporating the single chain constructs of the invention in the required amount in the appropriate

solvent, such as sodium phosphate-buffered saline, followed by filter sterilization. As used herein, "a physiologically acceptable carrier" includes any and all solvents, dispersion media, antibacterial and antifungal agents that are non-toxic to humans, and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. The media or agent must be compatible with maintenance of proper conformation of the single polypeptide chains, and its use in the therapeutic compositions. Supplementary active ingredients can also be incorporated into the compositions.

A bispecific single-chain Fv could also be fused to a toxin. For example, a bispecific sFv construct with specificity for c-erbB-2 and the transferrin receptor, a target that is rapidly internalized, would be an effective cytolytic agent due to internalization of the transferrin receptor/sFv-toxin complex. An sFv fusion protein may also include multiple protein domains on the same polypeptide chain, e.g., EGF-sFv-ricin A, where the EGF domain promotes internalization of toxin upon binding of sFv through interaction with the EGF receptor.

The single polypeptide chains of the invention can be labelled with radioisotopes such as Iodine-131, Indium-111, and Technetium-99m, for example. Beta emitters such as Technetium-99m and Indium-111 are preferred because they are detectable with a gamma camera and have favorable half-lives for imaging in vivo. The single polypeptide chains can be labelled, for example, with radioactive atoms and as Yttrium-90, Technetium-99m, or Indium-111 via a conjugated metal chelator (see, e.g., Khaw et al. (1980) Science 209:295; Gansow et al., U.S. Patent No. 4,472,509;

Hnatowich, U.S. Patent No. 4,479,930), or by other standard means of isotope linkage to proteins known to those with skill in the art.

The invention thus provides intact binding sites for c-erbB-2 or related antigens that are analogous to V_H-V_L dimers linked by a polypeptide sequence to form a composite $(V_H\text{-linker-}V_L)_n$ or $(V_L\text{-linker-}V_H)_n$ polypeptide, where n is equal to or greater than 1, which is essentially free of the remainder of the antibody molecule, and which may include a detectable moiety or a third polypeptide sequence linked to each V_H or V_L.

FIGs. 2A-2E illustrate examples of protein structures embodying the invention that can be produced by following the teaching disclosed herein. All are characterized by at least one biosynthetic sFv single chain segment defining a binding site, and containing amino acid sequences including CDRs and FRs, often derived from different immunoglobulins, or sequences homologous to a portion of CDRs and FRs from different immunoglobulins.

FIG. 2A depicts single polypeptide chain sFv 100 comprising polypeptide 10 having an amino acid sequence analogous to the heavy chain variable region (V_H) of a given anti-c-erbB-2 monoclonal antibody, bound through its carboxyl end to polypeptide linker 12, which in turn is bound to polypeptide 14 having an amino acid sequence analogous to the light chain variable region (V_L) of the anti-c-erbB-2 monoclonal. Of course, the light and heavy chain domains may be in reverse order. Linker 12 should be at least long enough (e.g., about 10 to 15 amino acids or about 40 Angstroms) to permit chains 10 and 14 to assume their proper conformation and interdomain relationship.

Linker 12 may include an amino acid sequence homologous to a sequence identified as "self" by the species into which it will be introduced, if drug use is intended. Unstructured, hydrophilic amino acid sequences are preferred. Such linker sequences are set forth in the Sequence Listing as amino acid residue numbers 116 through 135 in SEQ ID NOS:3, 4, 5, and 6, which include part of the 16 amino acid linker sequences set forth in the Sequence Listing SEQ ID NOS:12 and 14.

Other proteins or polypeptides may be attached to either the amino or carboxyl terminus of protein of the type illustrated in FIG. 2A. As an example, leader sequence 16 is shown extending from the amino terminal end of V_H domain 10.

FIG. 2B depicts another type of reagent 200 including a single polypeptide chain 100 and a pendant protein 18. Attached to the carboxyl end of the polypeptide chain 100 (which includes the FR and CDR sequences constituting an immunoglobulin binding site) is a pendant protein 18 consisting of, for example, a toxin or toxic fragment thereof, binding protein, enzyme or active enzyme fragment, or site of attachment for an imaging agent (e.g., to chelate a radioactive ion such as Indium-111).

FIG. 2C illustrates single chain polypeptide 300 including second single chain polypeptide 110 of the invention having the same or different specificity and connected via peptide linker 22 to the first single polypeptide chain 100.

FIG. 2D illustrates single chain polypeptide 400 which includes single polypeptide chains 110 and 100 linked together by linker 22, and pendant protein 18 attached to the carboxyl end of chain 110.

FIG. 2E illustrates single polypeptide chain 500 which includes chain 400 of Fig. 2D and pendant protein 20 (EGF) attached to the amino terminus of chain 400.

As is evident from Figs. 2A-E, single chain proteins of the invention may resemble beads on a string by including multiple biosynthetic binding sites, each binding site having unique specificity, or repeated sites of the same specificity to increase the avidity of the protein. As is evidenced from the foregoing, the invention provides a large family of reagents comprising proteins, at least a portion of which defines a binding site patterned after the variable region or regions of immunoglobulins to c-erbB-2 or related antigens.

The single chain polypeptides of the invention are designed at the DNA level. The synthetic DNAs are then expressed in a suitable host system, and the expressed proteins are collected and renatured if necessary.

The ability to design the single polypeptide chains of the invention depends on the ability to identify monoclonal antibodies of interest, and then to determine the sequence of the amino acids in the variable region of these antibodies, or the DNA sequence encoding them. Hybridoma technology enables production of cell lines secreting antibody to essentially any desired substance that elicits an immune response. For example, U.S. Patent No. 4,753,894 describes some monoclonal antibodies of interest which recognize c-erbB-2 related antigens on breast cancer cells, and explains how such antibodies were obtained. One monoclonal antibody that is particularly useful for this purpose is 520C9 (Bjorn et al. (1985) Cancer Res. 45:124-1221; U.S. Patent

No. 4,753,894). This antibody specifically recognizes the c-erbB-2 antigen expressed on the surface of various tumor cell lines, and exhibits very little binding to normal tissues. Alternative sources of sFv sequences with the desired specificity can take advantage of phage antibody and combinatorial library methodology. Such sequences would be based on cDNA from mice which were preimmunized with tumor cell membranes or c-erb-B-2 or c-erbB-2-related antigenic fragments or peptides. (See, e.g., Clackson et al, Nature 352 624-628 (1991))

The process of designing DNA that encodes the single polypeptide chain of interest can be accomplished as follows. RNA encoding the light and heavy chains of the desired immunoglobulin can be obtained from the cytoplasm of the hyridoma producing the immunoglobulin. The mRNA can be used to prepare the cDNA for subsequent isolation of V_H and V_L genes by PCR methodology known in the art (Sambrook et al., eds., Molecular Cloning, 1989, Cold Spring Harbor Laboratories Press, NY). The N-terminal amino acid sequence of H and L chain may be independently determined by automated Edman sequencing; if necessary, further stretches of the CDRs and flanking FRs can be determined by amino acid sequencing of the H and L chain V region fragments. Such sequence analysis is now conducted routinely. This knowledge permits one to design synthetic primers for isolation of V_H and V_L genes from hybridoma cells that make monoclonal antibodies known to bind the c-erbB-2 or related antigen. These V genes will encode the Fv region that binds c-erbB-2 in the parent antibody.

Still another approach involves the design and construction of synthetic V genes that will encode an Fv binding site specific for c-erbB-2 or related

receptors. For example, with the help of a computer program such as, for example, Compugene, and known variable region DNA sequences, one may design and directly synthesize native or near-native FR sequences from a first antibody molecule, and CDR sequences from a second antibody molecule. The V_H and V_L sequences described above are linked together directly via an amino acid chain or linker connecting the C-terminus of one chain with the N-terminus of the other.

These genes, once synthesized, may be cloned with or without additional DNA sequences coding for, e.g., a leader peptide which facilitates secretion or intracellular stability of a fusion polypeptide, or a leader or trailing sequence coding for a second polypeptide. The genes then can be expressed directly in an appropriate host cell.

By directly sequencing an antibody to a c-erbB-2 or related antigen, or obtaining the sequence from the literature, in view of this disclosure, one skilled in the art can produce a single chain Fv comprising any desired CDR and FR. For example, using the DNA sequence for the 520C9 monoclonal antibody set forth in the Sequence Listing as SEQ ID NO:3, a single chain polypeptide can be produced having a binding affinity for a c-erbB-2 related antigen. Expressed sequences may be tested for binding and empirically refined by exchanging selected amino acids in relatively conserved regions, based on observation of trends in amino acid sequence data and/or computer modeling techniques. Significant flexibility in V_H and V_L design is possible because alterations in amino acid sequences may be made at the DNA level.

Accordingly, the construction of DNAs encoding the single-chain Fv and sFv fusion proteins of the

invention can be done using known techniques involving the use of various restriction enzymes which make sequence-specific cuts in DNA to produce blunt ends or cohesive ends, DNA ligases, techniques enabling enzymatic addition of sticky ends to blunt-ended DNA, construction of synthetic DNAs by assembly of short or medium length oligonucleotides, cDNA synthesis techniques, and synthetic probes for isolating immunoglobulin genes. Various promoter sequences and other regulatory RNA sequences used in achieving expression, and various type of host cells are also known and available. Conventional transfection techniques, and equally conventional techniques for cloning and subcloning DNA are useful in the practice of this invention and known to those skilled in the art. Various types of vectors may be used such as plasmids and viruses including animal viruses and bacteriophages. The vectors may exploit various marker genes which impart to a successfully transfected cell a detectable phenotypic property that can be used to identify which of a family of clones has successfully incorporated the recombinant DNA of the vector.

Of course, the processes for manipulating, amplifying, and recombining DNA which encode amino acid sequences of interest are generally well known in the art, and therefore, not described in detail herein. Methods of identifying the isolated V genes encoding antibody Fv regions of interest are well understood, and described in the patent and other literature. In general, the methods involve selecting genetic material coding for amino acid sequences which define the CDRs and FRs of interest upon reverse transcription, according to the genetic code.

One method of obtaining DNA encoding the single-chain Fv disclosed herein is by assembly of synthetic oligonucleotides produced in a conventional, automated, polynucleotide synthesizer followed by ligation with appropriate ligases. For example, overlapping, complementary DNA fragments comprising 15 bases may be synthesized semi-manually using phosphoramidite chemistry, with end segments left unphosphorylated to prevent polymerization during ligation. One end of the synthetic DNA is left with a "sticky end" corresponding to the site of action of a particular restriction endonuclease, and the other end is left with an end corresponding to the site of action of another restriction endonuclease. Alternatively, this approach can be fully automated. The DNA encoding the single chain polypeptides may be created by synthesizing longer single strand fragments (e.g., 50-100 nucleotides long) in, for example, a Biosearch oligonucleotide synthesizer, and then ligating the fragments.

Additional nucleotide sequences encoding, for example, constant region amino acids or a bioactive molecule may also be linked to the gene sequences to produce a bifunctional protein.

For example, the synthetic genes and DNA fragments designed as described above may be produced by assembly of chemically synthesized oligonucleotides. 15-100mer oligonucleotides may be synthesized on a Biosearch DNA Model 8600 Synthesizer, and purified by polyacrylamide gel electrophoresis (PAGE) in Tris-Borate-EDTA buffer (TBE). The DNA is then electroeluted from the gel. Overlapping oligomers may be phosphorylated by T4 polynucleotide kinase and ligated into larger blocks which may also be purified by PAGE.

The blocks or the pairs of longer oligonucleotides may be cloned in E. coli using a suitable cloning vector, e.g., pUC. Initially, this vector may be altered by single-strand mutagenesis to eliminate residual six base altered sites. For example, V_H may be synthesized and cloned into pUC as five primary blocks spanning the following restriction sites: (1) EcoRI to first NarI site; (2) first NarI to XbaI; (3) XbaI to SalI; (4) SalI to NcoI; and (5) NcoI to BamHI. These cloned fragments may then be isolated and assembled in several three-fragment ligations and cloning steps into the pUC8 plasmid. Desired ligations, selected by PAGE, are then transformed into, for example, E. coli strain JM83, and plated onto LB Ampicillin + Xgal plates according to standard procedures. The gene sequence may be confirmed by supercoil sequencing after cloning, or after subcloning into M13 via the dideoxy method of Sanger (Molecular Cloning, 1989, Sambrook et al., eds, 2d ed., Vol. 2, Cold Spring Harbor Laboratory Press, NY).

The engineered genes can be expressed in appropriate prokaryotic hosts such as various strains of E. coli, and in eucaryotic hosts such as Chinese hamster ovary cells (CHO), mouse myeloma, hybridoma, transfectoma, and human myeloma cells.

If the gene is to be expressed in E. coli, it may first be cloned into an expression vector. This is accomplished by positioning the engineered gene downstream from a promoter sequence such as Trp or Tac, and a gene coding for a leader polypeptide such as fragment B (FB) of staphylococcal protein A. The resulting expressed fusion protein accumulates in refractile bodies in the cytoplasm of the cells, and may be harvested after disruption of the cells by

French press or sonication. The refractile bodies are solubilized, and the expressed fusion proteins are cleaved and refolded by the methods already established for many other recombinant proteins (Huston et al, 1988, supra) or, for direct expression methods, there is no leader and the inclusion bodies may be refolded without cleavage (Huston et al, 1991, Methods in Enzymology, vol 203, pp 46-88).

For example, subsequent proteolytic cleavage of the isolated sFv from their leader sequence fusions can be performed to yield free sFvs, which can be renatured to obtain an intact biosynthetic, hybrid antibody binding site. The cleavage site preferably is immediately adjacent the sFv polypeptide and includes one amino acid or a sequence of amino acids exclusive of any one amino acid or amino acid sequence found in the amino acid structure of the single polypeptide chain.

The cleavage site preferably is designed for specific cleavage by a selected agent. Endopeptidases are preferred, although non-enzymatic (chemical) cleavage agents may be used. Many useful cleavage agents, for instance, cyanogen bromide, dilute acid, trypsin, Staphylococcus aureus V-8 protease, post-proline cleaving enzyme, blood coagulation Factor Xa, enterokinase, and renin, recognize and preferentially or exclusively cleave at particular cleavage sites. One currently preferred peptide sequence cleavage agent is V-8 protease. The currently preferred cleavage site is at a Glu residue. Other useful enzymes recognize multiple residues as a cleavage site, e.g., factor Xa (Ile-Glu-Gly-Arg) or enterokinase (Asp-Asp-Asp-Asp-Lys). Dilute acid preferentially leaves the peptide bond between Asp-Pro residues, and CNBr in acid cleaves after Met, unless it is followed by Tyr.

If the engineered gene is to be expressed in eucaryotic hybridoma cells, the conventional expression system for immunoglobulins, it is first inserted into an expression vector containing, for example, the immunoglobulin promoter, a secretion signal, immunoglobulin enhancers, and various introns. This plasmid may also contain sequences encoding another polypeptide such as all or part of a constant region, enabling an entire part of a heavy or light chain to be expressed, or at least part of a toxin, enzyme, cytokine, or hormone. The gene is transfected into myeloma cells via established electroporation or protoplast fusion methods. Cells so transfected may then express V_H-linker-V_L or V_L-linker-V_H single-chain Fv polypeptides, each of which may be attached in the various ways discussed above to a protein domain having another function (e.g., cytotoxicity).

For construction of a single contiguous chain of amino acids specifying multiple binding sites, restriction sites at the boundaries of DNA encoding a single binding site (i.e., V_H-linker-V_L) are utilized or created, if not already present. DNAs encoding single binding sites are ligated and cloned into shuttle plasmids, from which they may be further assembled and cloned into the expression plasmid. The order of domains will be varied and spacers between the domains provide flexibility needed for independent folding of the domains. The optimal architecture with respect to expression levels, refolding and functional activity will be determined empirically. To create bivalent sFv's, for example, the stop codon in the gene encoding the first binding site is changed to an open reading frame, and several glycine plus serine codons including a restriction site such as BamHI (encoding

Gly-Ser) or XhoI (encoding Gly-Ser-Ser) are put in place. The second sFv gene is modified similarly at its 5' end, receiving the same restriction site in the same reading frame. The genes are combined at this site to produce the bivalent sFv gene.

Linkers connecting the C-terminus of one domain to the N-terminus of the next generally comprise hydrophilic amino acids which assume an unstructured configuration in physiological solutions and preferably are free of residues having large side groups which might interfere with proper folding of the V_H, V_L, or pendant chains. One useful linker has the amino acid sequence $[(Gly)_4Ser]_3$ (see SEQ ID NOS:5 and 6, residue numbers 121-135). One currently preferred linker has the amino acid sequence comprising 2 or 3 repeats of $[(Ser)_4Gly]$, such as $[(Ser)_4Gly]_2$ and $[(Ser)_4Gly]_3$ (see SEQ ID NOS:3 and 4).

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

EXAMPLES

1. Antibodies to c-erbB-2 Related Antigens

Monoclonal antibodies against breast cancer have been developed using human breast cancer cells or membrane extracts of the cells for immunizing mice, as described in Frankel et al. (1985) J. Biol. Resp. Modif. 4:273-286, hereby incorporated by reference. Hybridomas have been made and selected for production of antibodies using a panel of normal and breast cancer cells. A panel of eight normal tissue membranes, a fibroblast cell line, and frozen sections of breast cancer tissues were used in the screening. Candidates that passed the first screening were further tested on 16 normal tissue sections, 5 normal blood cell types,

11 nonbreast neoplasm sections, 21 breast cancer sections, and 14 breast cancer cell lines. From this selection, 127 antibodies were selected. Irrelevant antibodies and nonbreast cancer cell lines were used in control experiments.

Useful monoclonal antibodies were found to include 520C9, 454C11 (A.T.C.C. Nos. HB8696 and HB8484, respectively) and 741F8. Antibodies identified as selective for breast cancer in this screen reacted against five different antigens. The sizes of the antigens that the antibodies recognize: 200 kD; a series of proteins that are probably degradation products with Mr's of 200 kD, 93kD, 60 kD, and 37 kD; 180 kD (transferrin receptor); 42 kD; and 55 kD, respectively. Of the antibodies directed against the five classes of antigens, the most specific are the ones directed against the 200 kD antigen, 520C9 being a representative antibody for that antigen class. 520C9 reacts with fewer breast cancer tissues (about 20-70% depending on the assay conditions) and it reacts with the fewest normal tissues of any of the antibodies. 520C9 reacts with kidney tubules (as do many monoclonal antibodies), but not pancreas, esophagus, lung, colon, stomach, brain, tonsil, liver, heart, ovary, skin, bone, uterus, bladder, or normal breast among some of the tissues tested.

2. Preparation of cDNA Library Encoding 520C9 Antibody.

Polyadenylated RNA was isolated from approximately 1 x 10^8 (520C9 hybridoma) cells using the "FAST TRACK" mRNA isolation kit from Invitrogen (San Diego, CA). The presence of immunoglobulin heavy chain RNA was confirmed by Northern analysis (Molecular Cloning, 1989, Sambrook et al., eds., 2d ed., Cold

Spring Harbor Laboratory Press, NY) using a recombinant probe containing the various J regions of heavy chain genomic DNA. Using 6 μg RNA for each, cDNA was prepared using the Invitrogen cDNA synthesis system with either random and oligo dT primers. Following synthesis, the cDNA was size-selected by isolating 0.5-3.0 Kilobase (Kb) fragments following agarose gel electrophoresis. After optimizing the cDNA to vector ratio, these fragments were then ligated to the pcDNA II Invitrogen cloning vector.

3. Isolation of V_H and V_L Domains

After transformation of the bacteria with plasmid library DNA, colony hybridization was performed using antibody constant (C) region and joining (J) region probes for either light or heavy chain genes. See Orlandi, R., et al., 1989, Proc. Nat. Aca. Sci. 86:3833. The antibody constant region probe can be obtained from any of light or heavy chain nucleotide sequences from an immunoglobulin gene using known procedures. Several potential positive clones were identified for both heavy and light chain genes and, after purification by a second round of screening, these were sequenced. One clone (M207) contained the sequence of non-functional Kappa chain which has a tyrosine substituted for a conserved cysteine, and also terminates prematurely due to a 4 base deletion which causes a frame-shift mutation in the variable-J region junction. A second light chain clone (M230) contained virtually the entire 520C9 light chain gene except for the last 18 amino acids of the constant region and approximately half of the signal sequence. The 520C9 heavy chain variable region was present on a clone of approximately 1,100 base pairs (F320) which ended near the end of the CH2 domain.

4. Mutagenesis of V_H AND V_L

In order to construct the sFv, both the heavy and light chain variable regions were mutagenized to insert appropriate restriction sites (Kunkel, T.A., 1985, Proc. Nat. Acad. Sci. USA 82:1373). The heavy chain clone (F320) was mutagenized to insert a BamH1 site at the 5' end of V_H (F321). The light chain was also mutagenized simultaneously by inserting an EcoRV site at the 5' end and a PstI site with a translation stop codon at the 3' end of the variable region (M231).

5. Sequencing

cDNA clones encoding light and heavy chain were sequenced using external standard pUC primers and several specific internal primers which were prepared on the basis of the sequences obtained for the heavy chain. The nucleotide sequences were analyzed in a Genbank homology search (program Nucscan of DNA-star) to eliminate endogenous immunoglobulin genes. Translation into amino acids was checked with amino acid sequences in the NIH atlas edited by E. Kabat.

Amino acid sequences derived from 520C9 immunoglobulin confirmed the identity of these V_H and V_L cDNA clones. The heavy chain clone pF320 started 6 nucleotides upstream of the first ATG codon and extended into the CH2-encoding region, but it lacked the last nine amino acid codons of the CH2 constant domain and all of the CH3 coding region, as well as the 3' untranslated region and the poly A tail. Another short heavy chain clone containing only the CH2 and CH3 coding regions, and the poly A tail was initially assumed to represent the missing part of the 520C9 heavy chain. However, overlap between both sequences was not identical. The 520C9 clone (pF320) encodes the CH1 and CH2 domains of murine IgG1, whereas the short clone pF315 encodes the CH2 and CH3 of IgG2b.

6. Gene Design

A nucleic acid sequence encoding a composite 520C9 sFv region containing a single-chain Fv binding site which recognizes c-erbB-2 related tumor antigens was designed with the aid of Compugene software. The gene contains nucleic acid sequences encoding the V_H and V_L regions of the 520C9 antibody described above linked together with a double-stranded synthetic oligonucleotide coding for a peptide with the amino acid sequence set forth in the Sequence Listing as amino acid residue numbers 116 through 133 in SEQ ID NOS:3 and 4. This linker oligonucleotide contains helper cloning sites EcoRI and BamHI, and was designed to contain the assembly sites SacI and EcoRV near its 5' and 3' ends, respectively. These sites enable match-up and ligation to the 3' and 5' ends of 520C9 V_H and V_L, respectively, which also contain these sites (V_H-linker-V_L). However, the order of linkage to the oligonucleotide may be reversed (V_L-linker-V_H) in this or any sFv of the invention. Other restriction sites were designed into the gene to provide alternative assembly sites. A sequence encoding the FB fragment of protein A was used as a leader.

The invention also embodies a humanized single-chain Fv, i.e., containing human framework sequences and CDR sequences which specify c-erbB-2 binding, e.g., like the CDRs of the 520C9 antibody. The humanized Fv is thus capable of binding c-erbB-2 while eliciting little or no immune response when administered to a patient. A nucleic acid sequence encoding a humanized sFv may be designed and constructed as follows. Two strategies for sFv design are especially useful. A homology search in the GenBank database for the most related human framework (FR) regions may be performed

and FR regions of the sFv may be mutagenized according to sequences identified in the search to reproduce the corresponding human sequence; or information from computer modeling based on x-ray structures of model Fab fragments may be used (Amit et al., 1986, Science 233:747-753; Colman et al., 1987, Nature 326:358-363; Sheriff et al., 1987, Proc. Nat. Aca. Sci., 84:8075-8079; and Satow et al., 1986, J. Mol. Biol. 190:593-604, all of which are hereby incorporated by reference). In a preferred case, the most homologous human V_H and V_L sequences may be selected from a collection of PCR-cloned human V regions. The FRs are made synthetically and fused to CDRs to make successively more complete V regions by PCR-based ligation, until the full humanized V_L and V_H are completed. For example, a humanized sFv that is a hybrid of the murine 520C9 antibody CDRs and the human myeloma protein NEW FRs can be designed such that each variable region has the murine binding site within a human framework (FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4). The Fab NEW crystal structure (Saul et al., 1978, J. Biol. Chem. 253:585-597) also may be used to predict the location of FRs in the variable regions. Once these regions are predicted, the amino acid sequence or the corresponding nucleotide sequence of the regions may be determined, and the sequences may be synthesized and cloned into shuttle plasmids, from which they may be further assembled and cloned into an expression plasmid; alternatively, the FR sequences of the 520C9 sFv may be mutagenized directly and the changes verified by supercoil sequencing with internal primers (Chen et al., 1985, DNA 4:165-170).

7. Preparation of and Purification 520C9 sFv

A. Inclusion Body Solubilization.

The 520C9 sFv plasmid, based on a T_7 promoter and vector, was made by direct expression in E. coli of the fused gene sequence set forth in the Sequence Listing as SEQ. ID NO:3. Inclusion bodies (15.8 g) from a 2.0 liter fermentation were washed with 25 mM Tris, 10 mM EDTA, pH 8.0 (TE), plus 1 M guanidine hydrochloride (GuHCl). The inclusion bodies were solubilized in TE, 6 M GuHCl, 10 mM dithiothreitol (DTT), pH 9.0, and yielded 3825 A_{280} units of material. This material was ethanol precipitated, washed with TE, 3M urea, then resuspended in TE, 8M urea, 10 mM DTT, pH 8.0. This precipitation step prepared the protein for ion exchange purification of the denatured sFv.

B. Ion Exchange Chromatography

The solubilized inclusion bodies were subjected to ion exchange chromatography in an effort to remove contaminating nucleic acids and E. coli proteins before renaturation of the sFv. The solubilized inclusion bodies in 8M urea were diluted with TE to a final urea concentration of 6M, then passed through 100 ml of DEAE-Sepharose Fast Flow in a radial flow column. The sFv was recovered in the unbound fraction (69% of the starting sample).

The pH of this sFv solution (A_{280} = 5.7; 290 ml) was adjusted to 5.5 with 1 M acetic acid to prepare it for application to an S-Sepharose Fast Flow column. When the pH went below 6.0, however, precipitate formed in the sample. The sample was clarified; 60% of the sample was in the pellet and 40% in the supernatant. The supernatant was passed through 100 ml S-Sepharose Fast Flow and the sFv recovered in the unbound fraction. The pellet was resolubilized in TE, 6 M

GuHCl, 10 mM DTT, pH 9.0, and was also found to contain primarily sFv in a pool of 45 ml volume with an absorbance at 280 nm of 20 absorbance units. This reduced sFv pool was carried through the remaining steps of the purification.

C. Renaturation of sFv

Renaturation of the sFv was accomplished using a disulfide-restricted refolding approach, in which the disulfides were oxidized while the sFv was fully denatured, followed by removal of the denaturant and refolding. Oxidation of the sFv samples was carried out in TE, 6 M GuHCl, 1 mM oxidized glutathione (GSSG), 0.1 mM reduced glutathione (GSH), pH 9.0. The sFv was diluted into the oxidation buffer to a final protein A_{280} = 0.075 with a volume of 4000 ml and incubated overnight at room temperature. After overnight oxidation this solution was dialyzed against 10 mM sodium phosphate, 1 mM EDTA, 150 mM NaCl, 500 mM urea, pH 8.0 (PENU) [4 x (20 liters X 24 hrs)]. Low levels of activity were detected in the refolded sample.

D. Membrane Fractionation and Concentration of Active sFv

In order to remove aggregated misfolded material before any concentration step, the dialyzed refolded 520C9 sFv (5050 ml) was filtered through a 100K MWCO membrane (100,000 mol. wt. cut-off) (4 x 60 cm^2) using a Minitan ultrafiltration device (Millipore). This step required a considerable length of time (9 hours), primarily due to formation of precipitate in the retentate and membrane fouling as the protein concentration in the retentate increased. 95% of the protein in the refolded sample was retained by the 100K membranes, with 79% in the form of insoluble material. The 100K retentate had very low activity and was discarded.

The 100K filtrate contained most of the soluble sFv activity for binding c-erbB-2, and it was next concentrated using 10K MWCO membranes (10,000 mol. wt. cut-off) (4 x 60 cm^2) in the Minitan, to a volume of 100 ml (50X). This material was further concentrated using a YM10 10K MWCO membrane in a 50 ml Amicon stirred cell to a final volume of 5.2 ml (1000X). Only a slight amount of precipitate formed during the two 10K concentration steps. The specific activity of this concentrated material was significantly increased relative to the initial dialyzed refolding.

E. Size Exclusion Chromatography of Concentrated sFv

When refolded sFv was fractionated by size exclusion chromatography, all 520C9 sFv activity was determined to elut at the position of folded monomer. In order to enrich for active monomers, the 1000X concentrated sFv sample was fractionated on a Sephacryl S-200 HR column (2.5 x 40 cm) in PBSA (2.7 mM KCl, 1.1 mM KH_2PO_4, 138 mM NaCl, 8.1 mM $Na_2HPO_4 \cdot 7H_2O$, 0.02% NaN_3) + 0.5 M urea. The elution profile of the column and SDS-PAGE analysis of the fractions showed two sFv monomer peaks. The two sFv monomer peak fractions were pooled (10 ml total) and displayed c-erbB-2 binding activity in competition assays.

F. Affinity Purification of 520C9 sFv

The extracellular domain of (ECD) c-erbB-2 was expressed in bacculovirus-infected insect cells. This protein (ECD c-erbB-2) was immobilized on an agarose affinity matrix. The sFv monomer peak was dialyzed against PBSA to remove the urea and then applied to a 0.7 x 4.5 cm ECD c-erbB-2-agarose affinity column in PBSA. The column was washed to baseline A_{280}, then eluted with PBSA + 3 M LiCl, pH = 6.1. The peak

fractions were pooled (4 ml) and dialyzed against PBSA to remove the LiCl. 72 μg of purified sFv was obtained from 750 μg of S-200 monomer fractions. Activity measurements on the column fractions were determined by a competitive assay. Briefly, sFv affinity purification fractions and HRP-conjugated 520C9 Fab fragments were allowed to compete for binding to SK-BR-3 membranes. Successful binding of the sFv preparation prevented the HRP-52069 Fab fragment from binding to the membranes, thus also reducing or preventing utilization of the HRP substrate, and no color development (see below for details of competition assay). The results showed that virtually all of the sFv activity was bound by the column and was recovered in the eluted peak (Figure 4). As expected, the specific activity of the eluted peak was increased relative to the column sample, and appeared to be essentially the same as the parent Fab control, within the experimental error of these measurements.

9. Yield After Purification.

Table I shows the yield of various 520C9 preparations during the purification process. Protein concentration (μg/ml) was determined by the BioRad protein assay. Under "Total Yield", 300 AU denatured sFv stock represents 3.15 g inclusion bodies from 0.4 liters fermentation. The oxidation buffer was 25 mM Tris, 10 mM EDTA, 6 M GdnHCl, 1 MM GSSG, 0.1 mM GSH, pH 9.0. Oxidation was performed at room temperature overnight. Oxidized sample was dialyzed against 10 mM sodium phosphate, 1 mM EDTA, 150 mM NaCl, 500 mM urea, pH 8.0. All subsequent steps were carried out in this buffer, except for affinity chromatography, which was carried out in PBSA.

Table I

Sample	Volume	Protein Concentration	Total Yield	% Yield
1. Refolding III (oxidation)	4000 ml	0.075 A_{280}	300 AU	-
2. Dialyzed Refolding III	5050 ml	38 µg/ml	191.9 mg	100
3. Minitan 100K Filtrate	5000 ml	2 µg/ml	10.0 mg	5.4
4. Minitan 10K Retentate	100 ml	45 µg/ml	4.5 mg	2.3
6. YM10 10K Retentate	5.2 ml	600 µg/ml	3.1 mg	1.6
7. S-200 sFv Monomer Peak	10.0 ml	58 µg/ml	0.58 mg	0.3
8. Affinity Purified sFv	5.5 ml	13 µg/ml	0.07 mg	0.04

10. Immunotoxin Construction

The ricin A-520C9 single chain fused immunotoxin (SEQ. ID NO:7) encoding gene was constructed by isolating the gene coding for ricin A on a HindIII to BamH1 fragment from pPL229 (Cetus Corporation, Emeryville, CA) and using it upstream of the 520C9 sFv in pH777, as shown in FIG. 3. This fusion contains the 122 amino acid natural linker present between the A and B domains of ricin. However, in the original pRAP229 expression vector the codon for amino acid 268 of ricin was converted to a TAA translation stop codon so that the expression of the resulting gene produces only ricin A. Therefore, in order to remove the translation stop codon, site-directed mutagenesis was performed to remove the TAA and restore the natural serine codon. This then allows translation to continue through the entire immunotoxin gene.

In order to insert the immunotoxin back into the pPL229 and pRAP229 expression vectors, the PstI site at the end of the immunotoxin gene had to be converted to a sequence that was compatible with the BamHI site in vector. A synthetic oligonucleotide adaptor containing a BclI site nested between PstI ends was inserted. BclI and BamHI ends are compatible and can be combined into a hybrid BclI/BamHI site. Since BclI nuclease is sensitive to dam methylation, the construction first was transformed into a dam(-) E. coli strain, Gm48, in order to digest the plasmid DNA with BclI (and HindIII), then insert the entire immunotoxin gene on a HindIII/BclI fragment back into both Hind III/BamHI-digested expression vectors.

When native 520C9 IgG1 is conjugated with native ricin A chain or recombinant ricin A chain, the resulting immunotoxin is able to inhibit protein

synthesis by 50% at a concentration of about 0.4×10^{-9} M against SK-Br-3 cells. In addition to reacting with SK-Br-3 breast cancer cells, native 520C9 IgG1 immunotoxin also inhibits an ovarian cancer cell line, OVCAR-3, with a ID_{50} of 2.0×10^{-9} M.

In the ricin A-sFv fusion protein described above, ricin acts as leader for expression, i.e., is fused to the amino terminus of sFv. Following direct expression, soluble protein was shown to react with antibodies against native 520C9 Fab and also to exhibit ricin A chain enzymatic activity.

In another design, the ricin A chain is fused to the carboxy terminus of sFv. The 520C9 sFv may be secreted via the PelB signal sequence with ricin A chain attached to the C-terminus of sFv. For this construct, sequences encoding the PelB-signal sequence, sFv, and ricin are joined in a bluescript plasmid via a HindIII site directly following sFv (in our expression plasmids) and the HindIII site preceding the ricin gene, in a three part assembly (RI-HindIII-BamHI). A new PstI site following the ricin gene is obtained via the Bluescript polylinker. Mutagenesis of this DNA removes the stop codon and the original PstI site at the end of sFv, and places several serine residues between the sFv and ricin genes. This new gene fusion, PelB signal sequence/sFv/ricin A, can be inserted into expression vectors as an EcoRI/PstI fragment.

In another design, the pseudomonas exotoxin fragment analogous to ricin A chain, PE40, is fused to the carboxy terminus of the anti-c-erbB-2 741F8 sFv (Seq ID NOS: 15 and 16). The resulting 741F8 sFv-PE40 is a single-chain Fv-toxin fusion protein, which was constructed with an 18 residue short FB leader which initially was left on the protein. E. coli expression

of this protein produced inclusion bodies that were refolded in a 3 M urea glutathione/redox buffer. The resulting sFv-PE40 was shown to specifically kill c-erbB-2 bearing cells in culture more fully and with apparently better cytotoxicity than the corresponding crosslinked immunotoxin. The sFv-toxin protein, as well as the 741F8 sFv, can be made in good yields by these procedures, and may be used as therapeutic and diagnostic agents for tumors bearing the c-erbB-2 or related antigens, such as breast and ovarian cancer.

11. Assays

A. Competition ELISA

SK-Br-3 extract is prepared as a source of c-erbB-2 antigen as follows. SK-Br-3 breast cancer cells (Ring et al. 1989, Cancer Research 49:3070-3080), are grown to near confluence in Iscove's medium (Gibco BRL, Gaithersburg, Md.) plus 5% fetal bovine serum and 2 mM glutamine. The medium is aspirated, and the cells are rinsed with 10 ml fetal bovine serum (FBS) plus calcium and magnesium. The cells are scraped off with a rubber policeman into 10 ml FBS plus calcium and magnesium, and the flask is rinsed out with another 5 ml of this buffer. The cells are then centrifuged at 100 rpm. The supernate is aspirated off, and the cells are resuspended at 10^7 cells/ml in 10 mM NaCl, 0.5% NP40, pH 8 (TNN buffer), and are pipetted up and down to dissolve the pellet. The solution is then centrifuged at 1000 rpm to remove nuclei and other insoluble debris. The extract is filtered through 0.45 Millex HA and 0.2 Millex Gv filters. The TNN extract is stored as aliquots in Wheaton freezing vials at -70°C.

A fresh vial of SK-Br-3 TNN extract is thawed and diluted 200-fold into deionized water. Immediately thereafter, 40ug per well are added to a Dynatech PVC

96 well plak, which is allowed to sit overnight in a 37°C dry incubator. The plates are washed four times in phosphate buffered saline (PBS), 1% skim milk, 0.05% Tween 20.

The non-specific binding sites are blocked as follows. When the plate is dry, 100 ug per well PBS is added containing 1% skim milk, and the incubation allowed to proceed for one hour at room temperature.

The single-chain Fv test samples and standard 520C9 whole antibody dilutions are then added as follows. 520C9 antibody and test samples are diluted in dilution buffer (PBS + 1% skim milk) in serial two-fold steps, initially at 50ug/ml and making at least 10 dilutions for 520C9 standards. A control containing only dilution buffer is included. The diluted samples and standards are added at 50ul per well and incubated for 30 minutes at room temperature.

The 520C9-horseradish peroxidase (HRP) probe is added as follows. 520C9-HRP conjugate (Zymed Labs., South San Francisco, California) is diluted to 14 ug/ml with 1% skim milk in dilution buffer. The optimum dilutions must be determined for each new batch of peroxidase conjugate without removing the previous steps. 20 ul per well of probe was added and incubated for one hour at room temperature. The plate is then washed four times in PBS. The peroxidase substrate is then added. The substrate solution should be made fresh for each use by diluting tetramethyl benzidine stock (TMB; 2mg/ml in 100% ethanol) 1:20 and 3% hydrogen peroxide stock 1:2200 in substrate buffer (10mM sodium acetate, 10mM Na, EDTA, pH 5.0). This is incubated for 30 minutes at room temperature. The wells are then quenched with 100 ul per well 0.8 M H_2SO_4 and the absorbance at 150 nm read.

FIG. 4 compares the binding ability of the parent refolded but unpurified 520C9 monoclonal antibody, 520C9 Fab fragments, and the 520C9 sFv single-chain binding site after binding and elution from an affinity column (eluted) or the unbound flow through fraction (passed). In Fig. 4, the fully purified 520C9 sFv exhibits an affinity for c-erbB-2 that is indistinguishable from the parent monoclonal antibody, within the error of measuring protein concentration.

B. In vivo testing

Immunotoxins that are strong inhibitors of protein synthesis against breast cancer cells grown in culture may be tested for their in vivo efficacy. The in vivo assay is typically done in a nude mouse model using xenografts of human MX-1 breast cancer cells. Mice are injected with either PBS (control) or different concentrations of sFv-toxin immunotoxin, and a concentration-dependent inhibition of tumor growth will be observed. It is expected that higher doses of immunotoxin will produce a better effect.

The invention may be embodied in other specific forms without departing from the spirit and scope thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalence of the claims are intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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Houston, L. L.
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(ii) TITLE OF INVENTION: Biosynthetic Binding Protein for Cancer Marker

(iii) NUMBER OF SEQUENCES: 16

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4299 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..4299
(D) OTHER INFORMATION: /note= "product = "c-erb-b-2""

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

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ATG GAG CTG GCG GCC TTG TGC CGC TGG GGG CTC CTC CTC GCC CTC TTG      48
Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
 1               5                  10                  15

CCC CCC GGA GCC GCG AGC ACC CAA GTG TGC ACC GGC ACA GAC ATG AAG      96
Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
            20                  25                  30

CTG CGG CTC CCT GCC AGT CCC GAG ACC CAC CTG GAC ATG CTC CGC CAC     144
Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
        35                  40                  45

CTC TAC CAG GGC TGC CAG GTG GTG CAG GGA AAC CTG GAA CTC ACC TAC     192
Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
    50                  55                  60

CTG CCC ACC AAT GCC AGC CTG TCC TTC CTG CAG GAT ATC CAG GAG GTG     240
Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65                  70                  75                  80

CAG GGC TAC GTG CTC ATC GCT CAC AAC CAA GTG AGG CAG GTC CCA CTG     288
Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
                85                  90                  95

CAG AGG CTG CGG ATT GTG CGA GGC ACC CAG CTC TTT GAG GAC AAC TAT     336
Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
            100                 105                 110

GCC CTG GCC GTG CTA GAC AAT GGA GAC CCG CTG AAC AAT ACC ACC CCT     384
Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
        115                 120                 125

GTC ACA GGG GCC TCC CCA GGA GGC CTG CGG GAG CTG CAG CTT CGA AGC     432
Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
    130                 135                 140

CTC ACA GAG ATC TTG AAA GGA GGG GTC TTG ATC CAG CGG AAC CCC CAG     480
Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145                 150                 155                 160

CTC TGC TAC CAG GAC ACG ATT TTG TGG AAG GAC ATC TTC CAC AAG AAC     528
Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
                165                 170                 175

AAC CAG CTG GCT CTC ACA CTG ATA GAC ACC AAC CGC TCT CGG GCC TGC     576
Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
            180                 185                 190
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CAC CCC TGT TCT CCG ATG TGT AAG GGC TCC CGC TGC TGG GGA GAG AGT        624
His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
        195                 200                 205

TCT GAG GAT TGT CAG AGC CTG ACG CGC ACT GTC TGT GCC GGT GGC TGT        672
Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
    210                 215                 220

GCC CGC TGC AAG GGG CCA CTG CCC ACT GAC TGC TGC CAT GAG CAG TGT        720
Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225                 230                 235                 240

GCT GCC GGC TGC ACG GGC CCC AAG CAC TCT GAC TGC CTG GCC TGC CTC        768
Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
                245                 250                 255

CAC TTC AAC CAC AGT GGC ATC TGT GAG CTG CAC TGC CCA GCC CTG GTC        816
His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
            260                 265                 270

ACC TAC AAC ACA GAC ACG TTT GAG TCC ATG CCC AAT CCC GAG GGC CGG        864
Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
        275                 280                 285

TAT ACA TTC GGC GCC AGC TGT GTG ACT GCC TGT CCC TAC AAC TAC CTT        912
Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
    290                 295                 300

TCT ACG GAC GTG GGA TCC TGC ACC CTC GTC TGC CCC CTG CAC AAC CAA        960
Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305                 310                 315                 320

GAG GTG ACA GCA GAG GAT GGA ACA CAG CGG TGT GAG AAG TGC AGC AAG       1008
Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
                325                 330                 335

CCC TGT GCC CGA GTG TGC TAT GGT CTG GGC ATG GAG CAC TTG CGA GAG       1056
Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
            340                 345                 350

GTG AGG GCA GTT ACC AGT GCC AAT ATC CAG GAG TTT GCT GGC TGC AAG       1104
Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
        355                 360                 365

AAG ATC TTT GGG AGC CTG GCA TTT CTG CCG GAG AGC TTT GAT GGG GAC       1152
Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
    370                 375                 380

CCA GCC TCC AAC ACT GCC CCG CTC CAG CCA GAG CAG CTC CAA GTG TTT       1200
Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385                 390                 395                 400

GAG ACT CTG GAA GAG ATC ACA GGT TAC CTA TAC ATC TCA GCA TGG CCG       1248
Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
                405                 410                 415
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GAC AGC CTG CCT GAC CTC AGC GTC TTC CAG AAC CTG CAA GTA ATC CGG      1296
Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
            420                 425                 430

GGA CGA ATT CTG CAC AAT GGC GCC TAC TCG CTG ACC CTG CAA GGG CTG      1344
Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
        435                 440                 445

GGC ATC AGC TGG CTG GGG CTG CGC TCA CTG AGG GAA CTG GGC AGT GGA      1392
Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
    450                 455                 460

CTG GCC CTC ATC CAC CAT AAC ACC CAC CTC TGC TTC GTG CAC ACG GTG      1440
Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465                 470                 475                 480

CCC TGG GAC CAG CTC TTT CGG AAC CCG CAC CAA GCT CTG CTC CAC ACT      1488
Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
                485                 490                 495

GCC AAC CGG CCA GAG GAC GAG TGT GTG GGC GAG GGC CTG GCC TGC CAC      1536
Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
            500                 505                 510

CAG CTG TGC GCC CGA GGG CAC TGC TGG GGT CCA GGG CCC ACC CAG TGT      1584
Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
        515                 520                 525

GTC AAC TGC AGC CAG TTC CTT CGG GGC CAG GAG TGC GTG GAG GAA TGC      1632
Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
    530                 535                 540

CGA GTA CTG CAG GGG CTC CCC AGG GAG TAT GTG AAT GCC AGG CAC TGT      1680
Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545                 550                 555                 560

TTG CCG TGC CAC CCT GAG TGT CAG CCC CAG AAT GGC TCA GTG ACC TGT      1728
Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
                565                 570                 575

TTT GGA CCG GAG GCT GAC CAG TGT GTG GCC TGT GCC CAC TAT AAG GAC      1776
Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
            580                 585                 590

CCT CCC TTC TGC GTG GCC CGC TGC CCC AGC GGT GTG AAA CCT GAC CTC      1824
Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
        595                 600                 605

TCC TAC ATG CCC ATC TGG AAG TTT CCA GAT GAG GAG GGC GCA TGC CAG      1872
Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
    610                 615                 620

CCT TGC CCC ATC AAC TGC ACC CAC TCC TGT GTG GAC CTG GAT GAC AAG      1920
Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625                 630                 635                 640
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GGC TGC CCC GCC GAG CAG AGA GCC AGC CCT CTG ACG TCC ATC ATC TCT      1968
Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
                645                 650                 655

GCG GTG GTT GGC ATT CTG CTG GTC GTG GTC TTG GGG GTG GTC TTT GGG      2016
Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
            660                 665                 670

ATC CTC ATC AAG CGA CGG CAG CAG AAG ATC CGG AAG TAC ACG ATG CGG      2064
Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
        675                 680                 685

AGA CTG CTG CAG GAA ACG GAG CTG GTG GAG CCG CTG ACA CCT AGC GGA      2112
Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
    690                 695                 700

GCG ATG CCC AAC CAG GCG CAG ATG CGG ATC CTG AAA GAG ACG GAG CTG      2160
Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
705                 710                 715                 720

AGG AAG GTG AAG GTG CTT GGA TCT GGC GCT TTT GGC ACA GTC TAC AAG      2208
Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
                725                 730                 735

GGC ATC TGG ATC CCT GAT GGG GAG AAT GTG AAA ATT CCA GTG GCC ATC      2256
Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
            740                 745                 750

AAA GTG TTG AGG GAA AAC ACA TCC CCC AAA GCC AAC AAA GAA ATC TTA      2304
Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
        755                 760                 765

GAC GAA GCA TAC GTG ATG GCT GGT GTG GGC TCC CCA TAT GTC TCC CGC      2352
Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
    770                 775                 780

CTT CTG GGC ATC TGC CTG ACA TCC ACG GTG CAG CTG GTG ACA CAG CTT      2400
Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785                 790                 795                 800

ATG CCC TAT GGC TGC CTC TTA GAC CAT GTC CGG GAA AAC CGC GGA CGC      2448
Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
                805                 810                 815

CTG GGC TCC CAG GAC CTG CTG AAC TGG TGT ATG CAG ATT GCC AAG GGG      2496
Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
            820                 825                 830

ATG AGC TAC CTG GAG GAT GTG CGG CTC GTA CAC AGG GAC TTG GCC GCT      2544
Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
        835                 840                 845

CGG AAC GTG CTG GTC AAG AGT CCC AAC CAT GTC AAA ATT ACA GAC TTC      2592
Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
    850                 855                 860
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GGG CTG GCT CGG CTG CTG GAC ATT GAC GAG ACA GAG TAC CAT GCA GAT      2640
Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
865                 870                 875                 880

GGG GGC AAG GTG CCC ATC AAG TGG ATG GCG CTG GAG TCC ATT CTC CGC      2688
Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
                885                 890                 895

CGG CGG TTC ACC CAC CAG AGT GAT GTG TGG AGT TAT GGT GTG ACT GTG      2736
Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
            900                 905                 910

TGG GAG CTG ATG ACT TTT GGG GCC AAA CCT TAC GAT GGG ATC CCA GCC      2784
Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
        915                 920                 925

CGG GAG ATC CCT GAC CTG CTG GAA AAG GGG GAG CGG CTG CCC CAG CCC      2832
Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
    930                 935                 940

CCC ATC TGC ACC ATT GAT GTC TAC ATG ATC ATG GTC AAA TGT TGG ATG      2880
Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
945                 950                 955                 960

ATT GAC TCT GAA TGT CGG CCA AGA TTC CGG GAG TTG GTG TCT GAA TTC      2928
Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
                965                 970                 975

TCC CGC ATG GCC AGG GAC CCC CAG CGC TTT GTG GTC ATC CAG AAT GAG      2976
Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
            980                 985                 990

GAC TTG GGC CCA GCC AGT CCC TTG GAC AGC ACC TTC TAC CGC TCA CTG      3024
Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu
        995                 1000                1005

CTG GAG GAC GAT GAC ATG GGG GAC CTG GTG GAT GCT GAG GAG TAT CTG      3072
Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr Leu
    1010                1015                1020

GTA CCC CAG CAG GGC TTC TTC TGT CCA GAC CCT GCC CCG GGC GCT GGG      3120
Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly Ala Gly
1025                1030                1035                1040

GGC ATG GTC CAC CAC AGG CAC CGC AGC TCA TCT ACC AGG AGT GGC GGT      3168
Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg Ser Gly Gly
                1045                1050                1055

GGG GAC CTG ACA CTA GGG CTG GAG CCC TCT GAA GAG GAG GCC CCC AGG      3216
Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala Pro Arg
            1060                1065                1070

TCT CCA CTG GCA CCC TCC GAA GGG GCT GGC TCC GAT GTA TTT GAT GGT      3264
Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val Phe Asp Gly
        1075                1080                1085
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GAC CTG GGA ATG GGG GCA GCC AAG GGG CTG CAA AGC CTC CCC ACA CAT      3312
Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln Ser Leu Pro Thr His
    1090                1095                1100

GAC CCC AGC CCT CTA CAG CGG TAC AGT GAG GAC CCC ACA GTA CCC CTG      3360
Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro Leu
1105            1110                1115                1120

CCC TCT GAG ACT GAT GGC TAC GTT GCC CCC CTG ACC TGC AGC CCC CAG      3408
Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu Thr Cys Ser Pro Gln
                1125                1130                1135

CCT GAA TAT GTG AAC CAG CCA GAT GTT CGG CCC CAG CCC CCT TCG CCC      3456
Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro Gln Pro Pro Ser Pro
            1140                1145                1150

CGA GAG GGC CCT CTG CCT GCT GCC CGA CCT GCT GGT GCC ACT CTG GAA      3504
Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu
        1155                1160                1165

AGG CCC AAG ACT CTC TCC CCA GGG AAG AAT GGG GTC GTC AAA GAC GTT      3552
Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
    1170                1175                1180

TTT GCC TTT GGG GGT GCC GTG GAG AAC CCC GAG TAC TTG ACA CCC CAG      3600
Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln
1185                1190                1195                1200

GGA GGA GCT GCC CCT CAG CCC CAC CCT CCT CCT GCC TTC AGC CCA GCC      3648
Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala
                1205                1210                1215

TTC GAC AAC CTC TAT TAC TGG GAC CAG GAC CCA CCA GAG CGG GGG GCT      3696
Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala
            1220                1225                1230

CCA CCC AGC ACC TTC AAA GGG ACA CCT ACG GCA GAG AAC CCA GAG TAC      3744
Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu Asn Pro Glu Tyr
        1235                1240                1245

CTG GGT CTG GAC GTG CCA GTG TGA ACC AGA AGG CCA AGT CCG CAG AAG      3792
Leu Gly Leu Asp Val Pro Val  *  Thr Arg Arg Pro Ser Pro Gln Lys
    1250                1255                1260

CCC TGA TGT GTC CTC AGG GAG CAG GGA AGG CCT GAC TTC TGC TGG CAT      3840
Pro  *  Cys Val Leu Arg Glu Gln Gly Arg Pro Asp Phe Cys Trp His
1265                1270                1275                1280

CAA GAG GTG GGA GGG CCC TCC GAC CAC TTC CAG GGG AAC CTG CCA TGC      3888
Gln Glu Val Gly Gly Pro Ser Asp His Phe Gln Gly Asn Leu Pro Cys
                1285                1290                1295
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CAG GAA CCT GTC CTA AGG AAC CTT CCT TCC TGC TTG AGT TCC CAG ATG      3936
Gln Glu Pro Val Leu Arg Asn Leu Pro Ser Cys Leu Ser Ser Gln Met
            1300                1305                1310

GCT GGA AGG GGT CCA GCC TCG TTG GAA GAG GAA CAG CAC TGG GGA GTC      3984
Ala Gly Arg Gly Pro Ala Ser Leu Glu Glu Glu Gln His Trp Gly Val
        1315                1320                1325

TTT GTG GAT TCT GAG GCC CTG CCC AAT GAG ACT CTA GGG TCC AGT GGA      4032
Phe Val Asp Ser Glu Ala Leu Pro Asn Glu Thr Leu Gly Ser Ser Gly
    1330                1335                1340

TGC CAC AGC CCA GCT TGG CCC TTT CCT TCC AGA TCC TGG GTA CTG AAA      4080
Cys His Ser Pro Ala Trp Pro Phe Pro Ser Arg Ser Trp Val Leu Lys
1345                1350                1355                1360

GCC TTA GGG AAG CTG GCC TGA GAG GGG AAG CGG CCC TAA GGG AGT GTC      4128
Ala Leu Gly Lys Leu Ala  *  Glu Gly Lys Arg Pro  *  Gly Ser Val
                1365                1370                1375

TAA GAA CAA AAG CGA CCC ATT CAG AGA CTG TCC CTG AAA CCT AGT ACT      4176
 *  Glu Gln Lys Arg Pro Ile Gln Arg Leu Ser Leu Lys Pro Ser Thr
            1380                1385                1390

GCC CCC CAT GAG GAA GGA ACA GCA ATG GTG TCA GTA TCC AGG CTT TGT      4224
Ala Pro His Glu Glu Gly Thr Ala Met Val Ser Val Ser Arg Leu Cys
        1395                1400                1405

ACA GAG TGC TTT TCT GTT TAG TTT TTA CTT TTT TTG TTT TGT TTT TTT      4272
Thr Glu Cys Phe Ser Val  *  Phe Leu Leu Phe Leu Phe Cys Phe Phe
    1410                1415                1420

AAA GAT GAA ATA AAG ACC CAG GGG GAG                                 4299
Lys Asp Glu Ile Lys Thr Gln Gly Glu
1425                1430
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1433 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
 1               5                  10                  15

Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
            20                  25                  30
```

```
Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
        35                  40                  45

Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
    50                  55                  60

Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
65                  70                  75                  80

Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
                85                  90                  95

Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
            100                 105                 110

Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
        115                 120                 125

Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
    130                 135                 140

Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
145                 150                 155                 160

Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
                165                 170                 175

Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
            180                 185                 190

His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
        195                 200                 205

Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
    210                 215                 220

Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
225                 230                 235                 240

Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
                245                 250                 255

His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
            260                 265                 270

Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
        275                 280                 285

Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
    290                 295                 300

Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
305                 310                 315                 320
```

Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
325 330 335

Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
340 345 350

Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
355 360 365

Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
370 375 380

Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385 390 395 400

Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
405 410 415

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
420 425 430

Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
435 440 445

Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
450 455 460

Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465 470 475 480

Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
485 490 495

Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
500 505 510

Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
515 520 525

Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
530 535 540

Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545 550 555 560

Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
565 570 575

Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
580 585 590

Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
595 600 605

Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
610 615 620

Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625 630 635 640

Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
645 650 655

Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
660 665 670

Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
675 680 685

Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
690 695 700

Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
705 710 715 720

Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
725 730 735

Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
740 745 750

Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
755 760 765

Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
770 775 780

Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
785 790 795 800

Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
805 810 815

Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
820 825 830

Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
835 840 845

Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
850 855 860

Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
865 870 875 880

Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
885 890 895

Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
900 905 910

Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
915 920 925

Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
930 935 940

Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
945 950 955 960

Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
965 970 975

Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
980 985 990

Asp Leu Gly Pro Ala Ser Pro Leu Asp Ser Thr Phe Tyr Arg Ser Leu
995 1000 1005

Leu Glu Asp Asp Asp Met Gly Asp Leu Val Asp Ala Glu Glu Tyr Leu
1010 1015 1020

Val Pro Gln Gln Gly Phe Phe Cys Pro Asp Pro Ala Pro Gly Ala Gly
1025 1030 1035 1040

Gly Met Val His His Arg His Arg Ser Ser Ser Thr Arg Ser Gly Gly
1045 1050 1055

Gly Asp Leu Thr Leu Gly Leu Glu Pro Ser Glu Glu Glu Ala Pro Arg
1060 1065 1070

Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val Phe Asp Gly
1075 1080 1085

Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln Ser Leu Pro Thr His
1090 1095 1100

Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro Leu
1105 1110 1115 1120

Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu Thr Cys Ser Pro Gln
1125 1130 1135

Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro Gln Pro Pro Ser Pro
1140 1145 1150

Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu
1155 1160 1165

Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
1170 1175 1180

```
Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln
1185                1190                1195                1200

Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala
                1205                1210                1215

Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala
            1220                1225                1230

Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu Asn Pro Glu Tyr
        1235                1240                1245

Leu Gly Leu Asp Val Pro Val  *  Thr Arg Arg Pro Ser Pro Gln Lys
    1250                1255                1260

Pro  *  Cys Val Leu Arg Glu Gln Gly Arg Pro Asp Phe Cys Trp His
1265                1270                1275                1280

Gln Glu Val Gly Gly Pro Ser Asp His Phe Gln Gly Asn Leu Pro Cys
                1285                1290                1295

Gln Glu Pro Val Leu Arg Asn Leu Pro Ser Cys Leu Ser Ser Gln Met
            1300                1305                1310

Ala Gly Arg Gly Pro Ala Ser Leu Glu Glu Glu Gln His Trp Gly Val
        1315                1320                1325

Phe Val Asp Ser Glu Ala Leu Pro Asn Glu Thr Leu Gly Ser Ser Gly
    1330                1335                1340

Cys His Ser Pro Ala Trp Pro Phe Pro Ser Arg Ser Trp Val Leu Lys
1345                1350                1355                1360

Ala Leu Gly Lys Leu Ala  *  Glu Gly Lys Arg Pro  *  Gly Ser Val
                1365                1370                1375

 *  Glu Gln Lys Arg Pro Ile Gln Arg Leu Ser Leu Lys Pro Ser Thr
            1380                1385                1390

Ala Pro His Glu Glu Gly Thr Ala Met Val Ser Val Ser Arg Leu Cys
        1395                1400                1405

Thr Glu Cys Phe Ser Val  *  Phe Leu Leu Phe Leu Phe Cys Phe Phe
    1410                1415                1420

Lys Asp Glu Ile Lys Thr Gln Gly Glu
1425                1430
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 739 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..739
(D) OTHER INFORMATION: /note= "product = "520C9sFv/ amino acid info: 520C9sFv protein""

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

```
GAG ATC CAA TTG GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT GGA GAG        48
Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1               5                  10                  15

ACA GTC AAG ATC TCC TGC AAG GCT TCT GGA TAT ACC TTC GCA AAC TAT        96
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Asn Tyr
            20                  25                  30

GGA ATG AAC TGG ATG AAG CAG GCT CCA GGA AAG GGT TTA AAG TGG ATG       144
Gly Met Asn Trp Met Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
        35                  40                  45

GGC TGG ATA AAC ACC TAC ACT GGA CAG TCA ACA TAT GCT GAT GAC TTC       192
Gly Trp Ile Asn Thr Tyr Thr Gly Gln Ser Thr Tyr Ala Asp Asp Phe
    50                  55                  60

AAG GAA CGG TTT GCC TTC TCT TTG GAA ACC TCT GCC ACC ACT GCC CAT       240
Lys Glu Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Thr Thr Ala His
65                  70                  75                  80

TTG CAG ATC AAC AAC CTC AGA AAT GAG GAC TCG GCC ACA TAT TTC TGT       288
Leu Gln Ile Asn Asn Leu Arg Asn Glu Asp Ser Ala Thr Tyr Phe Cys
                85                  90                  95

GCA AGA CGA TTT GGG TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC AGT       336
Ala Arg Arg Phe Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Ser
            100                 105                 110

GTC TCT GCA TCG ATA TCG AGC TCC TCC GGA TCT TCA TCT AGC GGT TCC       384
Val Ser Ala Ser Ile Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly Ser
        115                 120                 125

AGC TCG AGT GGA TCC GAT ATC CAG ATG ACC CAG TCT CCA TCC TCC TTA       432
Ser Ser Ser Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
    130                 135                 140

TCT GCC TCT CTG GGA GAA AGA GTC AGT CTC ACT TGT CGG GCA AGT CAG       480
Ser Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln
145                 150                 155                 160

GAC ATT GGT AAT AGC TTA ACC TGG CTT CAG CAG GAA CCA GAT GGA ACT       528
Asp Ile Gly Asn Ser Leu Thr Trp Leu Gln Gln Glu Pro Asp Gly Thr
                165                 170                 175
```

```
ATT AAA CGC CTG ATC TAC GCC ACA TCC AGT TTA GAT TCT GGT GTC CCC      576
Ile Lys Arg Leu Ile Tyr Ala Thr Ser Ser Leu Asp Ser Gly Val Pro
            180                 185                 190

AAA AGG TTC AGT GGC AGT CGG TCT GGG TCA GAT TAT TCT CTC ACC ATC      624
Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile
        195                 200                 205

AGT AGC CTT GAG TCT GAA GAT TTT GTA GTC TAT TAC TGT CTA CAA TAT      672
Ser Ser Leu Glu Ser Glu Asp Phe Val Val Tyr Tyr Cys Leu Gln Tyr
    210                 215                 220

GCT ATT TTT CCG TAC ACG TTC GGA GGG GGG ACC AAC CTG GAA ATA AAA      720
Ala Ile Phe Pro Tyr Thr Phe Gly Gly Gly Thr Asn Leu Glu Ile Lys
225                 230                 235                 240

CGG GCT GAT TAA TCT GCA G                                           739
Arg Ala Asp  *  Ser Ala
                245
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 246 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1               5                  10                  15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Asn Tyr
            20                  25                  30

Gly Met Asn Trp Met Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
        35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Gln Ser Thr Tyr Ala Asp Asp Phe
    50                  55                  60

Lys Glu Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Thr Thr Ala His
65                  70                  75                  80

Leu Gln Ile Asn Asn Leu Arg Asn Glu Asp Ser Ala Thr Tyr Phe Cys
                85                  90                  95

Ala Arg Arg Phe Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val Ser
            100                 105                 110

Val Ser Ala Ser Ile Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly Ser
        115                 120                 125
```

```
Ser Ser Ser Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
    130                 135                 140

Ser Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg Ala Ser Gln
145                 150                 155                 160

Asp Ile Gly Asn Ser Leu Thr Trp Leu Gln Gln Glu Pro Asp Gly Thr
                165                 170                 175

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

Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser Leu Thr Ile
        195                 200                 205

Ser Ser Leu Glu Ser Glu Asp Phe Val Val Tyr Tyr Cys Leu Gln Tyr
    210                 215                 220

Ala Ile Phe Pro Tyr Thr Phe Gly Gly Gly Thr Asn Leu Glu Ile Lys
225                 230                 235                 240

Arg Ala Asp  *  Ser Ala
                245
```

(2) INFORMATION FOR SEQ ID NO:5: DELETED ACCORDING TO PRELIMINARY AMENDMENT

(2) INFORMATION FOR SEQ ID NO:6: DELETED ACCORDING TO PRELIMINARY AMENDMENT

(2) INFORMATION FOR SEQ IS NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 807 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..807
(D) OTHER INFORMATION: /note= "product = "Ricin-A chain gene/ amino acid info: Ricin-A chain protein""

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

```
ATG ATA TTC CCC AAA CAA TAC CCA ATT ATA AAC TTT ACC ACA GCG GGT        48
Met Ile Phe Pro Lys Gln Tyr Pro Ile Ile Asn Phe Thr Thr Ala Gly
 1               5                  10                  15

GCC ACT GTG CAA AGC TAC ACA AAC TTT ATC AGA GCT GTT CGC GGT CGT        96
Ala Thr Val Gln Ser Tyr Thr Asn Phe Ile Arg Ala Val Arg Gly Arg
            20                  25                  30
```

```
TTA ACA ACT GGA GCT GAT GTG AGA CAT GAA ATA CCA GTG TTG CCA AAC      144
Leu Thr Thr Gly Ala Asp Val Arg His Glu Ile Pro Val Leu Pro Asn
         35                  40                  45

AGA GTT GGT TTG CCT ATA AAC CAA CGG TTT ATT TTA GTT GAA CTC TCA      192
Arg Val Gly Leu Pro Ile Asn Gln Arg Phe Ile Leu Val Glu Leu Ser
     50                  55                  60

AAT CAT GCA GAG CTT TCT GTT ACA TTA GCG CTG GAT GTC ACC AAT GCA      240
Asn His Ala Glu Leu Ser Val Thr Leu Ala Leu Asp Val Thr Asn Ala
 65                  70                  75                  80

TAT GTG GTA GGC TAC CGT GCT GGA AAT AGC GCA TAT TTC TTT CAT CCT      288
Tyr Val Val Gly Tyr Arg Ala Gly Asn Ser Ala Tyr Phe Phe His Pro
                 85                  90                  95

GAC AAT CAG GAA GAT GCA GAA GCA ATC ACT CAT CTT TTC ACT GAT GTT      336
Asp Asn Gln Glu Asp Ala Glu Ala Ile Thr His Leu Phe Thr Asp Val
            100                 105                 110

CAA AAT CGA TAT ACA TTC GCC TTT GGT GGT AAT TAT GAT AGA CTT GAA      384
Gln Asn Arg Tyr Thr Phe Ala Phe Gly Gly Asn Tyr Asp Arg Leu Glu
        115                 120                 125

CAA CTT GCT GGT AAT CTG AGA GAA AAT ATC GAG TTG GGA AAT GGT CCA      432
Gln Leu Ala Gly Asn Leu Arg Glu Asn Ile Glu Leu Gly Asn Gly Pro
    130                 135                 140

CTA GAG GAG GCT ATC TCA GCG CTT TAT TAT TAC AGT ACT GGT GGC ACT      480
Leu Glu Glu Ala Ile Ser Ala Leu Tyr Tyr Tyr Ser Thr Gly Gly Thr
145                 150                 155                 160

CAG CTT CCA ACT CTG GCT CGT TCC TTT ATA ATT TGC ATC CAA ATG ATT      528
Gln Leu Pro Thr Leu Ala Arg Ser Phe Ile Ile Cys Ile Gln Met Ile
                165                 170                 175

TCA GAA GCA GCA AGA TTC CAA TAT ATT GAG GGA GAA ATG CGC ACG AGA      576
Ser Glu Ala Ala Arg Phe Gln Tyr Ile Glu Gly Glu Met Arg Thr Arg
            180                 185                 190

ATT AGG TAC AAC CGG AGA TCT GCA CCA GAT CCT AGC GTA ATT ACA CTT      624
Ile Arg Tyr Asn Arg Arg Ser Ala Pro Asp Pro Ser Val Ile Thr Leu
        195                 200                 205

GAG AAT AGT TGG GGG AGA CTT TCC ACT GCA ATT CAA GAG TCT AAC CAA      672
Glu Asn Ser Trp Gly Arg Leu Ser Thr Ala Ile Gln Glu Ser Asn Gln
    210                 215                 220

GGA GCC TTT GCT AGT CCA ATT CAA CTG CAA AGA CGT AAT GGT TCC AAA      720
Gly Ala Phe Ala Ser Pro Ile Gln Leu Gln Arg Arg Asn Gly Ser Lys
225                 230                 235                 240

TTC AGT GTG TAC GAT GTG AGT ATA TTA ATC CCT ATC ATA GCT CTC ATG      768
Phe Ser Val Tyr Asp Val Ser Ile Leu Ile Pro Ile Ile Ala Leu Met
                245                 250                 255
```

```
GTG TAT AGA TGC GCA CCT CCA CCA TCG TCA CAG TTT TAA                    807
Val Tyr Arg Cys Ala Pro Pro Ser Ser Gln Phe
            260                 265
```

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 268 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Met Ile Phe Pro Lys Gln Tyr Pro Ile Ile Asn Phe Thr Thr Ala Gly
 1               5                  10                  15

Ala Thr Val Gln Ser Tyr Thr Asn Phe Ile Arg Ala Val Arg Gly Arg
            20                  25                  30

Leu Thr Thr Gly Ala Asp Val Arg His Glu Ile Pro Val Leu Pro Asn
        35                  40                  45

Arg Val Gly Leu Pro Ile Asn Gln Arg Phe Ile Leu Val Glu Leu Ser
    50                  55                  60

Asn His Ala Glu Leu Ser Val Thr Leu Ala Leu Asp Val Thr Asn Ala
65                  70                  75                  80

Tyr Val Val Gly Tyr Arg Ala Gly Asn Ser Ala Tyr Phe Phe His Pro
                85                  90                  95

Asp Asn Gln Glu Asp Ala Glu Ala Ile Thr His Leu Phe Thr Asp Val
            100                 105                 110

Gln Asn Arg Tyr Thr Phe Ala Phe Gly Gly Asn Tyr Asp Arg Leu Glu
        115                 120                 125

Gln Leu Ala Gly Asn Leu Arg Glu Asn Ile Glu Leu Gly Asn Gly Pro
    130                 135                 140

Leu Glu Glu Ala Ile Ser Ala Leu Tyr Tyr Tyr Ser Thr Gly Gly Thr
145                 150                 155                 160

Gln Leu Pro Thr Leu Ala Arg Ser Phe Ile Ile Cys Ile Gln Met Ile
                165                 170                 175

Ser Glu Ala Ala Arg Phe Gln Tyr Ile Glu Gly Glu Met Arg Thr Arg
            180                 185                 190

Ile Arg Tyr Asn Arg Arg Ser Ala Pro Asp Pro Ser Val Ile Thr Leu
        195                 200                 205
```

```
Glu Asn Ser Trp Gly Arg Leu Ser Thr Ala Ile Gln Glu Ser Asn Gln
    210                 215                 220

Gly Ala Phe Ala Ser Pro Ile Gln Leu Gln Arg Arg Asn Gly Ser Lys
225                 230                 235                 240

Phe Ser Val Tyr Asp Val Ser Ile Leu Ile Pro Ile Ile Ala Leu Met
                245                 250                 255

Val Tyr Arg Cys Ala Pro Pro Pro Ser Ser Gln Phe
            260                 265
```

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1605 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1605
(D) OTHER INFORMATION: /note= "product = "G-FIT""

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

```
AAG CTT ATG ATA TTC CCC AAA CAA TAC CCA ATT ATA AAC TTT ACC ACA      48
Lys Leu Met Ile Phe Pro Lys Gln Tyr Pro Ile Ile Asn Phe Thr Thr
 1               5                  10                  15

GCG GGT GCC ACT GTG CAA AGC TAC ACA AAC TTT ATC AGA GCT GTT CGC      96
Ala Gly Ala Thr Val Gln Ser Tyr Thr Asn Phe Ile Arg Ala Val Arg
            20                  25                  30

GGT CGT TTA ACA ACT GGA GCT GAT GTG AGA CAT GAA ATA CCA GTG TTG     144
Gly Arg Leu Thr Thr Gly Ala Asp Val Arg His Glu Ile Pro Val Leu
        35                  40                  45

CCA AAC AGA GTT GGT TTG CCT ATA AAC CAA CGG TTT ATT TTA GTT GAA     192
Pro Asn Arg Val Gly Leu Pro Ile Asn Gln Arg Phe Ile Leu Val Glu
    50                  55                  60

CTC TCA AAT CAT GCA GAG CTT TCT GTT ACA TTA GCG CTG GAT GTC ACC     240
Leu Ser Asn His Ala Glu Leu Ser Val Thr Leu Ala Leu Asp Val Thr
65                  70                  75                  80

AAT GCA TAT GTG GTA GGC TAC CGT GCT GGA AAT AGC GCA TAT TTC TTT     288
Asn Ala Tyr Val Val Gly Tyr Arg Ala Gly Asn Ser Ala Tyr Phe Phe
                85                  90                  95
```

```
CAT CCT GAC AAT CAG GAA GAT GCA GAA GCA ATC ACT CAT CTT TTC ACT      336
His Pro Asp Asn Gln Glu Asp Ala Glu Ala Ile Thr His Leu Phe Thr
            100                 105                 110

GAT GTT CAA AAT CGA TAT ACA TTC GCC TTT GGT GGT AAT TAT GAT AGA      384
Asp Val Gln Asn Arg Tyr Thr Phe Ala Phe Gly Gly Asn Tyr Asp Arg
        115                 120                 125

CTT GAA CAA CTT GCT GGT AAT CTG AGA GAA AAT ATC GAG TTG GGA AAT      432
Leu Glu Gln Leu Ala Gly Asn Leu Arg Glu Asn Ile Glu Leu Gly Asn
    130                 135                 140

GGT CCA CTA GAG GAG GCT ATC TCA GCG CTT TAT TAT TAC AGT ACT GGT      480
Gly Pro Leu Glu Glu Ala Ile Ser Ala Leu Tyr Tyr Tyr Ser Thr Gly
145                 150                 155                 160

GGC ACT CAG CTT CCA ACT CTG GCT CGT TCC TTT ATA ATT TGC ATC CAA      528
Gly Thr Gln Leu Pro Thr Leu Ala Arg Ser Phe Ile Ile Cys Ile Gln
                165                 170                 175

ATG ATT TCA GAA GCA GCA AGA TTC CAA TAT ATT GAG GGA GAA ATG CGC      576
Met Ile Ser Glu Ala Ala Arg Phe Gln Tyr Ile Glu Gly Glu Met Arg
            180                 185                 190

ACG AGA ATT AGG TAC AAC CGG AGA TCT GCA CCA GAT CCT AGC GTA ATT      624
Thr Arg Ile Arg Tyr Asn Arg Arg Ser Ala Pro Asp Pro Ser Val Ile
        195                 200                 205

ACA CTT GAG AAT AGT TGG GGG AGA CTT TCC ACT GCA ATT CAA GAG TCT      672
Thr Leu Glu Asn Ser Trp Gly Arg Leu Ser Thr Ala Ile Gln Glu Ser
    210                 215                 220

AAC CAA GGA GCC TTT GCT AGT CCA ATT CAA CTG CAA AGA CGT AAT GGT      720
Asn Gln Gly Ala Phe Ala Ser Pro Ile Gln Leu Gln Arg Arg Asn Gly
225                 230                 235                 240

TCC AAA TTC AGT GTG TAC GAT GTG AGT ATA TTA ATC CCT ATC ATA GCT      768
Ser Lys Phe Ser Val Tyr Asp Val Ser Ile Leu Ile Pro Ile Ile Ala
                245                 250                 255

CTC ATG GTG TAT AGA TGC GCA CCT CCA CCA TCG TCA CAG TTT TCT CTT      816
Leu Met Val Tyr Arg Cys Ala Pro Pro Pro Ser Ser Gln Phe Ser Leu
            260                 265                 270

CTT ATA AGG CCA GTG GTA CCA AAT TTT AAT GCT GAT GTT TGT ATG GAT      864
Leu Ile Arg Pro Val Val Pro Asn Phe Asn Ala Asp Val Cys Met Asp
        275                 280                 285

CCT GAG ATC CAA TTG GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT GGA      912
Pro Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly
    290                 295                 300
```

```
GAG ACA GTC AAG ATC TCC TGC AAG GCT TCT GGA TAT ACC TTC GCA AAC      960
Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Asn
305                 310                 315                 320

TAT GGA ATG AAC TGG ATG AAG CAG GCT CCA GGA AAG GGT TTA AAG TGG     1008
Tyr Gly Met Asn Trp Met Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp
                325                 330                 335

ATG GGC TGG ATA AAC ACC TAC ACT GGA CAG TCA ACA TAT GCT GAT GAC     1056
Met Gly Trp Ile Asn Thr Tyr Thr Gly Gln Ser Thr Tyr Ala Asp Asp
            340                 345                 350

TTC AAG GAA CGG TTT GCC TTC TCT TTG GAA ACC TCT GCC ACC ACT GCC     1104
Phe Lys Glu Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Thr Thr Ala
        355                 360                 365

CAT TTG CAG ATC AAC AAC CTC AGA AAT GAG GAC TCG GCC ACA TAT TTC     1152
His Leu Gln Ile Asn Asn Leu Arg Asn Glu Asp Ser Ala Thr Tyr Phe
    370                 375                 380

TGT GCA AGA CGA TTT GGG TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC     1200
Cys Ala Arg Arg Phe Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val
385                 390                 395                 400

AGT GTC TCT GCA TCG ATA TCG AGC TCT GGT GGC GGT GGC TCG GGC GGT     1248
Ser Val Ser Ala Ser Ile Ser Ser Ser Gly Gly Gly Gly Ser Gly Gly
                405                 410                 415

GGT GGG TCG GGT GGC GGC GGA TCG GAT ATC CAG ATG ACC CAG TCT CCA     1296
Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
            420                 425                 430

TCC TCC TTA TCT GCC TCT CTG GGA GAA AGA GTC AGT CTC ACT TGT CGG     1344
Ser Ser Leu Ser Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg
        435                 440                 445

GCA AGT CAG GAC ATT GGT AAT AGC TTA ACC TGG CTT TCA CAG GAA CCA     1392
Ala Ser Gln Asp Ile Gly Asn Ser Leu Thr Trp Leu Ser Gln Glu Pro
    450                 455                 460

GAT GGA ACT ATT AAA CGC CTG ATC TAC GCC ACA TCC AGT TTA GAT TCT     1440
Asp Gly Thr Ile Lys Arg Leu Ile Tyr Ala Thr Ser Ser Leu Asp Ser
465                 470                 475                 480

GGT GTC CCC AAA AGG TTC AGT GGC AGT CGG TCT GGG TCA GAT TAT TCT     1488
Gly Val Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser
                485                 490                 495

CTC ACC ATC AGT AGC CTT GAG TCT GAA GAT TTT GTA GTC TAT TAC TGT     1536
Leu Thr Ile Ser Ser Leu Glu Ser Glu Asp Phe Val Val Tyr Tyr Cys
            500                 505                 510

CTA CAA TAT GCT ATT TTT CCG TAC ACG TTC GGA GGG GGG ACC AAC CTG     1584
Leu Gln Tyr Ala Ile Phe Pro Tyr Thr Phe Gly Gly Gly Thr Asn Leu
        515                 520                 525
```

GAA ATA AAA CGG GCT GAT TAA 1605
Glu Ile Lys Arg Ala Asp
530 535

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 534 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

Lys Leu Met Ile Phe Pro Lys Gln Tyr Pro Ile Ile Asn Phe Thr Thr
1 5 10 15

Ala Gly Ala Thr Val Gln Ser Tyr Thr Asn Phe Ile Arg Ala Val Arg
20 25 30

Gly Arg Leu Thr Thr Gly Ala Asp Val Arg His Glu Ile Pro Val Leu
35 40 45

Pro Asn Arg Val Gly Leu Pro Ile Asn Gln Arg Phe Ile Leu Val Glu
50 55 60

Leu Ser Asn His Ala Glu Leu Ser Val Thr Leu Ala Leu Asp Val Thr
65 70 75 80

Asn Ala Tyr Val Val Gly Tyr Arg Ala Gly Asn Ser Ala Tyr Phe Phe
85 90 95

His Pro Asp Asn Gln Glu Asp Ala Glu Ala Ile Thr His Leu Phe Thr
100 105 110

Asp Val Gln Asn Arg Tyr Thr Phe Ala Phe Gly Gly Asn Tyr Asp Arg
115 120 125

Leu Glu Gln Leu Ala Gly Asn Leu Arg Glu Asn Ile Glu Leu Gly Asn
130 135 140

Gly Pro Leu Glu Glu Ala Ile Ser Ala Leu Tyr Tyr Tyr Ser Thr Gly
145 150 155 160

Gly Thr Gln Leu Pro Thr Leu Ala Arg Ser Phe Ile Ile Cys Ile Gln
165 170 175

Met Ile Ser Glu Ala Ala Arg Phe Gln Tyr Ile Glu Gly Glu Met Arg
180 185 190

Thr Arg Ile Arg Tyr Asn Arg Arg Ser Ala Pro Asp Pro Ser Val Ile
195 200 205

Thr Leu Glu Asn Ser Trp Gly Arg Leu Ser Thr Ala Ile Gln Glu Ser
210 215 220

Asn Gln Gly Ala Phe Ala Ser Pro Ile Gln Leu Gln Arg Arg Asn Gly
225 230 235 240

Ser Lys Phe Ser Val Tyr Asp Val Ser Ile Leu Ile Pro Ile Ile Ala
245 250 255

Leu Met Val Tyr Arg Cys Ala Pro Pro Pro Ser Ser Gln Phe Ser Leu
260 265 270

Leu Ile Arg Pro Val Val Pro Asn Phe Asn Ala Asp Val Cys Met Asp
275 280 285

Pro Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly
290 295 300

Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ala Asn
305 310 315 320

Tyr Gly Met Asn Trp Met Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp
325 330 335

Met Gly Trp Ile Asn Thr Tyr Thr Gly Gln Ser Thr Tyr Ala Asp Asp
340 345 350

Phe Lys Glu Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Thr Thr Ala
355 360 365

His Leu Gln Ile Asn Asn Leu Arg Asn Glu Asp Ser Ala Thr Tyr Phe
370 375 380

Cys Ala Arg Arg Phe Gly Phe Ala Tyr Trp Gly Gln Gly Thr Leu Val
385 390 395 400

Ser Val Ser Ala Ser Ile Ser Ser Ser Gly Gly Gly Gly Ser Gly Gly
405 410 415

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
420 425 430

Ser Ser Leu Ser Ala Ser Leu Gly Glu Arg Val Ser Leu Thr Cys Arg
435 440 445

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

Asp Gly Thr Ile Lys Arg Leu Ile Tyr Ala Thr Ser Ser Leu Asp Ser
465 470 475 480

Gly Val Pro Lys Arg Phe Ser Gly Ser Arg Ser Gly Ser Asp Tyr Ser
485 490 495

```
Leu Thr Ile Ser Ser Leu Glu Ser Glu Asp Phe Val Val Tyr Tyr Cys
            500                 505                 510

Leu Gln Tyr Ala Ile Phe Pro Tyr Thr Phe Gly Gly Gly Thr Asn Leu
        515                 520                 525

Glu Ile Lys Arg Ala Asp
    530
```

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..45
(D) OTHER INFORMATION: /note= "product = "new linker/
info: new linker""

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

```
TCG AGC TCC TCC GGA TCT TCA TCT AGC GGT TCC AGC TCG AGT GGA          45
Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly
  1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly Ser Ser Ser Ser Gly
  1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..45
(D) OTHER INFORMATION: /note= "product = "old linker/
protein info: old linker""

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

```
GGA GGA GGA GGA TCT GGA GGA GGA GGA TCT GGA GGA GGA GGA TCT          45
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2001 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..2001
(D) OTHER INFORMATION: /note= "product = "741sFv-PE40""

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

```
GAT CCT GAG ATC CAA TTG GTG CAG TCT GGA CCT GAG CTG AAG AAG CCT      48
Asp Pro Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro
 1               5                  10                  15

GGA GAG ACA GTC AAG ATC TCC TGC AAG GCT TCT GGG TAT ACC TTC ACA      96
Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
            20                  25                  30
```

```
AAC TAT GGA ATG AAC TGG GTG AAG CAG GCT CCA GGA AAG GGT TTA AAG      144
Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys
         35                  40                  45

TGG ATG GGC TGG ATA AAC ACC AAC ACT GGA GAG CCA ACA TAT GCT GAA      192
Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr Ala Glu
     50                  55                  60

GAG TTC AAG GGA CGG TTT GCC TTC TCT TTG GAA ACC TCT GCC AGC ACT      240
Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr
 65                  70                  75                  80

GCC TAT TTG CAG ATC AAC AAC CTC AAA AAT GAG GAC ACG GCT ACA TAT      288
Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr
                 85                  90                  95

TTC TGT GGA AGG CAA TTT ATT ACC TAC GGC GGG TTT GCT AAC TGG GGC      336
Phe Cys Gly Arg Gln Phe Ile Thr Tyr Gly Gly Phe Ala Asn Trp Gly
            100                 105                 110

CAA GGG ACT CTG GTC ACT GTC TCT GCA TCG AGC TCC TCC GGA TCT TCA      384
Gln Gly Thr Leu Val Thr Val Ser Ala Ser Ser Ser Ser Gly Ser Ser
        115                 120                 125

TCT AGC GGT TCC AGC TCG AGC GAT ATC GTC ATG ACC CAG TCT CCT AAA      432
Ser Ser Gly Ser Ser Ser Ser Asp Ile Val Met Thr Gln Ser Pro Lys
    130                 135                 140

TTC ATG TCC ACG TCA GTG GGA GAC AGG GTC AGC ATC TCC TGC AAG GCC      480
Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Ile Ser Cys Lys Ala
145                 150                 155                 160

AGT CAG GAT GTG AGT ACT GCT GTA GCC TGG TAT CAA CAA AAA CCA GGG      528
Ser Gln Asp Val Ser Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
                165                 170                 175

CAA TCT CCT AAA CTA CTG ATT TAC TGG ACA TCC ACC CGG CAC ACT GGA      576
Gln Ser Pro Lys Leu Leu Ile Tyr Trp Thr Ser Thr Arg His Thr Gly
            180                 185                 190

GTC CCT GAT CCG TTC ACA GGC AGT GGA TCT GGG ACA GAT TAT ACT CTC      624
Val Pro Asp Pro Phe Thr Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu
        195                 200                 205

ACC ATC AGC AGT GTG CAG GCT GAA GAC CTG GCA CTT CAT TAC TGT CAG      672
Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Leu His Tyr Cys Gln
    210                 215                 220

CAA CAT TAT AGA GTG GCC TAC ACG TTC GGA AGG GGG ACC AAG CTG GAG      720
Gln His Tyr Arg Val Ala Tyr Thr Phe Gly Arg Gly Thr Lys Leu Glu
225                 230                 235                 240

ATA AAA CGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC      768
Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser
                245                 250                 255
```

```
AGT GAG CAG TTT GAG GGC GGC AGC CTG GCC GCG CTG AAC GCG CAC CAG        816
Ser Glu Gln Phe Glu Gly Gly Ser Leu Ala Ala Leu Asn Ala His Gln
            260                 265                 270

GCT TGC CAC CTG CCG CTG GAG ACT TTC ACC CGT CAT CGC CAG CCG CGC        864
Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
        275                 280                 285

GGC TGG GAA CAA CTG GAG CAG TGC GGC TAT CCG GTG CAG CGG CTG GTC        912
Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
    290                 295                 300

GCC CTC TAC CTG GCG GCG CGG CTG TCG TGG AAC CAG GTC GAC CAG GTG        960
Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
305                 310                 315                 320

ATC CGC AAC GCC CTG GCC AGC CCC GGC AGC GGC GGC GAC CTG GGC GAA       1008
Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
                325                 330                 335

GCG ATC CGC GAG CAG CCG GAG CAG GCC CGT CTG GCC CTG ACC CTG GCC       1056
Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
            340                 345                 350

GCC GCC GAG AGC GAG CGC TTC GTC CGG CAG GGC ACC GGC AAC GAC GAG       1104
Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
        355                 360                 365

GCC GGC GCG GCC AAC GCC GAC GTG GTG AGC CTG ACC TGC CCG GTC GCC       1152
Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
    370                 375                 380

GCC GGT GAA TGC GCG GGC CCG GCG GAC AGC GGC GAC GCC CTG CTG GAG       1200
Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
385                 390                 395                 400

CGC AAC TAT CCC ACT GGC GCG GAG TTC CTC GGC GAC GGC GGC GAC GTC       1248
Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
                405                 410                 415

AGC TTC AGC AAC CGC GGC ACG CAG AAC TGG ACG GTG GAG CGG CTG CTC       1296
Ser Phe Ser Asn Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu
            420                 425                 430

CAG GCG CAC CGC CAA CTG GAG GAG CGC GGC TAT GTG TTC GTC GGC TAC       1344
Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr
        435                 440                 445

CAC GGC ACC TTC CTC GAA GCG GCG CAA AGC ATC GTC TTC GGC GGG GTG       1392
His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val
    450                 455                 460

CGC GCG CGC AGC CAG GAC CTC GAC GCG ATC TGG CGC GGT TTC TAT ATC       1440
Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile
465                 470                 475                 480
```

```
GCC GGC GAT CCG GCG CTG GCC TAC GGC TAC GCC CAG GAC CAG GAA CCC      1488
Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro
                485                 490                 495

GAC GCA CGC GGC CGG ATC CGC AAC GGT GCC CTG CTG CGG GTC TAT GTG      1536
Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val
            500                 505                 510

CCG CGC TCG AGC CTG CCG GGC TTC TAC CGC ACC AGC CTG ACC CTG GCC      1584
Pro Arg Ser Ser Leu Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala
        515                 520                 525

GCG CCG GAG GCG GCG GGC GAG GTC GAA CGG CTG ATC GGC CAT CCG CTG      1632
Ala Pro Glu Ala Ala Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu
    530                 535                 540

CCG CTG CGC CTG GAC GCC ATC ACC GGC CCC GAG GAG GAA GGC GGG CGC      1680
Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg
545                 550                 555                 560

CTG GAG ACC ATT CTC GGC TGG CCG CTG GCC GAG CGC ACC GTG GTG ATT      1728
Leu Glu Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile
                565                 570                 575

CCC TCG GCG ATC CCC ACC GAC CCG CGC AAC GTC GGC GGC GAC CTC GAC      1776
Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp
            580                 585                 590

CCG TCC AGC ATC CCC GAC AAG GAA CAG GCG ATC AGC GCC CTG CCG GAC      1824
Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp
        595                 600                 605

TAC GCC AGC CAG CCC GGC AAA CCG CCG CGC GAG GAC CTG AAG TAA CTG      1872
Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Leu Lys  *  Leu
    610                 615                 620

CCG CGA CCG GCC GGC TCC CTT CGC AGG AGC CGG CCT TCT CGG GGC CTG      1920
Pro Arg Pro Ala Gly Ser Leu Arg Arg Ser Arg Pro Ser Arg Gly Leu
625                 630                 635                 640

GCC ATA CAT CAG GTT TTC CTG ATG CCA GCC CAA TCG AAT ATG AAT TGA      1968
Ala Ile His Gln Val Phe Leu Met Pro Ala Gln Ser Asn Met Asn  *
                645                 650                 655

TCC TCT AGA GTC GAC CTG CAG GCA TGC AAG CTT                          2001
Ser Ser Arg Val Asp Leu Gln Ala Cys Lys Leu
            660                 665
```

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 667 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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

```
Asp Pro Glu Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro
 1               5                  10                  15

Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
            20                  25                  30

Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys
        35                  40                  45

Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr Ala Glu
    50                  55                  60

Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr
65                  70                  75                  80

Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr
                85                  90                  95

Phe Cys Gly Arg Gln Phe Ile Thr Tyr Gly Gly Phe Ala Asn Trp Gly
            100                 105                 110

Gln Gly Thr Leu Val Thr Val Ser Ala Ser Ser Ser Ser Gly Ser Ser
        115                 120                 125

Ser Ser Gly Ser Ser Ser Ser Asp Ile Val Met Thr Gln Ser Pro Lys
    130                 135                 140

Phe Met Ser Thr Ser Val Gly Asp Arg Val Ser Ile Ser Cys Lys Ala
145                 150                 155                 160

Ser Gln Asp Val Ser Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly
                165                 170                 175

Gln Ser Pro Lys Leu Leu Ile Tyr Trp Thr Ser Thr Arg His Thr Gly
            180                 185                 190

Val Pro Asp Pro Phe Thr Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu
        195                 200                 205

Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Leu His Tyr Cys Gln
    210                 215                 220

Gln His Tyr Arg Val Ala Tyr Thr Phe Gly Arg Gly Thr Lys Leu Glu
225                 230                 235                 240

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

Ser Glu Gln Phe Glu Gly Gly Ser Leu Ala Ala Leu Asn Ala His Gln
            260                 265                 270
```

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Ala Cys His Leu Pro Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg
        275                 280                 285

Gly Trp Glu Gln Leu Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val
    290                 295                 300

Ala Leu Tyr Leu Ala Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val
305                 310                 315                 320

Ile Arg Asn Ala Leu Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu
                325                 330                 335

Ala Ile Arg Glu Gln Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala
            340                 345                 350

Ala Ala Glu Ser Glu Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu
        355                 360                 365

Ala Gly Ala Ala Asn Ala Asp Val Val Ser Leu Thr Cys Pro Val Ala
    370                 375                 380

Ala Gly Glu Cys Ala Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu
385                 390                 395                 400

Arg Asn Tyr Pro Thr Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val
                405                 410                 415

Ser Phe Ser Asn Arg Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu
            420                 425                 430

Gln Ala His Arg Gln Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr
        435                 440                 445

His Gly Thr Phe Leu Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val
    450                 455                 460

Arg Ala Arg Ser Gln Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile
465                 470                 475                 480

Ala Gly Asp Pro Ala Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro
                485                 490                 495

Asp Ala Arg Gly Arg Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val
            500                 505                 510

Pro Arg Ser Ser Leu Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala
        515                 520                 525

Ala Pro Glu Ala Ala Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu
    530                 535                 540

Pro Leu Arg Leu Asp Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg
545                 550                 555                 560
```

```
Leu Glu Thr Ile Leu Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile
                565                 570                 575

Pro Ser Ala Ile Pro Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp
            580                 585                 590

Pro Ser Ser Ile Pro Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp
        595                 600                 605

Tyr Ala Ser Gln Pro Gly Lys Pro Pro Arg Glu Asp Leu Lys  *  Leu
    610                 615                 620

Pro Arg Pro Ala Gly Ser Leu Arg Arg Ser Arg Pro Ser Arg Gly Leu
625                 630                 635                 640

Ala Ile His Gln Val Phe Leu Met Pro Ala Gln Ser Asn Met Asn  *
                645                 650                 655

Ser Ser Arg Val Asp Leu Gln Ala Cys Lys Leu
            660                 665
```

CLAIMS

1. A single-chain Fv (sFv) polypeptide defining a binding site which exhibits the immunological binding properties of an immunoglobulin molecule which binds c-erbB-2 or a c-erbB-2-related tumor antigen, said sFv comprising at least two polypeptide domains connected by a polypeptide linker spanning the distance between the C-terminus of one domain and the N-terminus of the other, the amino acid sequence of each of said polypeptide domains comprising a set of complementarity determining regions (CDRs) interposed between a set of framework regions (FRs), said CDRs conferring immunological binding to said c-erbB-2 or c-erbB-2-related tumor antigen.

2. The single-chain Fv polypeptide of claim 1 wherein said CDRs are substantially homologous with the CDRs of the c-erbB-2-binding immunoglobulin molecules selected from the group consisting of 520C9, 741F8, and 454C11 monoclonal antibodies.

3. The single-chain Fv polypeptide of claim 2 wherein the amino acid sequence of each of said sFv CDRs and each of said FRs are substantially homologous with the amino acid sequence of CDRs and FRs of the variable region of 520C9 antibody.

4. The single-chain Fv polypeptide of claim 1 wherein said polypeptide linker comprises the amino acid sequence as set forth in the Sequence Listing as amino acid residue numbers 118 through 133 in SEQ ID NO:4.

5. The single-chain Fv polypeptide of claim 1 wherein said polypeptide linker comprises an amino acid sequence selected from the group of sequences set forth as amino acid residues 116-135 in SEQ ID NO:6, or 122-135 in SEQ. ID NO:15 and the amino acid sequences set forth in SEQ ID NO: 12 and SEQ ID NO: 14.

6. The single-chain Fv polypeptide of claim 1 further comprising a remotely detectable moiety bound thereto to permit imaging of a cell bearing said c-erbB-2-related tumor antigen.

7. The single-chain Fv polypeptide of claim 6 wherein said remotely detectable moiety comprises a radioactive atom.

8. The single-chain Fv polypeptide of claim 1 further comprising, linked to the N or C terminus of said linked domains, a third polypeptide domain comprising an amino acid sequence defining CDRs interposed between FRs and defining a second immunologically active site.

9. The single-chain Fv polypeptide of claim 8, further comprising a fourth polypeptide domain, wherein said third and fourth polypeptide domains together comprise a second site which immunologically binds a c-erbB-2-related tumor antigen.

10. The single-chain Fv polypeptide of claim 1 or 7 further comprising a toxin linked to the N or C terminus of said linked domain.

11. The single-chain Fv polypeptide of claim 10 wherein said toxin comprises a toxic portion selected from the group: Pseudomonas exotoxin, ricin, ricin A chain, phytolaccin and diphtheria toxin.

12. The single-chain Fv polypeptide of claim 10 wherein said toxin comprises at least a portion of the ricin A chain.

13. A DNA sequence encoding the polypeptide chain of claim 1.

14. A method of producing a single chain polypeptide having specificity for a c-erbB-2-related tumor antigen, said method comprising the steps of:

(a) transfecting the DNA of claim 13 into a host cell to produce a transformant; and

(b) culturing said transformant to produce said single-chain polypeptide.

15. A method of imaging a tumor expressing a c-erbB-2-related antigen, said method comprising the steps of:

(a) providing an imaging agent comprising the polypeptide of claim 7;

(b) administering to a mammal harboring said tumor an amount of said imaging agent together with a physiologically-acceptable carrier sufficient to permit extracorporeal detection of said tumor after allowing said agent to bind to said tumor; and

(c) detecting the location of said remotely detectable moiety in said subject to obtain an image of said tumor.

16. A host cell transfected with a DNA of claim 13.

17. A method of inhibiting in vivo growth of a tumor expressing a c-erbB-2-related antigen, said method comprising:

administering to a patient harboring the tumor a tumor inhibiting amount of a therapeutic agent comprising a single-chain Fv of claim 1 and at least a first moiety peptide bonded thereto, said first moiety having the ability to limit the proliferation of a tumor cell.

18. The method of claim 17 wherein said first moiety comprises a cell toxin or a toxic fragment thereof.

19. The method of claim 17 wherein said first moiety comprises a radioisotope sufficiently radioactive to inhibit proliferation of said tumor cell.

20. A DNA sequence encoding the polypeptide chain of claim 10.

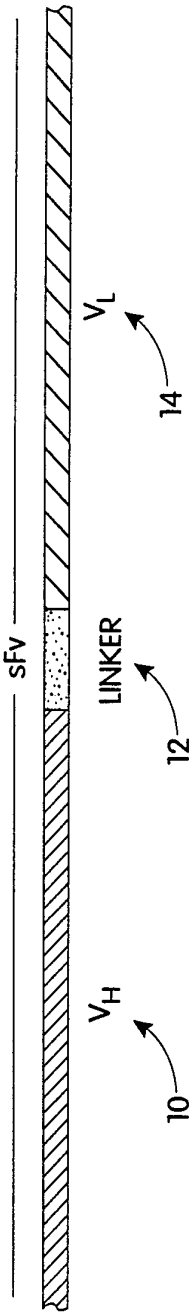


Fig. 1A

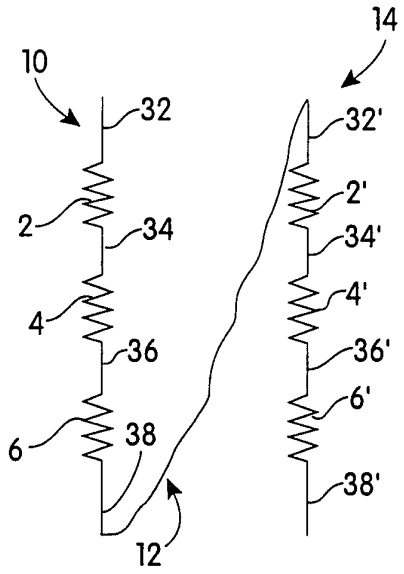


Fig. 1B

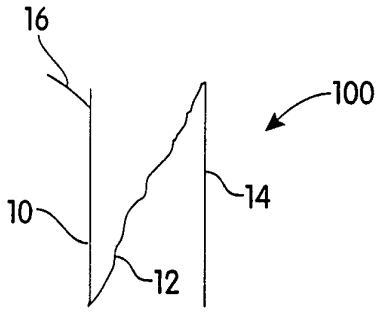


Fig. 2A

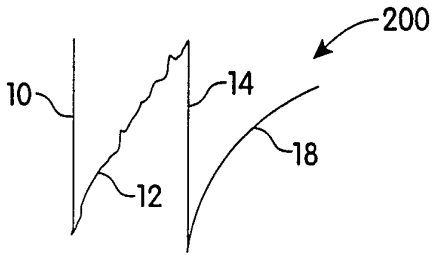


Fig. 2B

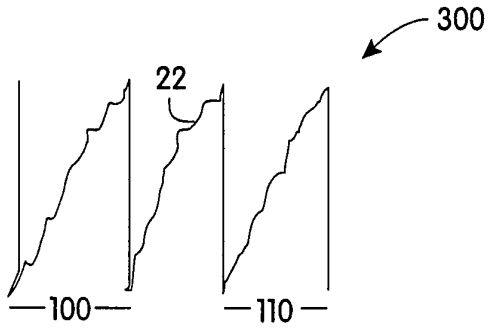
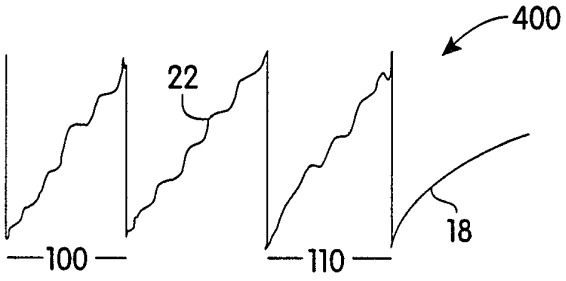


Fig. 2C



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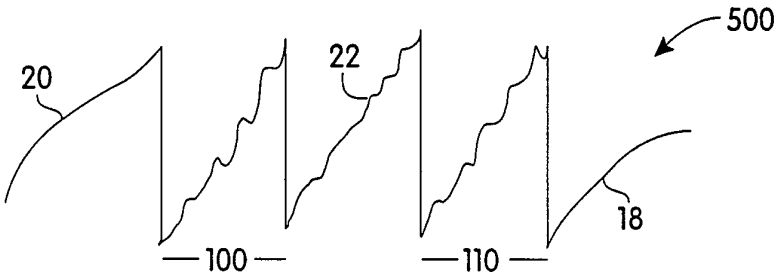
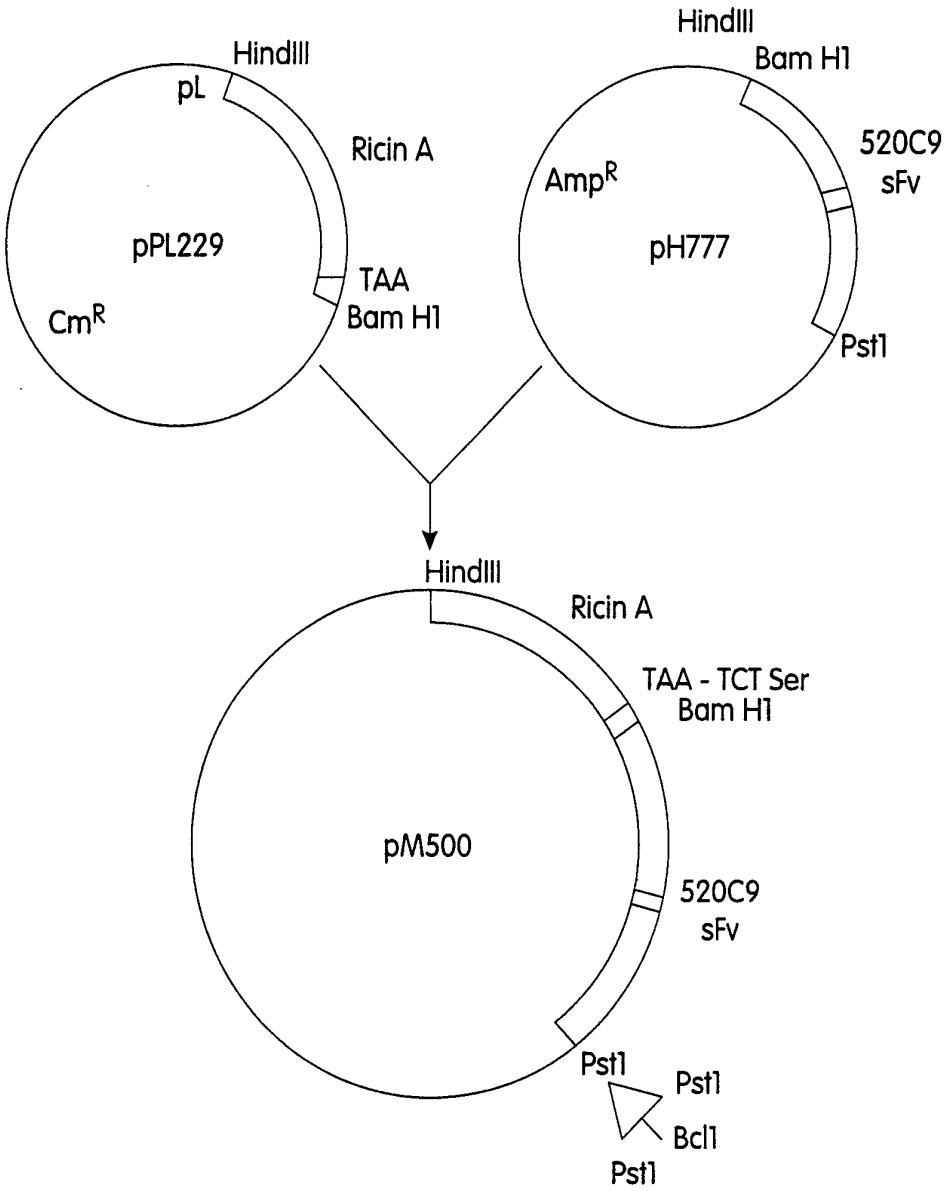


Fig. 2E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pPL229
TAA
Bam H1
Cm^R
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Pst1
HindIII
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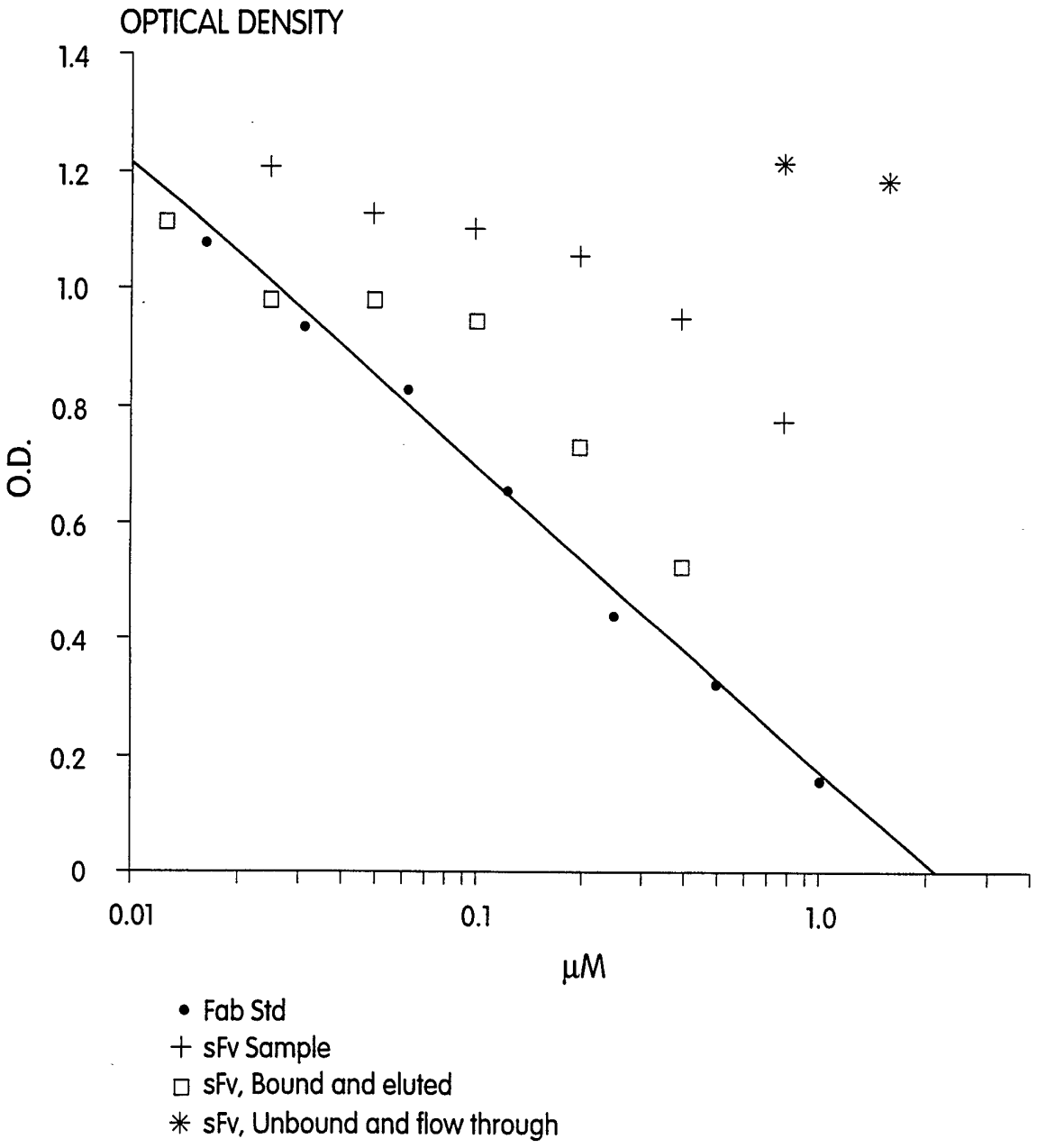
sFv
Pst1
Pst1
Bcl1
Pst1

Fig. 3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1.0
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0.6
0.4
0.2
0
O.D.
0.01
0.1
1.0
μM
• Fab Std
+ sFv Sample
□ sFv, Bound and eluted
✳ sFv, Unbound and flow through

Fig. 4