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(54) Title: SPECIFIC HUMAN ANTIBODIES FOR SELECTIVE CANCER THERAPY

(57) Abstract: The present invention is directed to a peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment having enhanced binding characteristics so as to bind selectively and/or specifically to a target cell in favor of other cells, wherein the binding selectivity or specificity is primarily determined by a first hypervariable region, and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags. The enhanced binding is directed to a substantially exposed and/or over-expressed binding site on or in a target comprising a cell in favor of other cells on or in which the binding site is not substantially available and/or expressed. The invention is further directed to a method for isolating such peptides and polypeptides from a phage display library and to the nucleic acid molecules encoding them. The invention provides for a pharmaceutical composition comprising the peptide or polypeptide and kits for diagnosis and treatment of disease, specifically cancer, most specifically acute myeloid leukemia.

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SPECIFIC HUMAN ANTIBODIES FOR SELECTIVE CANCER THERAPY

FIELD OF THE INVENTION

[1.] The present invention relates to the field of tissue targeting and identification, with the aid of phage display technology, of peptides and polypeptides that specifically bind to target cells. Such peptides and polypeptides are Fv molecules, constructs thereof, fragments of either or constructs of a fragment. More particularly, the peptides and polypeptides may have anti-cancer activity, and/or are associated with, or conjugated to, anti-cancer agents, especially against blood-related cancers.

BACKGROUND OF THE INVENTION

[2.] Tissue-selective targeting of therapeutic agents is an emerging discipline in the pharmaceutical industry. New cancer treatments based on targeting have been designed to increase the specificity and potency of the treatment, while reducing toxicity, thereby enhancing overall efficacy. Mouse monoclonal antibodies (MAb's) to tumor-associated antigens have been employed in an attempt to target toxin, radionucleotide, and chemotherapeutic conjugates to tumors. In addition, differentiation antigens, such as CD19, CD20, CD22 and CD25, have been exploited as cancer specific targets in treating hematopoietic malignancies. Although extensively studied, this approach has several limitations. One limitation is the difficulty of isolating appropriate monoclonal antibodies that display selective binding. A second limitation is the need for high antibody immunogenicity as a prerequisite for successful antibody isolation. A third limitation is the elicitation in the patient of an immune response against murine antibodies (human anti-mouse antibody-HAMA response) that often results in a shorter serum half-life, and prevents repetitive treatments, thus diminishing the therapeutic value of the antibody. This latter limitation has stimulated interest both in engineering chimeric or humanized monoclonal antibodies of murine origin, and in discovering human antibodies.

[3.] There are many factors that influence the therapeutic efficacy of monoclonal antibodies (Mabs) for treating cancer. These factors include specificity of antigen expression on tumor cells, level of expression, antigenic heterogeneity, and accessibility of the tumor mass. Leukemia and lymphoma have been generally more responsive to treatment with antibodies than solid tumors, such as carcinomas. MABs rapidly bind to leukemia and lymphoma cells in the bloodstream and easily penetrate to malignant cells in lymphatic tissue, thus making lymphoid tumors excellent candidates for MAb-based therapy. An ideal system would entail identifying a MAb that recognizes a marker on the cell surface of stem cells that produce malignant progeny cells.

[4.] To aid in the discovery/production of Mabs, phage libraries have been used to select random single chain Fvs (scFvs) that bind to isolated, pre-determined target proteins such as antibodies, hormones and receptors. In addition, the use of antibody display libraries in general, and phage scFv libraries in particular, facilitates an alternative means of discovering unique molecules for targeting specific, yet unrecognized and undetermined, cell surface moieties.

[5.] Leukemia, lymphoma, and myeloma are cancers that originate in the bone marrow and lymphatic tissues and are involved in uncontrolled growth of cells. Acute lymphoblastic leukemia ("ALL") is a heterogeneous disease that is defined by specific clinical and immunological characteristics. Like other forms of ALL, the definitive cause of most cases of B-cell ALL ("B-ALL") is not known, although in many cases, the disease results from acquired genetic alterations in the DNA of a single cell, causing it to become abnormal and multiply continuously

[6.] AML is a heterogeneous group of neoplasms with a progenitor cell that, under normal conditions, gives rise to terminally differentiated cells of the myeloid series (erythrocytes, granulocytes, monocytes, and platelets). As in other forms of neoplasia, AML is associated with acquired genetic alterations that result in replacement of normally differentiated myeloid cells with relatively undifferentiated blasts, exhibiting one or more type of early myeloid differentiation. AML generally evolves in the bone marrow and, to a lesser degree, in the secondary hematopoietic

organs. AML primarily affects adults, peaking in incidence between the ages of 15-40 years, but it is also known to affect both children and older adults. Nearly all patients with AML require treatment immediately after diagnosis to achieve clinical remission, in which there is no evidence of abnormal levels of circulating undifferentiated blast cells.

[7.] To date, a variety of monoclonal antibodies have been developed that induce cytolytic activity against tumor cells. A humanized version of the monoclonal antibody MuMAb4D5, directed to the extracellular domain of P185 - growth factor receptor (HER2) - was approved by the FDA and is being used to treat human breast cancer (US Patent No.'s 5,821,337 and 5,720,954). Following binding, the antibody is capable of inhibiting tumor cell growth that is dependent on the HER2 growth factor receptor. In addition, a chimeric antibody against CD20, which causes rapid depletion of peripheral B cells, including those associated with lymphoma, was recently approved by the FDA (US Patent No. 5,843,439). The binding of this antibody to target cells results in complement-dependent lysis. This product has recently been approved and is currently being used in the clinic to treat low-grade B-cell non-Hodgkin's lymphoma.

[8.] Several other humanized and chimeric antibodies are under development or are in clinical trials. In addition, a humanized Ig that specifically reacts with CD33 antigen, expressed both on normal myeloid cells, as well as on most types of myeloid leukemic cells, was conjugated to the anti-cancer drug calicheamicin, CMA-676 (Sievers *et al.*, *Blood*, 90 (10 Suppl. 1 Part 1), 504A (1997)). This conjugate, known as the drug Mylotarg, has recently been approved (Caron *et al.*, *Cancer Supplement*, 73, 1049-1056 (1994)). In light of its cytolytic activity, an additional anti-CD33 antibody (HuM195), currently in clinical trials, was conjugated to several cytotoxic agents, including the gelonin toxin (McGraw *et al.*, *Cancer Immunol. Immunother*, 39, 367-374 (1994)) and radioisotopes ¹³¹I (Caron *et al.*, *Blood* 83, 1760-1768 (1994)), ⁹⁰Y (Jurcic *et al.*, *Blood*, 92, (10 Suppl. Part 1-2), 613A (1998)) and ²¹³Bi (Sgouros *et al.*, *J. Nucl. Med.*, 38 (5 Suppl.), 231P (1997)).

[9.] A chimeric antibody against the leukocyte antigen CD-45 (cHuLym3) is in preclinical phase for treatment of human leukemia and lymphoma (Sun *et al.*, *Cancer Immunol. Immunother.*, 48, 595-602 (2000)) as a conditioning for bone marrow transplantation. In *in vitro* assays, specific cell lysis was observed in ADCC (antibody dependent cell-mediated cytotoxicity) assays (Henkart, *Immunity*, 1, 343-346 (1994); Squier and Cohen, *Current Opin. Immunol.*, 6, 447-452 (1994)).

[10.] Although these preliminary results seem promising, they have the following limitations. The final product comprises non-human sequences, resulting in a problematic immune response to non-human material, such as HAMA. This HAMA response prevents repetitive treatments and results in a shorter serum half-life for the product. In addition, the above methods allow for the isolation of a single antibody species only, and only allow for the isolation of antibodies against known and purified antigens. Further, these methods are not selective insofar as they allow for the isolation of antibodies against cell surface markers that are present on normal cells as well as on malignant cells.

[11.] Thus, a method, which overcomes these above discussed limitations, would be desirable. Further, such method would ideally enable the identification of target ligands or markers on cancer cells or cells involved in mediating metastasis of cancer cells, for example. Additionally, such method would also enable the production of antibodies to such targets. Phage display technology appears to offer such abilities.

[12.] The use of phage display technology has enabled the isolation of scFvs comprising fully human sequences. For example, fully human antibody against the human TGFb2-receptor based on a scFv clone derived from phage display technology was recently developed. This scFv, converted into a fully human IgG4 that is capable of competing with the binding of TGFb2 (Thompson *et al.*, *J. Immunol Methods*, 227, 17-29 (1999)), has strong anti-proliferative activity. This technology, known to one skilled in the art, is more specifically described in the following publications: Smith, *Science*, 228, 1315 (1985); Scott *et al*, *Science*, 249, 386-390 (1990); Cwirla *et al.*, *PNAS*, 87, 6378-6382 (1990); Devlin *et al.*, *Science*, 249, 404-406 (1990); Griffiths *et*

al., *EMBO J.*, 13(14), 3245-3260 (1994); Bass *et al.*, *Proteins*, 8, 309-314 (1990); McCafferty *et al.*, *Nature*, 348, 552-554(1990); Nissim *et al.*, *EMBO J.*, 13, 692 -698 (1994); U.S. Patent Nos 5,427,908, 5,432,018, 5,223,409 and 5,403,484, *lib*.

[13.] Using this phage display technology, the inventors of the present invention have identified cell markers present on or cells in diseased or malignant state. Therefore, it is an objective of the present invention to identify peptides and polypeptides that recognize cell markers that are substantially exposed or over-expressed, particularly on or in cells in a diseased or malignant state.

[14.] It is a further objective of the present invention to use and expand phage display technology as an aid to identify such peptides and polypeptides.

[15.] It is a further objective of the present invention to identify such peptides and polypeptides by immuno-cross-reactivity.

[16.] It is a still further objective of the present invention that such peptides and polypeptides be of fully human origin.

[17.] It is a still further objective of the present invention that such peptides and polypeptides be isolated against antigens that may not necessarily be immunogenic.

[18.] It is a still further objective of the present invention to provide peptides or polypeptides that prevent, retard or cure cancer, particularly blood-related cancers including leukemia or lymphoma.

[19.] It is a still further objective of the present invention to provide for local targeting of cancerous cells with such peptides and polypeptides alone, or associated with, or coupled to, an anti-cancer agent and/or a diagnostic label or marker.

[20.] It is a still further objective of the present invention to provide a method for producing a targeting agent against desired ligands.

[21.] It is a still further objective of the present invention to identify specific motifs that provide for the recognition of cell markers that are over-expressed in the malignant state and that can be used in the construction of a targeting or diagnostic label or marker for an anti-cancer agent.

[22.] It is a still further objective of the present invention to provide a composition comprising an effective amount of such peptides, polypeptides or motifs associated with, or coupled to, an anti-cancer agent or to a diagnostic label or marker.

[23.] It has been established that scFv penetrate tissues and are cleared from the blood more rapidly than a full size antibody because they are smaller in size. Adams, G.P., et al., Br. J. Cancer 77, 1405-1412 (1988); Hudson, P.J., Curr. Opin. Immunol. 11(5), 548-557 (1999); Wu, A.M., et al., Tumor Targeting 4, 47 (1999). Thus, scFv are often employed in diagnostics involving radioactive labels such as tumor imaging to allow for a more rapid clearance of the radioactive label from the body. A number of cancer targeting scFv multimers have recently undergone pre-clinical evaluation for in vivo stability and efficacy. Adams, G.P., et al., Br. J. Cancer 77, 1405-1412 (1988); Wu, A.M., et al., Tumor Targeting 4, 47 (1999).

[24.] Single chain Fv (scFv) fragments are comprised of the variable domains of the heavy (V_H) and light (V_L) chains of an antibody tethered together by a polypeptide linker. The linker is long enough to allow the (V_H) and the (V_L) domains to fold into a functional Fv domain enabling the scFv to recognize and bind its target with the similar or increased affinity of the parent antibody. A commonly used linker comprises glycine and serine residues to provide flexibility and protease resistance.

[25.] Typically, scFv monomers are designed with the C-terminal end of the V_H domain tethered by a polypeptide linker to the N-terminal residue of the V_L . Optionally an inverse orientation is employed: the C-terminal end of the V_L domain is tethered to the N-terminal residue of V_H through a polypeptide linker. Power, B., et al., J. Immun. Meth. 242, 193-204 (2000). The polypeptide linker is typically around twelve amino acids in length. When the linker is reduced to about three to twelve amino acids, the scFvs can not fold into a functional Fv domain and instead associate

with a second scFv to form a diabody. Further reducing the length of the linker to less than three amino acids forces the scFv association into trimers or tetramers, depending on the linker length, composition and Fv domain orientations. B.E. Powers, P.J. Hudson, J. Immun. Meth. 242 (2000) 193-194.

[26.] Recently, it has been discovered that multivalent antibody fragments such as scFv dimers, trimers, and tetramers often provide higher apparent affinity over the binding of the parent antibody to the target. This higher affinity offers many advantages including ideal pharmaco-kinetics for tumor targeting applications.

[27.] The greater binding affinity of these multivalent forms is therefore desirable in diagnostics and therapeutic regimens. For example, a scFv may be employed as a blocking agent to bind a target receptor and thus block the binding of the "natural" ligand. In such instances, it is desirable to have a high affinity association between the scFv and the receptor to decrease chances for disassociation, which may allow an undesirable binding of the natural ligand to the target. In addition, this high affinity is especially critical when the target receptors are involved in adhesion and rolling or when the target receptors are on cells present in areas of high sheer flow, such as platelets.

[28.] Therefore, an object of the invention is multivalent forms of Y1 and Y17 scFv. These multivalent forms include, but are not limited to dimers, trimers and tetramers, sometimes referred to herein as diabodies, triabodies, and tetrabodies, respectively.

SUMMARY OF THE INVENTION

[29.] The present invention provides for the identification of peptides and polypeptides that bind selectively and/or specifically to target cells especially against blood related cancer cells, their construction, their use on their own, or in association with, or combined, conjugated or fused to one or more pharmaceutical agents.

[30.] One embodiment of the present invention provides for a peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment having enhanced binding characteristics so as to bind selectively and/or specifically to a target cell in favor of other cells, wherein the binding selectivity or specificity is primarily determined by a first hypervariable region, and wherein the Fv is a single chain Fv ("scFv") or a disulfide Fv ("dsFv"), and optionally having one or more tags.

[31.] In another embodiment of the present invention there is provided a peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment having enhanced binding characteristics so as to bind selectively and/or specifically to a substantially exposed and/or overexpressed binding site on, or in, a target comprising a cell in favor of other cells on, or in which, the binding site is not substantially available and/or expressed, wherein the binding selectivity or specificity is primarily determined by a first hypervariable region, and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags.

[32.] In a further embodiment of the present invention there is provided a peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment having enhanced binding characteristics so as to bind selectively and/or specifically to a target cell in favor of other cells, wherein the Fv molecule comprises a first chain having a first, a second and a third hypervariable region and a second chain having a first, a second and a third hypervariable region, wherein one of the hypervariable regions of the first chain has a sequence selected from the group comprising SEQ ID NOs:8-24, and wherein one of the hypervariable regions of the second chain has a sequence selected from the group comprising SEQ ID NOs:1-6 and 125-202, and wherein the first, second, and third hypervariable regions are a CDR3, CDR2 and CDR1 region, respectively, and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags.

[33.] In a further embodiment of the invention,

- (a) the first and second chain each comprises a first hypervariable region selected from the group comprising SEQ ID NOs:8-24,
- (b) the first hypervariable regions of the first and second chains are identical and are selected from the group comprising SEQ ID NOs:8-24,
- (c) the first hypervariable region of the first chain is selected from the group comprising SEQ ID NOs:8-24, and the first hypervariable region of the second chain is selected from the group comprising SEQ ID NOs:1-6 and 125-202, or
- (d) the first hypervariable region of the first chain is selected from the group comprising SEQ ID NOs:1-6 and 125-202, and the first hypervariable region of the second chain is selected from the group comprising SEQ ID NOs:8-24.

[34.] In a still further embodiment of the present invention there is provided a peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either or a construct of a fragment that binds to an unknown ligand on a first cell having a first and a second state, wherein the binding is effective in the second state but not substantially in the first state and, by virtue of immuno-cross-reactivity, binds specifically or selectively to a ligand on a second cell, and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags.

[35.] In a still further embodiment of the present invention there is provided a method for identifying a targeting molecule which binds to unknown immuno-cross-reactive binding site on first and second cells comprising

- (a) one or more biopanning steps that are performed on a first target cell that, in a second state but not in a first state, substantially exposing or displaying a binding site comprising an unknown ligand, so as to produce a first population of recognition molecules;
- (b) subsequent biopanning and/or selection steps commencing with the resultant stock of recognition molecules of step (a) that are performed on a second cell that displays a binding site comprising an unknown ligand having immuno-cross-

reactivity to the the unknown ligand of the first cell so as to produce a second population of recognition molecules;

- (c) amplification and purification of the second population of recognition molecules of step (b); and
- (d) construction from the recognition sites of the purified recognition molecules of step (c) peptides or polypeptides that comprise targeting molecules that are selective and/or specific for unknown ligands on the second cell.

[36.] In a still further embodiment of the present invention there is provided a binding motif comprising the amino acid sequence of R_1 -X Phe Pro- R_2 wherein R_1 and R_2 each comprises 0-15 amino acid residues, and wherein X is either Arg, Gly or Lys.

[37.] In yet another embodiment of the present invention there is provided a method of production of a targeting agent comprising the following steps:

- a) isolating and selecting one or more targeting molecules comprising a primary recognition site by a biopanning procedure directly on a target cell or by a biopanning procedure indirectly on a first target cell in a second but not in a first state, and subsequently by a biopanning procedure directly on a second target cell to produce one or more said targeting molecules;
- b) amplification, purification and identification of the one or more targeting molecules;
- c) construction of a targeting agent from the one or more targeting molecules or recognition sites thereof;

wherein the targeting agent can be a peptide, polypeptide, antibody or antibody fragment or a multimer thereof.

[38.] In another embodiment of the present invention there is provided a peptide or polypeptide having the formula or structure:

A - X - B

wherein X is a hypervariable CDR3 region of 3 to 30 amino acids; A and B can each be amino acid chains from 1 to 1000 amino acids in length, wherein A is the amino end and B is the carboxy end.

BRIEF DESCRIPTION OF THE DRAWINGS

[39.] The invention is herein described in more detail, by way of example only, and not by way of limitation, with reference to the accompanying drawings described below, wherein:

[40.] **Figure 1** presents phage clone binding to fixed platelets, as determined by the EIA assay. Data are presented as a function of absorbance at 405 nm.

[41.] **Figures 2a, 2b and 2c** present the binding of mononuclear cell samples obtained from three individual AML patients to scFvs, as determined by FACS analysis. Fluorescence intensity of cells bound by the two FITC-labeled tested samples (control scFv and scFv clone Y1) is presented.

[42.] **Figure 3** presents the binding of Y-I to platelets (3a) and monocytes (3b) that have been Ficoll-purified, as determined by FACS analysis. Fluorescence intensity of cells bound by the two FITC-labeled tested samples (control scFv and scFv clone Y1) is presented.

[43.] **Figure 4** presents the binding of FITC-labeled scFv clone Y1 to cord-blood CD34+ stem cells. CD34+ gated cells, in the FL3-H channel, were analyzed in the FLI-H channel for their binding to FITC-labeled negative control scFv (figure 4a) or FITC-labeled scFv clone Y1 (figure 4b). Figure 4c presents a FSC and SSC dot plot analysis of the same FITC-labeled scFv clone Y1 sample as in 4b. The circled areas in figures 4b and 4c delineate the sub-population of CD34+ cells that bind scFv clone Y1.

[44.] **Figure 5:** FACS analyses of samples obtained from two patients with pre-B-ALL cells are presented: one from a child (5a, 5c, 5e) and the other from an

adult (5b, 5d, 5f). A double staining procedure, using either a commercially available PE-labeled CD19 (a marker for normal peripheral B-cells; Figure 5a, 5c) or a PE-labeled CD34 (a marker for stem cells; Figure 5d) was employed, together with a FITC-labeled negative control scFv (5a, 5b) or FITC-labeled Y-I scFv (5c, 5d). Figure 5b is a double negative control. Fluorescence intensity (x-axis) of cells bound by the FITC-labeled sample (scFv clone Y1), relative to the negative control staining pattern, is presented (5e and 5f).

[45.] **Figure 6:** This figure provides results of a binding comparison study performed using Jurkat cells. FACS analysis of binding to Jurkat cells of FITC-labeled Y-I scFv monomers, diabodies and triabodies, together with a negative control, is presented.

[46.] **Figure 7:** This figure provides results of a study comparing the binding of IgG- Y-I and scFv-Y1. A double staining procedure was employed to compare the binding of full sized IgG-Y1 to that of the scFv-YI form. Five nanograms of IgG-YI were used for FACS analysis on RAJI cell (YI negative cells; Figure 7a) and on Jurkat cells (Y1 positive cells; Figure 7b). For detection, PE labeled goat anti-human IgG was used. For the binding of the scFv-YI -I ~1 μ g (200-fold) was used, followed by staining with PE-labeled rabbit anti-scFv antibodies and FACS analysis (Figure 7c).

[47.] **Figure 8:** This figure shows a binding comparison between a YI dimer, the Y1 scFv (CONY1), and Y1 IgG.

[48.] **Figure 9:** This figure shows a binding comparison between a Y1 sulfide bridge dimer with the Y1 scFv (CONY1).

[49.] **Figure 10:** This figure is a graph of the Superdex 75 profile of Y1-cys-kak.

[50.] **Figure 11:** This figure reveals the size of the dimers compared to the monomer in reducing and non-reducing conditions.

[51.] **Figure 12:** This figure provides results of an ELISA assay.

[52.] **Figure 13:** This figure is a chart of the epitopes of anti-GPIIb α antibodies.

[53.] **Figure 14:** This figure is the amino acid SEQ ID NO:.

DETAILED DESCRIPTION OF THE INVENTION

[54.] Specificity is herein defined as the recognition, by one or more domains in the peptide or polypeptide of the invention, of a target ligand and subsequent binding thereto.

[55.] Selectivity is herein defined as the ability of a targeting molecule to choose and bind one cell type or cell state from a mixture of cell types or cell states, all cell types or cell states of which may be specific for the targeting molecule.

[56.] Conservative amino acid substitution is defined as a change in the amino acid composition by way of changing one or two amino acids of a peptide, polypeptide or protein, or fragment thereof. The substitution is of amino acids with generally similar properties (e.g., acidic, basic, aromatic, size, positively or negatively charged, polar, non-polar) such that the substitutions do not substantially in a major way alter peptide, polypeptide or protein characteristics (e.g., charge, IEF, affinity, avidity, conformation, solubility) or activity. Typical substitutions that may be performed for such conservative amino acid substitution may be among the groups of amino acids as follows:

- (i) glycine (G), alanine (A), valine (V), leucine (L) and isoleucine (I)
- (ii) aspartic acid (D) and glutamic acid (E)
- (iii) alanine (A), serine (S) and threonine (T)
- (iv) histidine (H), lysine (K) and arginine (R)
- (v) asparagine (N) and glutamine (Q)

(vi) phenylalanine (F), tyrosine (Y) and tryptophan (W)

[57.] Conservative amino acid substitutions can be made in, as well as, flanking the hypervariable regions primarily responsible for the selective and/or specific binding characteristics of the molecule, as well as other parts of the molecule, e.g., variable heavy chain cassette. Additionally or alternatively, modification can be accomplished by reconstructing the molecules to form full-size antibodies, diabodies (dimers), triabodies (timers) and/or tetrabodies (tetramers) or to form minibodies or microbodies.

[58.] As used herein in the specification and in the claims, an Fv is defined as a molecule that is made up of a variable region of a heavy chain of a human antibody and a variable region of a light chain of a human antibody, which may be the same or different, and in which the variable region of the heavy chain is connected, linked, fused or covalently attached to, or associated with, the variable region of the light chain.

[59.] A fragment of an Fv molecule is defined as any molecule smaller than the original Fv that still retains the selective and/or specific binding characteristics of the original Fv. Examples of such fragments include but are limited to (1) a minibody, which comprises a fragment of the heavy chain only of the Fv, (2) a microbody, which comprises a small fractional unit of antibody heavy chain variable region (PCT Application No. PCT/IL99/00581), (3) similar bodies comprising a fragment of the light chain, and (4) similar bodies comprising a functional unit of a light chain variable region.

[60.] An anti-cancer agent is an agent with anti-cancer activity, i.e., any activity that inhibits the growth or differentiation of cancerous or immature pre-cancerous cells, or any activity that inhibits metastasis of cancerous cells. In the present invention, an anti-cancer agent is also an agent with anti-angiogenic activity that prevents, inhibits, retards or halts angiogenesis of tumor tissue or is also an agent with anti-adhesion activities that inhibits, retards or halts adhesion and metastatic invasion of cancerous and pre-cancerous cells.

[61.] Inhibition of growth of a cancer cell is herein defined as the (i) prevention of cancerous or metastatic growth, (ii) slowing down of the cancerous or metastatic growth, (iii) the total prevention of the growth process of the cancer cell or the metastatic process, while leaving the cell intact and alive, or (iv) killing the cancer cell. More specifically, inhibition of cancerous growth can be applied especially against blood-related cancers, e.g., AML, multiple myeloma, or chronic lymphatic leukemia.

[62.] A phagemid is defined as a phage particle that carries plasmid DNA. Because it carries plasmid DNA, the phagemid particle does not have sufficient space to contain the full complement of the phage genome. The component that is missing from the phage genome is information essential for packaging the phage particle. In order to propagate the phage, therefore, it is necessary to culture the desired phage particles together with a helper phage strain that complements the missing packaging information.

[63.] A cassette, as applied to polypeptides and as defined in the present invention, refers to a given sequence of consecutive amino acids that serves as a framework and is considered a single unit and is manipulated as such. Amino acids can be replaced, inserted into, removed, or attached at one or both ends. Likewise, stretches of amino acids can be replaced, inserted into, removed or attached at one or both ends.

[64.] As used herein, an immunoglobulin (Ig) molecule is defined as any one of five classes, i.e., IgG, IgA, IgD, IgE, or IgM. The IgG class encompasses several sub-classes including, but not restricted to, IgG1, IgG2, IgG3, and IgG4.

[65.] A pharmaceutical composition refers to a formulation which comprises a peptide or polypeptide of the invention and a pharmaceutically acceptable carrier, excipient or diluent thereof.

[66.] A pharmaceutical agent refers to an agent that is useful in the prophylactic treatment or diagnosis of a mammal including, but not restricted to, a human, bovine, equine, porcine, murine, canine, feline, or any other warm-blooded

animal. The pharmaceutical agent is selected from the group comprising radioisotope, toxin, oligonucleotide, recombinant protein, antibody fragment, and anti-cancer agent. Examples of such pharmaceutical agents include, but are not limited to anti-viral agents including acyclovir, ganciclovir and zidovudine; anti-thrombosis/restenosis agents including cilostazol, dalteparin sodium, reviparin sodium, and aspirin; anti-inflammatory agents including zaltoprofen, pranoprofen, droxicam, acetyl salicylic 17, diclofenac, ibuprofen, dexibuprofen, sulindac, naproxen, amtolmetin, celecoxib, indomethacin, rofecoxib, and nimesulid; anti-autoimmune agents including leflunomide, denileukin diftitox, subreum, WinRho SDF, defibrotide, and cyclophosphamide; and anti-adhesion/anti-aggregation agents including limaprost, clorcromene, and hyaluronic acid.

[67.] An anti-leukemia agent is an agent with anti-leukemia activity. For example, anti-leukemia agents include agents that inhibit or halt the growth of leukemic or immature pre-leukemic cells, agents that kill leukemic or pre-leukemic, agents that increase the susceptibility of leukemic or pre-leukemic cells to other anti-leukemia agents, and agents that inhibit metastasis of leukemic cells. In the present invention, an anti-leukemia agent may also be agent with anti-angiogenic activity that prevents, inhibits, retards or halts vascularization of tumors.

[68.] The term "affinity" as used herein is a measure of the binding strength (association constant) between a receptor (e.g., one binding site on an antibody) and a ligand (e.g., antigenic determinant). The strength of the sum total of noncovalent interactions between a single antigen-binding site on an antibody and a single epitope is the affinity of the antibody for that epitope. Low affinity antibodies bind antigen weakly and tend to dissociate readily, whereas high-affinity antibodies bind antigen more tightly and remain bound longer. The term "avidity" differs from affinity because the former reflects the valence of the antigen-antibody interaction.

[69.] Specificity of antibody-antigen interaction: Although the antigen-antibody reaction is specific, in some cases antibody elicited by one antigen can cross-react with another unrelated antigen. Such cross-reactions occur if two different antigens share an homologous or similar epitope or an anchor region thereof or if

antibodies specific for one epitope bind to an unrelated epitope possessing similar chemical properties.

[70.] Blast cells are cells in an immature stage of cellular development distinguished by a higher cytoplasm-to-nucleus ratio than a resting cell.

[71.] A platelet is a disc like cytoplasmic fragment of a megakaryocyte that is shed in the marrow sinus and subsequently are circulating in the peripheral blood stream. Platelets have several physiological functions including a major role in clotting. A platelet contains granules in the central part and peripherally, clear protoplasm, but no definite nucleus.

[72.] The term "epitope" is used herein to mean the antigenic determinant or antigen site that interacts with an antibody, antibody fragment, antibody complex or a complex comprising a binding fragment thereof or T-cell receptor. The term epitope is used interchangeably herein with the terms ligand, domain, and binding region.

[73.] A given cell may express on its surface a protein having a binding site (or epitope) for a given antibody, but that binding site may exist in a cryptic form (*e.g.*, be sterically hindered or be blocked, or lack features needed for binding by the antibody) in the cell in a state, which may be called a first stage (stage I). Stage I may be, for example, a normal, healthy, non-diseased status. When the epitope exists in cryptic form, it is not recognized by the given antibody, *i.e.*, there is no binding of the antibody to this epitope or to the given cell at stage I. However, the epitope may be exposed by, *e.g.*, undergoing modifications itself, or being unblocked because nearby or associated molecules are modified or because a region undergoes a conformational change. Examples of modifications include changes in folding, changes in post-translational modifications, changes in phospholipidation, changes in sulfation, changes in glycosylation, and the like. Such modifications may occur when the cell enters a different state, which may be called a second stage (stage II). Examples of second states, or stages, include activation, proliferation, transformation, or in a malignant status. Upon being modified, the epitope may then be exposed, and the antibody may bind.

[74.] As used herein the term "Fab fragment" is a monovalent antigen-binding fragment of an immunoglobulin. A Fab fragment is composed of the light chain and part of the heavy chain.

[75.] Polyclonal antibodies are the product of an immune response and are formed by a number of different B-lymphocytes. Monoclonal antibodies are derived from a single cell.

[76.] Agglutination as used herein means the process by which suspended bacteria, cells, discs, or other particles of similar size are caused to adhere and form into clumps. The process is similar to precipitation but the particles are larger and are in suspension rather than being in solution.

[77.] The term aggregation means the clumping of platelets induced in vitro, and thrombin and collagen, as part of a sequential mechanism leading to the formation of a thrombus or hemostatic plug.

[78.] The expression pattern of a gene can be studied by analyzing the amount of gene product produced under various conditions, at specific times, in various tissues, etc. A gene is considered to be "over expressed" when the amount of gene product is higher than that found in a normal control, e.g., non-diseased control.

[79.] A promoter is a region on DNA at which RNA polymerase binds and initiates transcription.

[80.] Antibodies, or immunoglobulins, are protein molecules that bind to antigen. They are composed of units of four polypeptide chains (2 heavy and 2 light) linked together by disulfide bonds. Each of the chains has a constant and variable region. They can be divided into five classes, IgG, IgM, IgA, IgD, and IgE, based on their heavy chain component. They are produced by B lymphocytes and recognize a particular foreign antigenic determinant and facilitate clearing of that antigen.

[81.] Antibodies may be produced and used in many forms, including antibody complexes. As used herein, the term "antibody complex" or "antibody complexes" is used to mean a complex of one or more antibodies with another

antibody or with an antibody fragment or fragments, or a complex of two or more antibody fragments.

[82.] F(ab')₂ fragment is a bivalent antigen binding fragment of an immunoglobulin obtained by pepsin digestion. It contains both light chains and part of both heavy chains.

[83.] Fc fragment is a non-antigen-binding portion of an immunoglobulin. It contains the carboxy-terminal portion of heavy chains and the binding sites for the Fc receptor.

[84.] Fd fragment is the variable region and first constant region of the heavy chain of an immunoglobulin.

[85.] Contaminating proteins are those proteins that are not specifically being selected for and which may be present in a sample.

[86.] Peptido-mimetics are small molecules, peptides, polypeptides, lipids, polysaccharides or conjugates thereof that have the same functional effect or activity of another entity such as an antibody.

[87.] Phagemids are plasmid vectors designed to contain an origin of replication from a filamentous phage, such as m13 or fd.

[88.] A wide spectrum of diseases exists that involves diseased, altered, or otherwise modified cells that express cell-specific and/or disease-specific ligands on their surfaces. These ligands can be utilized to effect recognition, selection, diagnosis and treatment of specific diseases through recognition, selection, diagnosis and treatment of each individual cell. The subject invention provides for peptides or polypeptides that comprise an Fv molecule, a construct thereof, a fragment thereof, a construct of a fragment thereof, or a fragment of a construct, all of which have enhanced binding characteristics. These binding characteristics allow the peptide or polypeptide molecule to bind selectively and/or specifically to a target cell in favor of other cells, the binding specificity and/or selectivity being primarily determined by a first hypervariable region. The Fv can be a scFv or a dsFv.

[89.] The Fv molecule described above can be used to target the diseased cell. The diseased cell can be, for example, a cancer cell. Examples of types of cancer that are amenable to diagnosis and/or treatment by specific targeting include, but are not limited to, carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma. Leukemia, lymphoma, and myeloma are cancers that originate in the bone marrow and lymphatic tissues and are involved in uncontrolled growth of cells.

[90.] New approaches for diagnosing and treating diseases, particularly cancer, have been developed in recent years. Among them is the tumor targeting approach, using targeting molecules that can be selected and produced in a variety of ways. One approach for identifying possible targeting molecules is phage display. Phage display is a technique in which peptides, polypeptides, antibodies or proteins are generated and selected by their expression and display on the surface of a filamentous bacteriophage by fusion to a phage coat protein, with the DNA encoding the displayed protein residing within the phage virion. The scFv that is produced by the phage display technique is comprised of the variable domains of each of the antibody heavy and light chains, linked by a flexible amino-acid polypeptide spacer (Nissim *et al.*, *EMBO J*, 13, 692-698 (1994)).

[91.] A phage display library (also termed phage peptide/antibody library, phage library, or peptide/antibody library) comprises a large population of phage (generally 10^8 - 10^9), each phage particle displaying a different peptide or polypeptide sequence. These peptide or polypeptide fragments may be constructed to be of variable length. The displayed peptide or polypeptide can be derived from, but need not be limited to, human antibody heavy or light chains.

[92.] In the present invention, an scFv antibody library produced by the phage display technique was utilized to obtain and produce targeting molecules. Flow cytometry, particularly fluorescence-activated cell sorting ("FACS"), was used for identifying and isolating specific phage clones, the peptide or polypeptide of which recognizes target cells. Phage-expressed scFv antibody fragments are amenable to *in vitro* screening, enrichment and selection of high affinity clones (U.S. patent

5,821,337; U.S. patent 5,720,954). Thus, a library of this type offers a powerful means for generating new tools for research and clinical applications, and has numerous advantages over the conventional approach (Caron *et al.*, *Cancer Supplement*, 73, 1049-1056 (1994)). The library contains the potential for a high diversity of antibody molecules (Nissim *et al.*, *EMBO J.*, 692-698 (1994)). In the present instance, stable human cDNA can be used as a continuous source of material for antibody production (U.S. patent 5,843,439). Molecule recognition and selection are not influenced by the *in vivo* immunogenicity of candidate target proteins.

[93.] While affinity selection of phage displayed antibodies provides a useful method for enriching antigen-reactive scFvs from large libraries, it requires multiple steps to isolate a single clone and to characterize soluble scFv. The scFvs themselves can be modified to improve their affinities and/or avidity by performing conservative amino acid substitutions, or by producing fragments of the scFv, or constructs of said fragments.

[94.] The scFvs of the subject invention, specific for different human cells and tissues, can be associated with, combined, fused or linked to various pharmaceutical agents and/or radioactive isotopes in a pharmaceutically effective amount with, optionally, a pharmaceutically effective carrier, to form drug-peptide compositions, fusions or conjugates having anti-disease and/or anti-cancer activity, and/or for diagnostic purposes thereof.

[95.] Phage clones are selected by and identified through a multi-step procedure known as biopanning. Biopanning is carried out by incubating phage displaying protein ligand variants (a phage display library) with a target, removing unbound phage by a washing technique, and specifically eluting the bound phage. The eluted phage are optionally amplified before being taken through additional cycles of binding and optional amplification that enriches the pool of specific sequences in favor of those phage clones bearing antibody fragments that display the best binding to the target. After several rounds, individual phage clones are characterized, and the sequences of the peptides displayed by the clones are determined by sequencing the corresponding DNA of the phage virion.

[96.] The scFv obtained in this manner is also referred to a lead compound. A lead compound is defined as a compound, the final format of which comprises a core peptide or polypeptide. The lead compound can be modified and/or expanded, but it must retain the core peptide or polypeptide or some conservatively modified form thereof. Modifications by way of amino acid substitution can be made at the N-terminus, at the carboxy terminus, or in any of the CDR regions of an Fv or in the regions upstream or downstream thereof, for example. Modifications also include but are not limited to, fused proteins, coupling to drugs or toxins, construction of multimers, and expansion to full antibody molecules. One preferred category of lead compound, as provided for in the present patent, is an scFv obtained as the final product of the biopanning procedure.

[97.] An embodiment of the invention provides for at least one non-natural modification of the peptide or polypeptide of the invention. The non-natural modification can render the peptide or polypeptide more immunogenic or more stable. Non-natural modifications include, but are not limited to peptoid modification, sernipeptoid modification, cyclic peptide modification, N-terminus modification, C-terminus modification, peptide bond modification, backbone modification, and residue modification.

[98.] The selection of antigen-specific phage antibodies has largely relied on biopanning against an immobilized single antigen. There has been limited selection using whole cells as a target. In the present invention, whole cells were used to select specific antibodies that recognize leukemia cell surface determinants, wherein the specific receptor was not previously known or characterized. This method does not permit facile adjustment of antigen concentration or the removal of undesired dominant antibody reactivities. Additionally, the phage may enrich for those that display multiple copies of scFv, as opposed to those with higher affinity clones. Nevertheless, the advantages of this approach make it an invaluable tool for isolating novel human antibody molecules.

[99.] An embodiment of the invention provides for a peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either or a construct of

a fragment that binds to an unknown ligand on a first cell having a first and a second state, wherein the binding is effective in the second state but not substantially in the first state and, by virtue of immuno-cross-reactivity, binds specifically or selectively to a ligand on a second cell, and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags.

[100.] A further embodiment provides for the peptide or polypeptide of the invention, wherein the selective and/or specific binding of the peptide or polypeptide to the ligand of the second cell is determined primarily by a first hypervariable region.

[101.] A yet further embodiment provides for the peptide or polypeptide of the invention, wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24.

[102.] A yet further embodiment provides for the peptide or polypeptide of the invention, wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24, and wherein the binding selectivity or specificity is secondarily influenced by a second hypervariable region and/or by a third hypervariable region and/or by one or more of the upstream and/or by one or more of the downstream regions flanking the first, the second and the third hypervariable regions, respectively.

[103.] A further embodiment provides for the ligand of the second cell bound by the peptide or polypeptide of the invention. One such two-cell selection protocol was based on the following: Megakaryocytes are large multinucleated cells derived from hematopoietic stem cells in the bone marrow. Platelets break off the megakaryocyte cytoplasm and enter the peripheral blood. *In vitro*, a wide range of cytokines directly affects stem cells. For example, thrombopoietin increases platelet count by directly increasing the differentiation of stem cells into megakaryocytes. Thus, these cells express several cell surface markers that are also found in premature cells.

[104.] Malignant blood cells (leukemia and lymphoma) are characterized as immature cells that express cell surface proteins normally found in partially

differentiated hematopoietic progenitors. Thus, platelets are an attractive source for the identification of premature cell surface markers expressed on diseased or malignant blood cells. In one protocol discussed below, specific cells such as, but not limited to platelets, carrying unknown ligands, were used for initial biopanning steps. Subsequent clone selection was performed with a desired target cell, of which the targeted cell surface markers are unknown, such as but not limited to AML cells. In this method, phage clones obtained by biopanning on platelets can provide tools for recognizing and binding to ligands on diseased or malignant blood cells of interest.

[105.] The target as described above includes cells derived from an isolated tissue. The isolated tissue can be a diseased tissue and, more specifically, a cancer tissue. Cancer tissue can be derived from any form of malignancy including, but not limited to, carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma.

[106.] In addition to the biopanning method described above, another approach is based on isolation of a peptide or polypeptide that binds a ligand on a cell as determined by direct panning on that ligand.

[107.] The present invention provides for a peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment. A construct may be a multimer (e.g., diabody, triabody, tetrabody) or a full-size Ig molecule; a fragment might be a minibody or a microbody. All derived constructs and fragments retain enhanced binding characteristics so as to bind selectively and/or specifically to a target cell in favor of other cells. The binding selectivity and/or specificity is primarily determined by a first hypervariable region, and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags.

[108.] In one embodiment of the invention, a tag is inserted or attached to the Fv peptide or polypeptide to aid in the preparation and identification thereof, and in diagnostics. The tag can later be removed from the molecule. The tag may be, but is not limited to, the following tags: AU1, AU5, BTag, c-myc, FLAG, Glu-Glu, HA, His6, HSV, HTTPHH, IRS, KT3, Protein C, S•Tag[®], T7, V5, VSV-G (Jarvik and

Telmer, *Ann. Rev. Gen.*, 32, 601-618 (1998)), and KAK (lysine-alanine-lysine). The tag is preferably c-myc or KAK.

[109.] The two variable chains of the Fv molecule of the present invention may be connected or linked together by a spacer of 0-20 amino acid residues in length. The spacer may be branched or unbranched. Preferably, the linker is 0-15 amino acid residues, and most preferably the linker is (Gly₄Ser)₃ to yield a single chain Fv ("scFv"). The scFv is obtainable from a phage display library.

[110.] The Fv molecule itself is comprised of a first chain and a second chain, each chain comprising a first, second and third hypervariable region. The hypervariable loops within the variable domains of the light and heavy chains are termed Complementary Determining Regions (CDR). There are CDR1, CDR2 and CDR3 regions in each of the heavy and light chains. These regions are believed to form the antigen binding site and can be specifically modified to yield enhanced binding activity. The most variable of these regions in nature being the CDR3 region of the heavy chain. The CDR3 region is understood to be the most exposed region of the Ig molecule and as shown and provided herein is the site primarily responsible for the selective and/or specific binding characteristics observed.

[111.] An embodiment of the invention provides for a peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment having enhanced binding characteristics so as to bind selectively and/or specifically to a substantially exposed and/or over-expressed binding site on or in a target comprising a cell in favor of other cells on or in which the binding site is not substantially available and/or expressed, wherein the binding selectivity or specificity is primarily determined by a first hypervariable region, and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags.

[112.] A further embodiment of the invention provides for a peptide or polypeptide wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24.

[113.] A yet further embodiment provides for the peptide or polypeptide of the invention, wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24, and wherein the binding selectivity or specificity is secondarily influenced by a second hypervariable region and/or by a third hypervariable region and/or by one or more of the upstream regions and/or by one or more of the downstream regions flanking the first, the second and the third hypervariable regions, respectively, wherein the second and third hypervariable regions are a CDR2 and a CDR1 region, respectively.

[114.] An embodiment of the invention provides for peptide or polypeptide that binds to a target cell that is an activated, excited, modified, changed, disturbed or diseased cell. A further embodiment of the invention provides for the target cell being a cancer cell. The target cell can be selected from the group comprised of, but is not limited to, carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma. In a preferred embodiment, the cancer cell is a leukemia cell. In a most preferred embodiment, the leukemia cell is an AML cell.

[115.] The peptide or polypeptide of the present invention is also any construct or modified construct of the Fv that retains one or more of the hypervariable regions of the heavy and/or light chains and has selective and/or specific binding characteristics. Construct or modified construct includes, but is not limited to, scFv, dsFv, multimers of scFv such as dimers, trimers, tetramers and the like (also referred to as diabody, triabody, tetrabody), and full antibody, and any other multimer that can be constructed thereof, and that incorporates one or more of the hypervariable domains of the antibody. The peptide or polypeptide of the present invention is also a fragment of any construct or modified construct having some or all of the binding characteristics of the original construct.

[116.] The peptide or polypeptide of the present invention is also a construct of a fragment having some or all of the selective and/or specific binding characteristics of the original construct. Fvs herein described selectively and/or specifically bind to target cells and can be associated with, or conjugated to, anti-cancer agents or anti-disease agents.

[117.] Peptides, polypeptides, fragments thereof, constructs thereof and fragments of constructs thereof of Fv molecules of the invention can be prepared in either prokaryotic or eukaryotic expression systems. In one embodiment of the invention, the eukaryotic expression system is a mammalian system, and the peptide or polypeptide produced in the mammalian expression system, after purification, is substantially free of mammalian contaminants. A eukaryotic cell system, as defined in the present invention refers to an expression system for producing peptides or polypeptides by genetic engineering methods, wherein the host cell is a eukaryote. In another embodiment of the invention, a prokaryotic system for production of the peptide or polypeptide of the invention uses *E. coli* as the host for the expression vector. The peptide or polypeptide produced in the *E. coli* system, after purification, is substantially free of *E. coli* contaminating proteins. Use of a prokaryotic expression system may result in the addition of a methionine residue to the N-terminus of some or all of the sequences provided for in the present invention. Removal of the N-terminal methionine residue after peptide or polypeptide production to allow for full expression of the peptide or polypeptide can be performed by methods commonly known in the art, such as, but not limited to, the use of *Aeromonas* aminopeptidase under suitable conditions (U.S. Patent No.; 5,763,215).

[118.] The subject invention provides for production of a scFv based on the Fv peptide of the invention. Promoters incorporated into the vectors used for the cloning and amplification of the scFv in prokaryotic cells can be chosen from a wide selection. A promoter is a DNA sequence that is situated upstream of structural genes and is capable of controlling the expression of genes. Promoters are found in the natural state in the chromosome(s) of the organism and can also be engineered into prokaryotic or eukaryotic expression vectors. Promoters engineered into specific loci on the desired DNA fragment provide for finely tuned and precisely controlled expression of the gene of interest. In the present invention, several promoters were used in constructs that include the gene coding for the Fv of choice. Promoters include, but are not limited to the following: deo, P1-P2, osmB, λ P_L, β -lac-U5, SR α 5, and CMV early promoter. Deo is a double stranded DNA plasmid which, upon introduction into a suitable *E. coli* host, renders the host capable of effecting

expression of DNA encoding a desired naturally-occurring polypeptide or polypeptide analog thereof under the control of the constitutive *E. coli*-derived deoxyribonucleotide promoter, deo P1-P2. A fuller description is provided in U.S. Patent No. 5,795,776 (Fischer, August 18, 1998) and U.S. Patent No. 5,945,304 (Fischer, August 31, 1999).

[119.] Expression of the *E. coli* *osmB* promoter is regulated by osmotic pressure. Vectors carrying this promoter can be used to produce high levels of a wide variety of recombinant eukaryotic and prokaryotic polypeptides under control of the *osmB* promoter in an *E. coli* host. A fuller description is provided in U.S. Patent No. 5,795,776 (Fischer, August 18, 1998) and U.S. Patent No. 5,945,304 (Fischer, August 31, 1999).

[120.] λP_L is a thermoinducible λ bacteriophage promoter regulated by the thermolabile repressor cI^{857} . For A fuller discussion, see Hendrix *et al. Lambda II*, Cold Spring Harbor Laboratory (1983).

[121.] β -lac-U5 is a *lacZ* promoter (Gilbert and Muller-Hill, *PNAS (US)*, 58, 2415 (1967)).

[122.] $SR_{\alpha 5}$ is a mammalian cDNA expression system composed of the simian virus 40 (SV40) early promoter and the R-U5 segment of the human T-cell leukemia virus type 1 long terminal repeat. This expression system is 1 or 2 orders of magnitude more active than the SV40 early promoter in a wide variety of cell types (Takebe *et al.*, *Molecular and Cellular Biology*, 8, 466-472 (1988)).

[123.] The human cytomegalovirus promoter, known as the CMV intermediate/early enhancer/promoter is most preferably used in the present invention to promote constitutive expression of clone DNA inserts in mammalian cells. The CMV promoter is described in Schmidt, E.V. *et al.*, (1990) *Mol. Cell. Biol.*, 10, 4406, and is U.S. Patent Nos. 5,168,062 and 5,385,839.

[124.] In a preferred embodiment of the invention, the promoter for induction of the phagemid system in prokaryotes is selected from a group comprising deo,

osmB, λP_L , β -lac-U5, and CMV promoters. In a more preferred embodiment of the invention, the β -lac-U5 promoter was used for induction of the phagemid system in *E. coli*. In a most preferred embodiment, the CMV promoter is used.

[125.] In an embodiment of the invention, a peptide or polypeptide of the subject invention comprises: (a) a leader sequence that is present only in the encoded sequence but is lacking in the mature protein; (b) a variable regions of a heavy chain of the order of 135-145 amino acids, including a first hypervariable region of 4-12 amino acids that is subject to modifications; (c) a spacer region of ≤ 20 amino acids that may be shortened or eliminated; (d) variable region of a light chain that is also subject to specific modifications described herein followed by; (e) a tag sequence for follow-up, that is optionally not present in the final injectable product. The spacer, being generally about 15 amino acid residues long in the scFv, allows the two variable chains (heavy and light) to fold into functional Fv domain. The functional Fv domain retains selective and/or specific enhanced binding activity.

[126.] In another embodiment, (d) above is followed by a tag sequence or label that can be used for conjugation, diagnostic and/or identification purposes. In this embodiment, the tag is designed to connect between the peptide or polypeptide of the invention and an agent for treatment or diagnosis of the target cell.

[127.] The spacer region of the scFv may be linear or branched, and is generally comprised of glycine and serine residues, in multiples of the formula $(Gly_4Ser)_n$, and is generally between a total of 0-20 amino acids in length, preferably 0-15 amino acids long and linear. By changing the spacer length as appropriate, a variety of multimers can be obtained. In an embodiment of the invention, the spacer is 0-5 amino acids in length. In another embodiment, the spacer is < 3 amino acids long (as detailed below).

[128.] An example of an amino acid sequence of a scFv molecule of the subject invention follows:

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1      ATGAAATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCGGCCAGCCGGCC
      M K Y L L P T A A A G L L L L A A Q P A
61     ATGGCCGAGGTGCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTG
21     M A E V Q L V E S G G G V V R P G G S L
121    AGACTCTCCTGTGCAGCCTCTGGATTACCTTTGATGATTATGGCATGAGCTGGGTCCGC
41     R L S C A A S G F T F D D Y G M S W V R
181    CAAGCTCCAGGGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACA
61     Q A P G K G L E W V S G I N W N G G S T
241    GGTATGCAGACTCTGTGAGGGGCCGATTACCATCTCCAGAGACAACGCCAAGAACTCC
81     G Y A D S V K G R F T I S R D N A K N S
301    CTGTATCTGCAAATGAACAGTCTGAGAGCCGAGGACCGCCGGTGTATTACGTGGCAAGA
101    L Y L Q M N S L R A E D T A V Y Y C A R
361    ATGAGGGCTCCTGTGATTTGGGCCCAAGTAACCCTGGTCACCGTGTGAGAGTGGGAGGC
121    M R A P V I W G Q G T L V T V S R G G G
421    GGTTCAGGCGGAGGTGGCTCTGGCGGTGGCGGATCGTCTGAGCTGACACAGGACCCTGCT
141    G S G G G G S G G G G S S E L T Q D P A
481    GTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACATGCCAAGGAGACAGCCTCAGAAGC
161    V S V A L G Q T V R I T C Q G D S L R S
541    TATTATGCAAGCTGGTACCAGCAGAAGCAGGACCAGGCCCCCTTGTCTTGTGCATCATGGGT
181    Y Y A S W Y Q Q K P G Q A P V L V I Y G
601    AAAAACAACCGGCCCTCAGGGATCCAGACCGATTCTCTGGCTCCAGCTCAGGAAACACA
201    K N N R P S G I P D R F S G S S S G N T
661    GCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGATGAGGCTGACTATTACTGTAACCTC
221    A S L T I T G A Q A E D E A D Y Y C N S
721    CGGGACAGCAGTGGTAACCATGTGGTATTCGGCGGAGGGACCAAGCTGACCGTCCTAGGT
241    R D S S G N H V V F G G G T K L T V L G
781    GCGGCCGCAGAACAAAACTCATCTCAGAAGAGGATCTGAATGGGGCCGCATAG
261    A A A E Q K L I S E E D L N G A A *

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[129.] The leader sequence is underlined with a dashed line. The V_H region is encoded by the bolded amino acid sequence. This specific clone is derived from the germline V_H3-DP32; however, the germline of each clone is dependent on its particular origin (see below). The amino acid sequence enclosed in a box encodes for the V_H-CDR3 sequence, the hypervariable region among all clones derived from this library. The spacer region joining the V_H and the V_L regions is a flexible polypeptide, encoded by amino acids shown by italics. Finally the V_L region is presented. The

fused V_L fragment in all the clones is derived from a single unmutated V gene of germline IGLV3SI, and is here followed by the c-myc tag, underlined with a wavy line. The full amino acid sequence is identical to SEQ ID NO:25.

[130.] Repertoires of V_H fragments (from 49 germ lines) were first generated by PCR from rearranged V-genes of peripheral blood lymphocytes of non-immunized human (referred to as a "naive repertoire") by the supplier of the library. The origin (germline) of the V_H-sequence can be identified by a homology test (Blast search), using one of the following web sites:

[131.] The binding characteristics of an antibody can be optimized in one of several ways. One way of optimizing an antibody to obtain a higher binding affinity relative to the original lead-compound is based on replacing the amino acid residues in the lead-compound, to introduce higher variability, or to extend the sequence. For example, the entire original V_L region can be replaced with a V_L region from a different antibody subtype.

[132.] An additional way to optimize binding affinity is to construct a phagemid display mutagenesis library. In a phagemid display mutagenesis library, oligonucleotides are synthesized so that each amino acid of the core sequence within the V_H and the V_L CDR3 is independently substituted by any other amino acid, preferably in a conservative manner known in the art. The subject invention provides for a set of specific antibody scFv displayed on phage, wherein the displayed antibody fragments and the soluble antibody fragments that can be extracted from the phage virions have the same biological activity.

[133.] The phage display library used herein was constructed from peripheral blood lymphocytes of non-immunized human, and the Fv peptide was selected against previously uncharacterized and unpurified antigens on the surface of a target cell. As used herein, previously uncharacterized and unpurified antigens refer to ligands presented on the surface of cells that have not been identified, characterized, isolated or purified by biochemical or molecular means previous to the current work, and that

are observed or predicted in the present work by virtue of the selective and/or specific binding to isolated antibody fragments observed.

[134.] The scFv of the present invention displays enhanced binding to a target cell. The enhanced binding is directed to specific surface markers. Specific surface markers are molecules that are sequestered in the cellular membrane and are accessible to circulating recognition molecules. The presence of surface markers allowed for the development of the phage display technology via the biopanning technology described herein. In the present invention specific surface markers are employed to characterize and differentiate among various cell types, as well as to serve as the binding site for Fvs in their various forms. A variety of hematopoietic cell types can be differentiated according to their characteristic surface markers and, similarly, diseased or cancerous cells display surface markers that are unique to their type and stage.

[135.] Selection of the scFv clone can be accomplished by two different biopanning strategies:

1. selection directly, by using the diseased or cancer cell as the target cell, and
2. step-wise selection, by using a first e.g., normal cell in a second, e.g., activated, excited, modified, changed, or disturbed state, whereby a binding site of the first cell in the second state comprises an unknown ligand that is substantially exposed or displayed. By virtue of immuno-cross-reactivity, the resulting clone may bind, after subsequent biopanning or selection steps, selectively and/or specifically to a novel and unknown ligand on a second cell. Following further optional amplification and subsequent purification, targeting molecules may be constructed from the recognition sites of the purified recognition molecules selective and/or specific for an unknown ligand on a second cell.

[136.] In one embodiment of the invention, the first cell may be a normal cell, the first state a non-activated state and the second state an activated, excited, modified, changed or disturbed state. The second cell in the step-wise selection may be a human cell. In another embodiment of the invention, the second cell in the step-

wise selection is a diseased cell. In a more preferred embodiment, the second cell in the step-wise selection is a cancer cell such as, but not limited to, carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma. In a more preferred embodiment, the second cell is a leukemia cell. In a most preferred embodiment, the second cell is an AML cell.

[137.] A more preferred embodiment of the invention provides for a peptide or polypeptide wherein the selective and/or specific binding of the peptide or polypeptide to the ligand of the second cell is determined primarily by a first hypervariable region. In a yet more preferred embodiment, the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24.

[138.] In another embodiment of the present invention herein provides for the ligand of the second cell bound by the peptide or polypeptide of the invention. A further embodiment provides for any molecule that recognizes and binds the ligand bound by the peptide or polypeptide of the invention.

[139.] The enhanced binding to a cancer cell is most likely due to overexpression of the ligand and/or exposure of binding site in the cancer cell relative to expression in the normal cell. As used herein, the term overexpression of the ligand is defined as the expression of a gene or its product normally silent in the particular cell type and/or in a particular stage of the cell cycle, or of increased expression of a gene that is expressed at basal levels under normal, non-malignant conditions for that particular cell type.

[140.] In a more preferred embodiment of the invention, the target cell of the biopanning procedure is contained in a cell suspension. Hematopoietic cells are obtained in suspension, and biopanning may be carried out by mixing a phage library with a blood cell suspension, followed by washing with several buffers. Phage are extracted from the human cells, amplified, and the displayed antibody fragment sequence is determined.

[141.] In a yet more preferred embodiment of the invention, the blood cell suspension comprises leukemic cells. In a most preferred embodiment, the blood cell suspension comprises AML cells. In another embodiment of the subject invention, the target cell is derived from an isolated organ or part thereof.

[142.] In another embodiment of the subject invention, the target cell or the second cell is derived from a cell line. Cell lines can be cultured and manipulated such that they can aid in determination of the binding characteristics of the Fv clones. In addition, cell lines can be useful in the development of diagnostic kits.

[143.] In a preferred embodiment, the cell line is a hematopoietic cell line, such as but not limited to the following lines: Jurkat, Molt-4, HS-602, U937, TF-I, THP-1, KG-1, ML-2, and HUT-78 cell lines.

[144.] In a preferred embodiment of the invention the CDR3 region is built, inserted, coupled or fused into or onto any one of 84 cassettes (SEQ ID NOs:30-113). In a more preferred embodiment, the CDR3 region is built, inserted, coupled or fused into or onto any one of 49 cassettes (SEQ ID NOs:30-32, 35, 37-39, 41, 43, 45, 46, 48, 51, 54, 57, 59-68, 70, 71, 76-85, 87, 89-92, 94, 97, 99, 103, 106, 112, and 113). In a most preferred embodiment, the CDR3 region is built, inserted, coupled or fused to the C-terminus of cassette of SEQ ID NO:61, or any of the above sequences having at least 90% sequence similarity therewith.

[145.] In one embodiment, the amino acid sequence of the cassette is ostensibly fixed, whereas the replaced, inserted or attached sequence can be highly variable. The cassette can be comprised of several domains, each of which encompasses a function crucial to the final construct. The cassette of a particular embodiment of the present invention comprises, from the N-terminus, framework region 1 (FR1), CDRI, framework region 2 (FR2), CDR2, and framework region 3 (FR3).

[146.] In an embodiment of the invention, it is possible to replace distinct regions within the cassette. For example, the CDR2 and CDR1 hypervariable regions of the cassette may be replaced or modified by non-conservative or, preferably,

conservative amino acid substitutions. More specifically, the CDR2 and CDR1 regions of a cassette of consecutive amino acids selected from the group comprising of SEQ ID NOs:30-113 or a fragment thereof can be replaced by SEQ ID NOs:115 and 114, respectively. Even more specifically, the CDR2 and CDR1 regions of a cassette of consecutive amino acids selected from the group comprising of SEQ ID NOs:30-32, 35, 37-39, 41, 43, 45, 46, 48, 51, 54, 57, 59-68, 70, 71, 76-85, 87, 89-92, 94, 97, 99, 103, 106, 112, and 113 or fragment thereof can be replaced by SEQ DD NOs:115 and 114, respectively.

[147.] In a preferred embodiment of the invention, the peptide or polypeptide comprises a heavy and a light chain, and each chain comprises a first, second and third hypervariable region which are the CDR3, CDR2 and CDR1 regions, respectively. The binding selectivity and specificity are determined particularly by the CDR3 region of a chain, possibly by the CDR3 region of the light chain and, preferably, by the CDR3 region of the heavy chain, and secondarily by the CDR2 and CDR1 regions of the light chain and, preferably, of the heavy chain. The binding selectivity and specificity may also be secondarily influenced by the upstream or downstream regions flanking the first, second, and/or third hypervariable regions.

[148.] In a preferred embodiment, the CDR3 region of the peptide or polypeptide has an amino acid sequence selected from the group comprising SEQ ID NOs:8-24.

[149.] In a more preferred embodiment, the CDR3 region of the heavy chain has an amino acid sequence selected from the group comprising SEQ ID NOs:8-24, the CDR2 has an amino acid sequence identical to SEQ ID NO:115, and the CDR1 region has an amino acid sequence identical to SEQ ID NO: 114.

[150.] In a most preferred embodiment of the invention, the CDR3 region has an amino acid sequence identical to SEQ ID NO:8.

[151.] In addition to the heavy and light chain, the Fv comprises a flexible spacer of 0-20 amino acid residues. The spacer can be a branched chain or a straight

chain. Two possible sequences of the spacer are identical to SEQ ID NOs:123 and 124.

[152.] A preferred embodiment of the invention is a scFv with a CDR3 sequence identical to SEQ ID NO: 8 and a full scFv sequence identical to SEQ ID NO: 25.

[153.] Another preferred embodiment of the invention is a scFv with a CDR3 sequence identical to SEQ ID NO: 20 and a full scFv sequence identical to SEQ ID NO: 203.

[154.] In a most preferred embodiment of the invention the CDR3, CDR2 and CDR1 regions have the amino acid SEQ ID NOs:8, 115 and 114, respectively.

[155.] In an embodiment of the invention, the Fv peptide comprises a CDR1 and CDR2 region of the variable heavy chain, which itself comprises a cassette with an amino acid sequence selected from the group comprising SEQ ID NOs:30-113; a CDR3 region, preferably of the variable heavy chain, which has an amino acid sequence selected from the group comprising SEQ ID NO: 8-24; an upstream region flanking the CDR3 region which has the amino acid sequence of SEQ ID NO: 117; a downstream region flanking the CDR3 region which has the amino acid sequence of SEQ ID NO:116; a spacer of 0-20 amino acid residues of SEQ ID NO:123 or 124; a variable light chain region the sequence of which is SEQ ID NO:7.

[156.] Similarly, in another embodiment the upstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO:119, the downstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO:118, the upstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO:121 and the downstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO:120.

[157.] A preferred embodiment of the invention provides for a peptide or polypeptide wherein the second and third hypervariable regions are a CDR2 and a CDR1 hypervariable region, respectively and wherein the CDR3 amino acid sequence

is SEQ ID NO:8, wherein the CDR2 amino acid sequence is SEQ ID NO:115, wherein the CDR1 amino acid sequence is SEQ ID NO:114, wherein the upstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO:117, wherein the downstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO:116, wherein the upstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 119, wherein the downstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 118, wherein the upstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 121 and wherein the downstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO:120.

[158.] Another preferred embodiment of the invention provides for an Fv molecule that comprises a first chain having a first, a second and a third hypervariable region and a second chain having a first, a second and a third hypervariable region, wherein one of the hypervariable regions of the first chain has a sequence selected from the group consisting of SEQ ID NOs:8-24, and wherein one of the hypervariable regions of the second chain has a sequence selected from the group consisting of SEQ ID NOs:1 -6 and 125-202, and wherein the first, second and third hypervariable regions are a CDR3, CDR2 and CDR1 region, respectively and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags.

[159.] Another embodiment of the invention provides for a peptide or polypeptide (i) wherein the first chain and the second chain each comprises a first hypervariable region selected from the group consisting of SEQ ID NOs:8-24; or (ii) wherein the first hypervariable region of the first and second chains are identical and selected from the group consisting of SEQ ID NOs:8-24; or (iii) wherein the first hypervariable region of the first chain is selected from the group consisting of SEQ ID NOs:8-24, and the first hypervariable region of the second chain is selected from the group consisting of SEQ ID NOs:1-6 and 125-202; or (iv) wherein the first hypervariable region of the first chain is selected from the group consisting of SEQ ID NOs:1-6 and 125-202, and the first hypervariable region of the second chain is selected from the group consisting of SEQ ID NOs:8-24.

[160.] A further embodiment provides for the peptide or polypeptide of the invention wherein the second and third hypervariable regions of the first chain are SEQ ID NOs:114 and 115, respectively.

[161.] For all the amino acid sequences of ≤ 25 amino acid residues described and detailed herein (e.g., CDR regions, CDR flanking regions), it is to be understood and considered as a further embodiment of the invention that these amino acid sequences include within their scope one or two amino acid substitution(s) and that preferably the substitutions are conservative amino acid substitutions. For all the amino acid sequences of > 25 amino acid residues described and detailed herein, it is to be understood and considered as an embodiment of the invention that these amino acid sequences include within their scope an amino acid sequence with $\geq 90\%$ sequence similarity to the original sequence (Altschul *et al.*, *Nucleic Acids Res.*, 25, 3389-3402 (1997)). Similar or homologous amino acids are defined as non-identical amino acids which display similar properties, e.g., acidic, basic, aromatic, size, positively or negatively charged, polar, non-polar.

[162.] Percentage amino acid similarity or homology or sequence similarity is determined by comparing the amino acid sequences of two different peptides or polypeptides. The two sequences are aligned, usually by use of one of a variety of computer programs designed for the purpose, and amino acid residues at each position are compared. Amino acid identity or homology is then determined. An algorithm is then applied to determine the percentage amino acid similarity. It is generally preferable to compare amino acid sequences, due to the greatly increased sensitivity to detection of subtle relationships between the peptide, polypeptide or protein molecules. Protein comparison can take into account the presence of conservative amino acid substitutions, whereby a mismatch may yet yield a positive score if the non-identical amino acid has similar physical and/or chemical properties (Altschul *et al.*, *Nucleic Acids Res.*, 25, 3389-3402 (1997)).

[163.] In an embodiment of the invention the three hypervariable regions of each of the light and heavy chains can be interchanged between the two chains and among the three-hypervariable sites within and/or between chains.

[164.] One skilled in the art will realize that demonstration of specific and/or selective binding of the peptide or polypeptide of the invention necessitates the use of a suitable negative control. A suitable negative control may be a peptide or polypeptide, the amino acid sequence of which is almost identical to the peptide or polypeptide of the invention, with the only difference being in the hypervariable CDR3 region. Another suitable negative control may be a peptide or polypeptide that is the same size and/or general three-dimensional structure as the peptide or polypeptide of the invention but has a totally unrelated amino acid sequence. Another suitable negative control may be a peptide or polypeptide with completely different physical and chemical characteristics, when compared to the peptide or polypeptide of the invention. The negative controls used in the development of the present invention are designated N14, having a CDR3 sequence identical to SEQ ID NO:28, and C181, having a CDR3 sequence identical to SEQ ID NO:29. Other negative controls, however, may likewise be suitable.

[165.] Another embodiment provides for a nucleic acid molecule, preferably a DNA molecule, encoding the Fv peptide or polypeptide of the invention.

[166.] In a preferred embodiment of the invention, and in order to optimize the selective binding of the Fv, the CDR3 sequences that confers primary binding selectivity and/or specificity to the Fv may be moved to any other heavy chain germline. More particularly they may be moved to one of 84 possible heavy chain germlines. These 84 germlines (SEQ ID NOs:30-113) comprise (a) the germline in which the claimed phage clone was originally isolated, (b) 48 additional germlines available in the phage display library and (c) 35 alternative germlines claimed herein (Tomlinson *et al*, *J. Mol. Biol.*, 227(3):776-798 (1992)). The local linear, or 3-dimensional environment of the CDR3 region, in concert with the CDR3 region itself, may potentially play a role in guiding or encouraging the proper CDR3 binding. For example, peptides having any of the CDR3 sequences recited herein as SEQ ID NOs:8-24, 125 and derived from any of the 49 germline sequences (SEQ ID NOs:30-32, 35, 37-39, 41, 43, 45, 46, 48, 51, 54, 57, 59-68, 70, 71, 76-85, 87, 89-92, 94, 97, 99, 103, 106, 112, and 113) are also encompassed by the subject invention.

[167.] Germline DP-32 is the cassette for several clones of the present invention. The C-terminus of this germline has been replaced with a consensus sequence to aid in phage display library preparation. The seven carboxy-terminal amino acids of SEQ ED NO: 61 have been replaced by the seven amino acid sequence of SEQ ID NO: 122.

[168.] CDR3 regions of Fvs of the present invention may contain the core sequence ^{Arg}/_{Gly/Lys}**Phe Pro** which binds specifically to AML cells. Eight examples of such CDR3 regions are presented in Table 2. Although the motif coincides with the three N-terminal amino acid residues of the CDR3 region in each case, it may also be located elsewhere in the CDR3 region. Alternatively, the motif is a binding motif that is used to build or construct an anchor or a binding region of part of a larger binding or targeting or recognition molecule or is used alone as a target vehicle.

[169.] In a further embodiment of the present invention there is provided a binding motif comprising the amino acid sequence of R₁-X Phe Pro-R₂ wherein R₁ and R₂ each comprises 0- 15, preferably 1-9, amino acid residues and wherein X is either Arg, Gly or Lys. Most preferably, the CDR3 comprises the amino acid sequence of R₁-X Phe Pro-R₂, wherein R₁ and R₂ each comprises 0-15 amino acid residues, and wherein X is either Arg, Gly, or Lys.

[170.] In another preferred embodiment of the peptide or polypeptide of the subject invention, 1-1000 amino acids may be added either to the C-terminus or to the N-terminus of the peptide, while the peptide maintains its biological activity. In a preferred embodiment of the invention, 150-500 amino acids may be added either to the C-terminus or to the N-terminus of the peptide or polypeptide, while the peptide maintains its biological activity. In another preferred embodiment of the invention, 800-1000 amino acids may be added either to the C-terminus or the N-terminus of the peptide or polypeptide, while the peptide or polypeptide maintains its biological activity.

[171.] An example for extending the core amino-acid sequence is by building a full-sized immunoglobulin Ig, using a lead compound as the core of the Ig. The

full-sized Ig may, for example, belong to the immunoglobulin class that can induce the endogenous cytolytic activity via complement or activation of cellular cytolytic activity (e.g., IgG1, IgG2, or IgG3). The full-sized Ig may belong to the immunoglobulin class of strongly binding antibodies (e.g., IgG4). On binding, the full-sized Ig may act in one or more of many ways, e.g., by acting as a flag for the body's defense mechanism to initiate an immune response, by transducing intracellular cell signaling, or by causing damage to a target cell.

[172.] One preferred embodiment of the present invention provides for an Ig molecule expressed as a recombinant polypeptide and produced in a eukaryotic cell system. In a preferred embodiment of the invention, the Ig polypeptide is an IgG polypeptide and it is produced in a mammalian cell system. In a more preferred embodiment the mammalian cell system comprises the CMV promoter.

[173.] In a preferred embodiment of the invention, the IgG molecule comprises a CDR3, CDR2 and CDR1 hypervariable region, both in the light and in the heavy chains. In a more preferred embodiment of the invention, the Fv molecule comprises a CDR3, CDR2 and a CDR1 region having SEQ ID NOs:8, 115 and 114, respectively. The CDR3, CDR2 and CDR1 regions can be of the heavy chain or of the light chain.

[174.] A further preferred embodiment of the invention provides for an IgG molecule having a light chain with a sequence identical to SEQ ID NO: 27 and a heavy chain with a sequence identical to SEQ ID NO: 26, or a heavy chain and a light chain having at least 90% sequence similarity therewith. In a most preferred embodiment of the invention the two heavy chains of the IgG are identical and the two light chains of the IgG are identical.

[175.] In another embodiment, the peptide of the subject invention is constructed to fold into multivalent Fv forms.

[176.] The present invention provides for a Y1 or Y17 peptide or polypeptide comprising an scFv molecule. As used herein a scFv is defined as a molecule which is made up of a variable region of a heavy chain of a human antibody and a variable

region of a light chain of a human antibody, which may be the same or different, and in which the variable region of the heavy chain is connected, linked, fused or covalently attached to, or associated with, the variable region of the light chain.

[177.] A Y1 and Y17 scFv construct may be a multimer (e.g., dimer, trimer, tetramer, and the like) of scFv molecules that incorporate one or more of the hypervariable domains of the Y1 or Y17 antibody. All scFv derived constructs and fragments retain enhanced binding characteristics so as to bind selectively and/or specifically to a target cell in favor of other cells. The binding selectivity and/or specificity is primarily determined by hypervariable regions.

[178.] The hypervariable loops within the variable domains of the light and heavy chains are termed Complementary Determining Regions (CDR). There are CDR1, CDR2 and CDR3 regions in each of the heavy and light chains. The most variable of these regions is the CDR3 region of the heavy chain. The CDR3 region is understood to be the most exposed region of the Ig molecule, and as provided herein, is the site primarily responsible for the selective and/or specific binding characteristics observed.

[179.] The Y1 and Y17 peptide of the subject invention can be constructed to fold into multivalent Fv forms. Y1 and Y17 multimeric forms were constructed to improve binding affinity and specificity and increased half-life in blood.

[180.] Multivalent forms of scFv have been produced by others. One approach has been to link two scFvs with linkers. Another approach involves using disulfide bonds between two scFvs for the linkage. The simplest approach to production of dimeric or trimeric Fv was reported by Holliger et al., *PNAS*, 90, 6444-6448 (1993) and A. Kortt, et al., *Protein Eng.*, 10, 423-433 (1997). One such method was designed to make dimers of scFvs by adding a sequence of the FOS and JUN protein region to form a leucine zipper between them at the c-terminus of the scFv. Kostelny SA et al., *J Immunol.* 1992 Mar 1;148(5):1547-53; De Kruif J et al., *J Biol Chem.* 1996 Mar 29;271(13):7630-4. Another method was designed to make tetramers by adding a streptavidin coding sequence at the c-terminus of the scFv.

Streptavidin is composed of 4 subunits so when the scFv-streptavidin is folded, 4 subunits accommodate themselves to form a tetramer. Kipriyanov SM et al., *Hum Antibodies Hybridomas*, 1995;6(3):93-101. In yet another method, to make dimers, trimers and tetramers, a free cysteine is introduced in the protein of interest. A peptide-based cross linker with variable numbers (2 to 4) of maleimide groups was used to cross link the protein of interest to the free cysteines. Cochran JR et al., *Immunity*, 2000 Mar;12(3):241-50.

[181.] In this system, the phage library (as described herein above) was designed to display scFvs, which can fold into the monovalent form of the Fv region of an antibody. Further, and also discussed herein above, the construct is suitable for bacterial expression. The genetically engineered scFvs comprise heavy chain and light chain variable regions joined by a contiguously encoded 15 amino acid flexible peptide spacer. The preferred spacer is (Gly₄Ser)₃. The length of this spacer, along with its amino acid constituents provides for a nonbulky spacer, which allows the VH and the VL regions to fold into a functional Fv domain that provides effective binding to its target.

[182.] The present invention is directed to Y1 and Y17 multimers prepared by any known method in the art. A preferred method of forming multimers, and especially dimers, employs the use of cysteine residues to form disulfide bonds between two monomers. In this embodiment, dimers are formed by adding a cysteine on the carboxyl terminus of the scFvs (referred to as Y1-cys scFv or Y1 dimer) in order to facilitate dimer formation. After the DNA construct was made (See Example 2D and 6D) and used for transfection, Y1 dimers were expressed in a production vector and refolded *in vitro*. The protein was analyzed by SDS-PAGE, HPLC, and FACS. Two-liter fermentation batches of the antibodies were run. After expressing Y1-cys in *E. coli* strain BL21, refolding was done in arginine. Following refolding, the protein was dialyzed and purified by Q-sepharose and gel filtration (sephadex 75). Two peaks were detected by SDS-PAGE (non-reduced) and by gel filtration. The peaks were collected separately and analyzed by FACS. Monomer and dimer binding to Jurkat cells was checked by FACS. The binding by dimers required only 1/100 the

amount of the monomeric antibody for the same level of staining, indicating that the dimer has greater avidity. Conditions for dimer refolding were determined, and material comprising >90% dimers (mg quantities) was produced after subsequent dialysis, chromatographic, and gel filtration steps. The purified dimer was characterized by gel filtration and by SDS-PAGE analysis under oxidizing conditions. The dimer's binding capacity was confirmed by radioreceptor assay, ELISA, and FACS analyses.

[183.] CONY1 scF antibody fragment is derived from Y1 scFv. The DNA sequence encoding the myc tag of Y1 scFv were removed and replaced by synthetic oligonucleotide DNA sequence encoding the amino acids lysine, alanine lysine (KAK).

[184.] To compare the binding of the scFv monomer (also referred to as CONY1) with the Y1 dimer, binding competition experiments were done *in vitro* on KG-1 cells. In addition, these experiments also compared the binding of the full Y1 IgG to the scFv Y1 monomers. To perform this study, Y1 IgG was labeled with biotin. This study revealed that Y1 IgG competed with IgG Y1-Biotin. Non-relevant human IgG did not compete with the labeled Y1 IgG. Y1 scFvs (5 µg and 10 µg) partially competed with Y1 IgG-Biotin (50ng). The studies also showed that 1ng of IgGY1-FITC bound to KG-1 cells (without serum) to the same extent as 1µg of scFv-FITC, but in the presence of serum, most of Y1 IgG binding was "blocked." These studies also showed that the binding of the Y1 dimer is at least 20-fold higher than that of the scFv monomer as analyzed by radioreceptor assay, ELISA or FACS.

[185.] In yet another embodiment, a lysine-alanine-lysine was added in addition to the cysteine at the carboxyl end (referred to as YI-cys-kak scFv). The amino acid sequence of this scFv construct is reproduced below.

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1 MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
  APGKGLEWVS GINWNGGSTG 60
61 YADSVKGRFT ISRDNAKNSL YLQMNSLRAE DTAVYYCARM
  RAPVIWGQGT LTVSRGGGG 120
121 SGGGGSGGGG SSELTDPAV SVALGQTVRI TCQGDSLRSY
  YASWYQQKPG QAPVLVIYGK 180

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181 NNRPSGIPDR FSGSSSGNTA SLTITGAQAE DEADYYCNSR
DSSGNHVVFVG GGTKLTVLGG 240
241 GGCKAK

[186.] The Y1-cys-kak was produced in a λ -pL vector in bacteria. Expression in the λ -pL vector was induced by increasing the temperature to 42°C. Inclusion bodies were obtained from induced cultures and semi-purified by aqueous solutions, to remove unwanted soluble proteins. The inclusion bodies were solubilized in guanidine, reduced by DTT, and refolded *in vitro* in a solution based on arginine/ox-glutathione. After refolding, the protein was dialyzed and concentrated by tangential flow filtration to a buffer containing Urea/phosphate buffer. The protein was repurified and concentrated by ionic-chromatography in an SP-column.

[187.] In order to obtain higher levels of expression in *E. coli* of the CONY1 scFv, as well as in the Y1-cys-KaK scFv, we introduced at position 2 of the N-terminal sequence of the scFv construct the amino acid encoding for the alanine residue. A four-fold level of expression was obtained with this newly modified construct.

[188.] An ELISA assay was performed to ascertain the differences in binding between the monomer (CONY1 scFv-also known as Y1-kak) and the dimer YI-cys kak (the cysteine dimer) for antigen GPIb (glycocalicin) derived from platelets. A polyclonal anti single chain antibody and/or a novel polyclonal anti-V_L (derived from rabbits) and anti-rabbit HRP, were used to detect the binding to GPIb. The dimer was approximately 20-100 fold more active than the monomer. For instance, to reach 0.8 OD units 12.8mg/ml of monomer was used compared to only 0.1mg/ml of dimer. See Figure 12.

[189.] The dimer was characterized by SDS-page electrophoresis, gel filtration chromatography, ELISA, radioreceptor binding, and FACS. The apparent affinity of the dimer was higher than the monomer due to the avidity effect. This

effect was confirmed by ELISA to glycolalicin, FACS to KG-1 cells, and competition in a radioreceptor assay.

[190.] HPLC was performed to profile the dimer after refolding and purification from a Superdex 75 gel filtration column. In Figure 10, the Y1-cys-kak (dimer) is the first peak on the left (~10.8 minutes) and the subsequent peak is the monomer (~12 minutes). The dimer is approximately 52kDa and the monomer 26kDa, according to protein size markers run on the same column. The balance between the dimer and monomer can be changed by varying the conditions of the refolding (concentration of the oxidized agent and the concentration of the protein in the refolding buffer). The dimer and monomer were separated by chromatography in a superdex 75 column.

[191.] In Figure 11, a gel is shown with a mixed population of dimers and monomers. In the reduced form, the monomers are seen due to the reduction between the two monomers and in the non-reduced form, two population are seen (as in the gel filtration experiment) a monomer fraction of about 30kDa and a dimer of about 60kDa.

[192.] In addition, FACS binding analysis to KG-1 cells showed that the dimer is more sensitive than the monomer when a two or three-step binding assay was performed. Dimers directly labeled by FITC showed a slight advantage (use of 10x fold less material) than the monomer. The radio receptor assay on KG-1 cells, where the dimer was used as competitor, showed that the dimer is 30x fold more efficient than the monomer.

[193.] Varying the length of the spacers is yet another preferred method of forming dimers, trimers, and tetramers (often referred to in the art as diabodies, triabodies and tetrabodies, respectively). Dimers are formed under conditions where the spacer joining the two variable chains of a scFv is shortened to generally. This shortened spacer prevents the two variable chains from the same molecule from folding into a functional Fv domain. Instead, the domains are forced to pair with complimentary domains of another molecule to create two binding domains. In a

preferred method, a spacer of only 5 amino acids (Gly₄Ser) was used for diabody construction. This dimer can be formed from two identical scFvs, or from two different populations of scFvs and retain the selective and/or specific enhanced binding activity of the parent scFv(s), and/ or show increased binding strength or affinity.

[194.] In a similar fashion, triabodies are formed under conditions where the spacer joining the two variable chains of a scFv is shortened to generally less than 5 amino acid residues, preventing the two variable chains from the same molecule from folding into a functional Fv domain. Instead, three separate scFv molecules associate to form a trimer. In a preferred method, triabodies were obtained by removing this flexible spacer completely. The triabody can be formed from three identical scFvs, or from two or three different populations of scFvs and retain the selective and/or specific enhanced binding activity of the parent scFv(s), and/or show increased binding strength or affinity.

[195.] Tetrabodies are similarly formed under conditions where the spacer joining the two variable chains of a scFv is shortened to generally less than 5 amino acid residues, preventing the two variable chains from the same molecule from folding into a functional Fv domain. Instead, four separate scFv molecules associate to form a tetramer. The tetrabody can be formed from four identical scFvs, or from 1-4 individual units from different populations of scFvs and should retain the selective and/or specific enhanced binding activity of the parent scFv(s), and/or show increased binding strength or affinity.

[196.] Whether triabodies or tetrabodies form under conditions where the spacer is generally less than 5 amino acid residues long depends on the amino acid sequence of the particular scFv(s) in the mixture and the reaction conditions.

[197.] In a preferred method, tetramers are formed via a biotin/streptavidin association. A novel fermentation construct that is capable of being enzymatically labeled with biotin (referred to herein as Y1-biotag or Y1-B) was created. A sequence that is a substrate for the BirA enzyme was added at the Y1 C-terminus.

The BirA enzyme adds a biotin to the lysine residue within the sequence. Y1-biotag was cloned and expressed in *E. coli*. The inclusion body material was isolated and refolded. The purity of the folded protein was >95%, and >100 mg were obtained from a 1-L culture (small-scale, non-optimized conditions). The molecular weight of this form was found to be similar to that of the scFv according to HPLC, SDS-PAGE, and mass spectroscopy. Y1-biotag was found to be the most consistent reagent for FACS analysis. However, when Y1-biotag binding to KG-1 cells was examined in the presence of serum, high concentrations (10-fold more) are required for comparable binding in the absence of serum. Nevertheless, this construct offered the advantage of specific biotinylation in which the binding site of the molecule remains intact. Further, each molecule is labeled by only one biotin -- each molecule receives one biotin on the carboxyl end.

[198.] Limiting labeling to one biotin/molecule in a desired location enabled production of tetramers with streptavidin. The tetramers were formed by incubating Y1-B with streptavidin-PE.

[199.] FACS analysis indicated that the tetramers made by Y1-biotag and streptavidin-PE were 100 to 1000 fold more sensitive than Y1 scFv monomers in the absence of serum. Y1-biotag tetramers with streptavidin-PE appear to specifically bind to one of the Y1-reactive cell lines (KG-1). The differential of this reaction, from background binding, was very high, and offered high sensitivity to detect low amounts of receptor. FACS evaluation of normal whole blood with Y1-SAV tetramers indicated that no highly reactive population is present. Monocytes and granulocytes were positive to a small extent. In cell lines where a positive result was present, such as with KG-1 cells, the tetramers were at least 100-fold more reactive.

[200.] Then, the tetramers were incubated with the cell samples. A low dose of the Y1 tetramers (5 ng) binds well to the cell line (KG-1) providing a 10 to 20-fold higher response than previously observed with other Y1 antibody forms. A minor reaction was observed when a negative cell line was examined with varying doses of the tetramers.

[201.] An embodiment of the invention provides for a method for identifying a targeting molecule which binds to unknown immuno-cross-reactive binding sites on first and second cells comprising (a) one or more biopanning steps that are performed on a first target cell that, in a second state but not in a first state, substantially exposes or displays a binding site comprising an unknown ligand so as to produce a first population of recognition molecules; (b) subsequent biopanning and/or selection steps, commencing with the resultant stock of recognition molecules of step (a), that are performed on a second cell that displays a binding site comprising an unknown ligand having immuno-cross-reactivity to the unknown ligand of the first cell so as to produce a second population of recognition molecules; (c) amplification and purification of the second population of recognition molecules of step (b); and (d) construction from the recognition sites of the purified recognition molecules of step (c) peptides or polypeptides that comprise targeting molecules that are selective and/or specific for unknown ligands on the second cell.

[202.] A preferred embodiment provides for the first cell to be a normal cell, the first state to be a non-activated state and the second state to be an activated, excited, modified, changed or disturbed state. In a more preferred embodiment the second cell is a diseased cell. In a yet more preferred embodiment the diseased cell is a cancer cell. The cancer cell may be, but is not limited to carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma. In a yet more preferred embodiment, the cancer cell is a leukemia cell. In a most preferred embodiment the leukemia cell is an AML cell.

[203.] An embodiment of the present invention provides for use of the peptide or polypeptide optionally in association with or attached, coupled, combined, linked or fused to a pharmaceutical agent, in the manufacture of a medicament. In a preferred embodiment the medicament has activity against a diseased cell. In yet a more preferred embodiment, the activity is against a cancer cell. The cancer cell be but is not limited to carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma. In yet amore preferred embodiment the cancer

cell is a leukemia cell. In a most preferred embodiment the leukemia cell is an AML cell.

[204.] An embodiment of the invention provides for a pharmaceutical composition comprising mixtures of different monomeric scFvs, and/or mixtures of diabodies or triabodies or tetrabodies constructed from different scFvs.

[205.] A further embodiment provides for use of the peptide or polypeptide of the invention, in association with, or attached, coupled, combined, linked or fused to a pharmaceutical agent, in the manufacture of a medicament. The medicament can have activity against diseased cells, and more specifically against cancer cells. The cancer cells may be, but are not limited to, carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma. In a more preferred embodiment, the medicament is active against leukemia cells. In a most preferred embodiment, the medicament is active against AML cells. Activity of the medicament against the said cells may cause retardation of cancerous growth, complete prevention of any growth, or killing of the cancerous cells.

[206.] In an embodiment of the invention, the activity of the medicament or of the pharmaceutical composition is by inhibiting cell growth.

[207.] The peptide or polypeptide of the invention can be used for preparing a composition, preferably a pharmaceutical composition, for use in inhibiting the growth of a cancer cell, preferably a leukemia cell, and most preferably an AML cell. In an embodiment of the invention, the peptide or polypeptide can be used for preparing a composition for use in inhibition of growth of a cancer cell, said composition comprising at least one compound having a pharmaceutical ligand selective and/or specific for the cancer cell.

[208.] A peptide or polypeptide of the subject invention may be administered alone to a patient, or as comprising a medicament or a pharmaceutical composition, in association with, conjugated, linked, or fused to a pharmaceutically effective amount of a pharmaceutical agent, a pharmaceutically effective carrier and, optionally, an adjuvant. Such pharmaceutical compositions may include proteins, diluents,

preservatives and anti-oxidants (see Osol *et al.* (eds.), *Remington's Pharmaceutical Sciences* (16th ed), Mack Publishing Company, (1980)).

[209.] In another embodiment, the pharmaceutical agent is an antibody or fragment thereof that is linked to a peptide or polypeptide of the invention by a peptide bond.

[210.] In a preferred embodiment, the toxin is, for example, gelonin, Pseudomonas exotoxin (PE), PE40, PE38, diphtheria toxin, ricin, or modifications or derivatives thereof.

[211.] In a preferred embodiment, the radioisotopes used include gamma-emitters, positron-emitters, and x-ray emitters that may be used for localization and/or therapy, and beta-emitters and alpha-emitters that may be used for therapy.

[212.] In a specific embodiment of the subject invention, the therapeutic radioisotope is selected from a group comprising ¹¹¹indium, ¹¹³indium, ^{99m}rhenium, ¹⁰⁵rhenium, ¹⁰¹rhenium, ^{99m}technetium, ^{121m}tellurium, ^{122m}tellurium, ^{125m}tellurium, ¹⁶⁵thulium, ¹⁶⁷thulium, ¹⁶⁸thulium, ¹²³iodine, ¹²⁶iodine, ¹³¹iodine, ¹³³iodine, ^{81m}krypton, ³³xenon, ⁹⁰yttrium, ²¹³bismuth, ⁷⁷bromine, ¹⁸fluorine, ⁹⁵ruthenium, ⁹⁷ruthenium, ¹⁰³ruthenium, ¹⁰⁵ruthenium, ¹⁰⁷mercury, ²⁰³mercury, ⁶⁷gallium and ⁶⁸gallium and the like.

[213.] In another specific embodiment of the subject invention, the anti-cancer agent is selected from the group comprising doxorubicin, adriamycin, cis-platinum, taxol, calicheamicin, vincristine, cytarabine (Ara-C), cyclophosphamide, prednisone, daunorubicin, idarubicin, fludarabine, chlorambucil, interferon alpha, hydroxyurea, temozolomide, thalidomide and bleomycin, and derivatives thereof.

[214.] An embodiment of the invention provides for a method of inhibiting the growth of a cancer cell that comprises contacting the cancer cell with an amount of the peptide or polypeptide of the invention. In a preferred embodiment, the cancer cells may be but are not limited to carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma. In a more preferred

embodiment the cancer cell is a leukemia cell. In a most preferred embodiment the leukemia cell is an AML cell. An embodiment of the invention allows for *in vivo* and *ex vivo* treatment of the patient. A more specific embodiment of the invention allows for *ex vivo* purging of autologous bone marrow to remove abnormal stem cells.

[215.] In a more specific embodiment of the invention, the blood of a leukemia patient can be circulated *ex vivo* through a system comprising a peptide or polypeptide of the invention conjugated to an anti-cancer agent. After removal of bound cells and unbound anti-cancer agent, the blood cells can be reintroduced into the body of a patient. Alternatively, the blood of a leukemia patient can be circulated *ex vivo* through a system comprising a peptide or polypeptide of the invention attached to a solid phase. The cells that pass through the system and that do not bind to the peptide or polypeptide of the invention attached to a solid phase can be reintroduced into the body of a patient.

[216.] In another preferred embodiment of the invention, the peptide or polypeptide is utilized for *ex vivo* autologous bone marrow in suspension in order to remove abnormal stem cells prior to implantation. Purging of abnormal stem cells can be performed by running the suspension over a solid support (such as, but not limited to, magnetic beads and affinity columns) to which the peptide or polypeptide of the invention (i.e., the targeting molecule), constructs, fragments, fragments of constructs, or constructs of fragments thereof are bound. Bone marrow thus purged *ex vivo* can then be used for autologous bone marrow transplantation. This preferred embodiment is based on the identification in the present invention of a phagemid clone (Y1) that binds to stem cells released from bone marrow of leukemia patients, but does not bind to stem cells released from the bone marrow of healthy donors. Similarly, the Y1 phagemid clone binds to blast cells that are determined by FACS analysis to be abnormal, as well as to leukemic cells.

[217.] Blast cells are herein defined as primary cells that are precursors for all the circulating cells in the mammalian organism. Due to their progenitor characteristics, blast cells are not found circulating in significant quantities in the adult organism. The presence of circulating blast cells without exogenous stimulation

can be an indication of malignancy, e.g., of the hematopoietic system, and their subsequent disappearance may indicate remission of the malignant disease.

[218.] In another embodiment of the invention, the pharmaceutical composition is used for prophylaxis.

[219.] In a preferred embodiment, two or more peptides or polypeptides of the invention are combined to form a mixture.

[220.] As used herein, a mixture is defined as two or more molecules or particles of different species that are contained in a single preparation. The different species of molecules form neither covalent nor non-covalent chemical bonds.

[221.] In one embodiment of the subject invention, the peptide or polypeptide of the subject invention is linked, fused or conjugated to a pharmaceutical agent.

[222.] In another embodiment of the subject invention, the link between the peptide and the pharmaceutical agent is a direct link. As used herein, a direct link between two or more neighboring molecules is obtained via a chemical bond between elements or groups of elements in the molecules. The chemical bond can be for example, an ionic bond, a covalent bond, a hydrophobic bond, a hydrophilic bond, an electrostatic bond or a hydrogen bond. The bonds can be selected from, but not limited to, a group comprising amine, carboxy, amide, hydroxyl, peptide and disulfide. The direct link could preferably be a protease resistant bond.

[223.] In yet another embodiment, the link between the peptide and the pharmaceutical agent is affected by a linker compound. As used herein in the specification and in the claims, a linker compound is defined as a compound that joins two or more moieties together. The linker can be straight-chained or branched. The branched linker compound can be composed of a double-branch, triple branch, or quadruple or more branched compound. The linker compound may be, but is not limited to, a dicarboxylic acid, a maleimido hydrazide, PDPH, a carboxylic acid hydrazide, and a small peptide. Examples of other linker compounds include: Dicarboxylic acids such as succinic acid, glutaric acid, and adipic acid; Maleimido

hydrazides such as N-[ε-maleimidocaproic acid] hydrazide, 4-[N-maleimidomethyl]cyclohexan-1-carboxylhydrazide, and N-[κ-maleimidoundcanoic acid] hydrazide]; PDPH linker such as (3-[2-pyridyldithio]propionyl hydrazide) conjugated to sulfurhydryl reactive protein; Carboxylic acid hydrazides selected from 2-5 carbon atoms; and direct coupling using small peptide linkers between the free sugar of, for example, the anti-cancer drug doxorubicin and a scFv. Small peptides include, but are not limited to AU1, AU5, BTag, c-myc, FLAG, Glu-Glu, HA, His6, HSV, HTTPHH, IRS, KT3, Protein C, S•Tag[®], T7, V5, VSV-G, and KAK Tag.

[224.] Any known method of administration of a peptide or polypeptide of the subject invention may be used such as: intravenous, intramuscular, subcutaneous, topical, intratracheal, intrathecal, intraperitoneal, intralymphatic, nasal, sublingual, oral, rectal, vaginal, respiratory, buccal, intradermal, transdermal or intrapleural.

[225.] For intravenous administration, the formulation preferably will be prepared so that the amount administered to the patient will be an effective amount from about 0.1 mg to about 1000mg of the desired composition. More preferably, the amount administered will be in the range of about 1mg to about 500mg of the desired composition. The compositions of the invention are effective over a wide dosage range, and depend on factors such as the particulars of the disease to be treated, the half-life of the peptide or polypeptide-based pharmaceutical composition in the body of the patient, physical and chemical characteristics of the pharmaceutical agent and of the pharmaceutical composition, mode of administration of the pharmaceutical composition, particulars of the patient to be treated or diagnosed, as well as other parameters deemed important by the treating physician.

[226.] The pharmaceutical composition for oral administration can be in the form of tablet, liquid, emulsion, suspension, syrup, pill, caplet, or capsule. The pharmaceutical composition may also be administered in a device.

[227.] The pharmaceutical composition for topical administration can be in the form of cream, ointment, lotion, patch, solution, suspension, or gel.

[228.] In addition, the pharmaceutical composition can be prepared for solid, liquid, or sustained release formulation.

[229.] The compositions comprising the antibody fragments produced in accordance with the invention may comprise conventional pharmaceutically acceptable diluents or carriers. Tablets, pills, caplets and capsules may include conventional excipients such as lactose, starch and magnesium stearate. Suppositories may include excipients such as waxes and glycerol. Injectable solutions comprise sterile pyrogen-free media such as saline, and may include buffering agents, stabilizing agents or preservatives. Conventional enteric coatings may also be used.

[230.] The subject invention also encompasses a method of producing the antibody fragment by synthetic means known in the art.

[231.] An embodiment of the invention comprises a pharmaceutical composition comprising at least one peptide or polypeptide of the invention, attached, coupled, combined, linked or fused to an imaging agent for use in the diagnostic localization and/or imaging of a tumor.

[232.] A further embodiment of the invention provides for a diagnostic kit for *in vitro* analysis of treatment efficacy before, during, or after treatment, comprising an imaging agent comprising a peptide of the invention linked to an indicative marker molecule. The invention further provides for a method of using the imaging agent for diagnostic localization and/or imaging of a cancer, more specifically a tumor, comprising the following steps:

- a) contacting the cells with the composition,
- b) measuring the radioactivity bound to the cells, and
- c) visualizing the tumor.

[233.] In a preferred embodiment of the invention, the imaging agent of the kit is a fluorescent dye and the kit provides for analysis of treatment efficacy of cancers, more specifically blood-related cancers, e.g., leukemia, lymphoma and

myeloma. FACS analysis is used to determine the percentage of cells stained by the imaging agent and the intensity of staining at each stage of the disease, e.g., upon diagnosis, during treatment, during remission and during relapse.

[234.] The invention further provides a composition comprising an effective amount of an imaging agent, the peptide of the invention and a physiologically acceptable carrier.

[235.] In a preferred embodiment, the indicative marker molecule is any known marker known in the art, which includes, but is not limited to, a radioactive isotope, an element that is opaque to X-rays, a paramagnetic ion, or a fluorescent molecule, and the like.

[236.] In a specific embodiment of the subject invention, the indicative radioactive isotope may be, but is not limited to, ¹¹¹indium, ¹¹³indium, ^{99m}rhenium, ¹⁰⁵rhenium, ¹⁰¹rhenium, ^{99m}technetium, ^{121m}tellurium, ^{122m}tellurium, ^{125m}tellurium, ¹⁶⁵thulium, ¹⁶⁷thulium, ¹⁶⁸thulium, ¹²³iodine, ¹²⁶iodine, ¹³¹iodine, ¹³³iodine, ^{81m}krypton, ³³xenon, ⁹⁰yttrium, ²¹³bismuth, ⁷⁷bromine, ¹⁸fluorine, ⁹⁵ruthenium, ⁹⁷ruthenium, ¹⁰³ruthenium, ¹⁰⁵ruthenium, ¹⁰⁷mercury, ²⁰³mercury, ⁶⁷gallium and ⁶⁸gallium.

[237.] According to another preferred embodiment the indicative marker molecule is a fluorescent marker molecule. According to a more preferred embodiment the fluorescent marker molecule is fluorescein, phycoerythrin, or rhodamine, or modifications or conjugates thereof.

[238.] The subject invention also envisages a composition comprising an effective amount of an imaging agent of the invention, a pharmaceutical agent linked thereto and a physiologically acceptable carrier.

[239.] The invention also provides a method for imaging an organ or cells that involves contacting the organ or cells to be imaged with an imaging agent of the invention under conditions such that the imaging agent binds to the organ and cells, imaging the bound imaging agent and, thereby, imaging the organ or cells.

[240.] The subject invention further provides a method of treating an organ *in vivo* that involves contacting the organ to be treated with a composition of the invention under conditions such that the composition binds to the organ, thereby treating the organ.

[241.] In a preferred embodiment of the invention, the peptide or polypeptide may be utilized to target malignant cells, more particularly, leukemia cells in whole blood, by monitoring and imaging the cells, e.g., by FACS analysis. Specimens receiving higher scores (e.g., four-fold higher) for tumor cells relative to normal cells are subject for treatment.

[242.] The invention provides for treating a patient suffering from a cancer, comprising administering to the patient an amount of the peptide or polypeptide of the invention effective to treat the cancer. In a preferred embodiment the cancer is selected from the group comprising carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma. In a more preferred embodiment the cancer is a leukemia and in a most specific embodiment the leukemia is AML.

[243.] In a most preferred embodiment the peptide or polypeptide of the invention specifically or selectively binds to AML cells. The invention provides for a ligand presented on AML cells bound the peptide or polypeptide of the invention, and further provides for a peptide or polypeptide that binds said ligand.

[244.] The novel antibody fragments of the subject invention or their corresponding peptidomimetics are used in the manufacture of compositions or medicaments to treat various diseases and conditions.

[245.] The subject invention provides a method for production of a targeting agent comprising the following steps:

- a) isolating and selecting one or more targeting molecules comprising a primary recognition site by a biopanning procedure directly on a target cell or by a biopanning procedure indirectly on a first target cell in a second but not in a first state and

subsequently by a biopanning procedure directly on a second target cell to produce one or more said targeting molecules;

b) amplification, purification and identification of the one or more targeting molecules; and

c) construction of a targeting agent from the one or more targeting molecules or recognition sites thereof wherein the targeting agent can be a peptide, polypeptide, antibody or antibody fragment or a multimer thereof.

[246.] The targeting agent can additionally be constructed so as to be coupled, attached, combined, linked or fused to or in association with a pharmaceutical agent.

[247.] In a preferred embodiment of the invention the targeting agent is an anti-disease or anti-cancer agent.

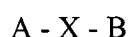
[248.] In another preferred embodiment of the invention the pharmaceutical agent is selected from the group comprising radioisotope, toxin, oligonucleotide, recombinant protein, antibody fragment, and anti-cancer agent. The radioisotope may be selected from a group comprising ¹¹¹indium, ¹¹³indium, ^{99m}rhenium, ¹⁰⁵rhenium, ¹⁰¹rhenium, ^{99m}technetium, ^{121m}tellurium, ^{122m}tellurium, ^{125m}tellurium, ¹⁶⁵thulium, ¹⁶⁷thulium, ¹⁶⁸thulium, ¹²³iodine, ¹²⁶iodine, ¹³¹iodine, ¹³³iodine, ^{81m}krypton, ³³xenon, ⁹⁰yttrium, ²¹³bismuth, ⁷⁷bromine, ¹⁸fluorine, ⁹⁵ruthenium, ⁹⁷ruthenium, ¹⁰³ruthenium, ¹⁰⁵ruthenium, ¹⁰⁷mercury, ²⁰³mercury, ⁶⁷gallium and ⁶⁸gallium.

[249.] In yet another embodiment the toxin may be selected from the group comprising gelonin, Pseudomonas exotoxin (PE), PE40, PE38, diphtheria toxin, ricin, or modifications or derivatives thereof.

[250.] In yet another embodiment of the invention the anti-cancer agent is selected from the group comprising doxorubicin, morpholino-doxorubicin (MDOX), adriamycin, cis-platinum, taxol, calicheamicin, vincristine, cytarabine (Ara-C), cyclophosphamide, prednisone, daunorubicin, idarubicin, fludarabine, chlorambucil, interferon alpha, hydroxyurea, temozolomide, thalidomide and bleomycin, and derivatives thereof.

[251.] The subject invention provides a method for the identification of antibody fragments by: (a) biopanning that involves incubating a phage display library with cells derived from blood; (b) washing to remove unbound phage; (c) eluting the bound phage from the blood cells; (d) amplifying the resulting bound phage; and (e) determining the displayed peptide sequence of the bound phage so as to identify the peptide.

[252.] The subject invention provides for a peptide or polypeptide having, the formula or structure:



wherein X is a hypervariable CDR3 region of 3 to 30 amino acids; and A and B can each be amino acid chains from 1 to 1000 amino acids in length, wherein A is the amino end and B is the carboxy end.

[253.] In a preferred embodiment of the invention A is 150-250 amino acid residues and B is 350-500 amino acid residues.

[254.] In another preferred embodiment the CDR3 region of the peptide is 5-13 amino acid residues.

[255.] In another preferred embodiment X in the formula above is an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24.

[256.] In another embodiment of the invention the peptide or polypeptide is part of a larger or full antibody or a multimer.

[257.] In yet another embodiment a dimeric molecule comprises two peptides or polypeptides, one of which is the peptide or polypeptide of the invention. The dimeric molecule may comprise two identical peptides or polypeptides of the invention.

[258.] In a preferred embodiment of the invention X is an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24 in said dimeric molecule.

[259.] Another embodiment provides for a nucleic acid molecule encoding the peptide or polypeptide or dimeric molecule of the invention.

[260.] The invention provides for the use of the peptide or polypeptide, optionally in association with or attached, coupled, combined, linked or fused to a pharmaceutical agent, in the manufacture of a medicament.

[261.] The invention further provides for use of the peptide or polypeptide in the manufacture of a medicament that has activity against a diseased cell, more specifically a cancer cell. The cancer cell may be selected from a group comprising carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma. More specifically, the cancer cell may be a leukemia cell and most specifically, the leukemia cell may be an AML cell.

[262.] An exchangeable system, as defined in the present invention and as discussed below in the examples, is a nucleic acid construct that has been designed to allow for exchange or replacement of a redefined variable region within said construct, without need for further manipulation or rebuilding of the molecule. Such a system allows for rapid and convenient preparation of the desired nucleic acid molecule.

EXAMPLES

[263.] The following examples are set forth to aid in understanding the invention but are not intended and should not be construed, to limit its scope in any way. Although specific reagents and reaction conditions are described, modifications can be made that are meant to be encompassed by the scope of the invention. The following examples, therefore, are provided to further illustrate the invention.

EXAMPLE 1:

[264.] **1.** Preparation of cells, bacterial strains, scFv phage display library, cellular membranes and protein purification for the biopanning procedure:

[265.] **1.1 Preparation of leukemia cells.** Blood samples were obtained from leukemia patients. Mononuclear cells (primary cells) were separated from other blood cells on a Ficoll cushion (Iso-prep, Robbins Scientific Corp., Sunnyvale, CA, USA). Centrifugation was performed at 110 x g for 25 minutes. Cells at the interface were collected and washed twice in PBS. Cells were then suspended in RPMI + 10% fetal calf serum (FCS) and enumerated. For long term storage, 10% FCS and 10% DMSO were added to the lymphocytes which were then frozen at -70°C.

[266.] **1.2 Preparation of fixed platelets.** Platelet concentrate obtained from a blood bank was incubated for 1 hour, at 37°C. An equal volume of 2.0% paraformaldehyde was added, and platelets were fixed for 18 hr, at 40°C. The platelets were washed twice with cold saline (centrifugation for 10 min, at 2500 x g), resuspended in 0.01% HEPES in saline, and counted using a microscope.

[267.] Platelet sensitivity to plasma von Willebrand factor and ristocetin was verified. Plasma von Willebrand factor (vWF; 18 µg/ml) and ristocetin (0.6 mg/ml) were added to fixed platelets, and platelet aggregation was induced and monitored by a chronolog lumi-aggregometer.

[268.] **1.3 Bacterial strains - TG-1 and HB2151:** Fresh bacterial cultures were prepared for infection by growing the cells to A₆₀₀ of 0.5-0.9 (exponentially growing cells). *E. coli* TG-1 cells were used for phage propagation and *E. coli* HB2151 cells were used for scFv protein production.

[269.] **1.4 scFv display phage library source.** The scFv library (Nissim *et al.*, *EMBO J.*, 13, 692-698 (1994)) was provided by Dr. A. Nissim with the agreement of the MRC. The library was originally constructed as a phagemid library displaying scFv fragments in which the V_H and the V_L domains were linked by a flexible polypeptide. The scFvs displayed in the phagemid library were fused to the

N-terminus of the minor coat protein pIII of the phage, which was then subcloned into the pHEN1 vector (Nissim *et al.*, *EMBO J.*, 13, 692-698 (1994)). Repertoires of antibody fragments were first generated by PCR from rearranged V-genes of peripheral blood lymphocytes of unimmunized human (referred to as "naive repertoires"). To diversify the repertoire, random nucleotide sequences encoding heavy chain CDR3 lengths of 4-12 residues were introduced into a bank of 49 cloned human V_H gene segments. The fused V_L fragment in all the clones it derived from a single unmutated V gene of germline IGLV3S1, creating a single pot library of approximately 10⁸ clones.

[270.] **1.5** Membrane preparation from AML cells. To the pellet containing 10⁸ washed cells, 1 ml cold lysis solution (0.3M sucrose, 5mM EDTA, 1mM PMSF) was added, then spun for 20 minutes at 11,000 x g at 4°C. The supernatant fluid was discarded, and the pellet was resuspended in TE (10mM Tris, 1mM EDTA, 1mM PMSF) and spun as above. The final pellet was resuspended in 6 ml PBS at an A₂₈₀ of 0.4 and was used to coat 3 Maxisorb immuno-tubes (NUNC), at 37°C, for 2 hr. Following coating, tubes were rinsed 3 times with PBS, then blocked with MPBS (2% skim milk in PBS), at RT, for 2 hours. Before biopanning, the tubes were rinsed an additional three times with PBS.

EXAMPLE 2:

[271.] **2.** Manipulation of phagemid particles re: biopanning procedure

[272.] **2.1** Phagemid selection and amplification: Phagemids that expressed epitopes of specific interest were selected from the library by a four-step biopanning procedure:

- a) Binding of the phagemid particles to a target, more particularly binding of the phagemid particles to washed target cells or cell membranes
- b) Removal of the non-bound phagemid particles, more particularly removal by extensive washing
- c) Elution of bound phagemid particles

- d) Propagation and amplification of the eluted phagemid particles, more particularly propagating and amplifying in *E. coli*

[273.] **2.2 Clone identification:** The four-step biopanning procedure was generally repeated 3-5 times. Selected phagemid clones were individually propagated, and further characterized by:

- a) DNA sequencing
- b) Comparison *ex-vivo* of phage binding to several cell types
- c) Infection of *E. coli* HB2151 to produce soluble scFv

[274.] **2.3 Sequence analysis:** The encoded scFv DNA of ~800bp within the phagemid particles was amplified by PCR using an upstream primer #203743 (5'-GAAATACCTATTGCCTACGG) and a downstream primer #181390 (5'-TGAATTTTCTGTATGAGG). DNA fragments were fully sequenced from both ends by the automatic ABI PRISM DNA sequencer (310 Genetic Analyzer, Perkin Elmer) using ABI PRISM Big Dye termination cycle sequencing kit and the above primers. Two additional primers, primer #191181 (5%-CGATCCGCCACCGCCAGAG) and its complementary primer # 191344 (5'-CTCTGGCGGTGGCGGATCG), which are located at the flexible polypeptide junction region between the heavy and light chains, were used for sequencing.

EXAMPLE 3:

[275.] **3. Biopanning protocols**

[276.] **3.1 Basic biopanning protocols:** The biopanning procedure is an integral part of the phage display technology described above. Three biopanning protocols were developed and used in the present work:

- a) **Protocol AM** (AML cell membrane panning/bacterial elution, followed by whole AML cell panning/trypsin elution)
- b) **Protocol YPR** (fixed human platelet panning/acid elution)

c) **Protocol YPNR** (fixed human platelet panning/acid elution)

[277.] These protocols are described in detail below:

[278.] **3.1.1 Protocol AM**

[279.] **3.1.1.1 Prewashing:** One ml aliquots containing 2×10^7 frozen AML cells from patients, stored at -70°C , were quick-thawed at 37°C and immediately diluted into 10 ml cold 2% PBS-Milk (MPBS). Cells were spun 5' at $120 \times g$ at room temperature (RT), washed twice, resuspended in MPBS and counted with a hemocytometer. Cell membranes were prepared as described in Section 1.5.

[280.] **3.1.1.2 Selection** was carried out on immobilized AML cell membranes by the addition of 2ml MPBS containing 10^{12} phagemids from the original Nissim library. The tube was slowly agitated for 30 minutes, then incubated for an extra 90 minutes without agitation, both steps at RT. Following three rounds of panning on AML cell membranes, one round of panning was carried out on whole AML cells.

[281.] **3.1.1.3 Wash:** To remove excess unbound phagemids, the tube contents were decanted and the tube was washed 10 times with PBS, 0.1 % Tween, followed by 10 washes with PBS only.

[282.] **3.1.1.4 Elution:** Exponentially growing *E. coli* TG-1 cells (2 ml) were added directly to the tube and incubated with slow agitation at 37°C for 30 minutes. As above, an aliquot was plated for titration and the remaining volume was plated for amplification.

[283.] **3.1.1.5 Amplification:** Colonies from the large plates were scraped and pooled. An aliquot ($\sim 10^7$) of ampicillin resistant *E. coli* TG-1 cells was grown in liquid culture to A_{600} of ~ 0.5 , then infected with helper phage (VSC-M13, Stratagene) to produce a large amplified phagemid stock. Phagemids were rescued by a PEG precipitation procedure (18a). The amplified T1 6MI stock above ($\sim 10^{11}$ phagemids/ml) was used for subsequent rounds of panning. The selection procedure was repeated for two additional rounds, using 10^{11} phagemids of the previously

amplified stock. The amplified stock of the third panning procedure on immobilized membranes was designated T16M3.

[284.] **3.1.1.6 Re-panning on whole cells:** The amplified stock of the third membrane panning, T16M3, was used to pan intact AML cells. Selection was carried out in a final volume of 0.5 ml MPBS containing 2×10^7 cells and 10^{10} Colony Forming Units (CFU) of phagemids (Nissim library), and 10^{13} wild-type bacteriophage M13, with slow agitation for 2 hours at 4°C. Bound phagemids were eluted from the washed cell pellet with 50 μ l of Trypsin:EDTA (0.25%:0.05%), then neutralized by the addition of 50 μ l of FCS. For titration and amplification, 1 ml of an *E. coli* TG-I culture ($A_{600} = 0.5$) was used. The amplified, and final, stock was designated T16M3.1.

[285.] **3.1.2 Protocol YPR**

[286.] **3.1.2.1 Selection:** Clone selection was accomplished by panning 10^8 fixed human platelets with 10^{11} phagemids (Nissim library) in 1 ml PBS/HEPES/1%BSA buffer. Binding was allowed to proceed for one hour at RT while mixing the sample by rotation.

[287.] **3.1.2.2 Cell wash:** Platelets were washed five times by low speed centrifugation (3500 x g) and resuspended as above.

[288.] **3.1.2.3 Elution:** First round bound phagemids were eluted from fixed platelets by the acid elution technique:

[289.] The platelets were incubated for 10 minutes at RT with 200 μ l 0.1 M glycine (pH 2.2). After neutralization with 0.5 M Tris-HCl, pH 8.0 and centrifugation, the remaining platelet-bound phage were eluted by addition of 200 μ l trypsin-EDTA (0.25%/0.05) and neutralization by the addition of 50 μ l FCS. The cells were removed by centrifugation, and the supernatant fluids containing eluted phage, from both acid and trypsin elution protocols, were collected and designated YPR(a)-I and YPR(t)-I stocks, respectively. These stocks were then amplified by adding 1 ml of exponentially growing TG-1 cells for 30 min., at 37°C. An aliquot

was plated for titration, and the remaining infected *E. coli* cells were plated on 2 x TV/AMP 15 cm plates. Plates were incubated overnight at 30°C. The output after each round of panning was determined by counting the colonies on a titration plate.

[290.] **3.1.2.4 Amplification:** The clones were amplified as described in section 3.1.1.5. The amplified stocks of $\sim 10^{12}$ phagemid/ml from the acid and trypsin elution protocols, designated R1(a) and R1(t) stocks, respectively, were combined and used for subsequent rounds of panning.

[291.] **3.1.2.5 Second and third rounds of panning** were carried out as described for the first panning round of the YPR procedure with the following modifications: (i) For the second panning 10^{12} of R1(a), combined with 10^{12} of R1(t), were used and (ii) elution was carried out with glycine (pH 2.2) only. The amplified eluate of the second round was designated R2. (iii) For the third round of biopanning, 10^{12} of R2 was used, and elution was carried out as in the second round. The amplified stock of round three was designated R3.

[292.] **3.1.3 YPNR protocol**

[293.] **3.1.3.1 Biopanning and washing** were carried out essentially as described in the YPR protocol. However, in this protocol, (i) elution was carried out after each of three rounds of panning with glycine (pH 2.2), and (ii) the first panning and amplification were followed by two subsequent rounds of panning without amplification. The first, second, and third rounds were designated YPNR1, YPNR2, and YPNR3, respectively.

[294.] **3.2 Selection of negative control scFv clones**

[295.] **3.2.1 N14 CDR3 sequence:** For all binding experiments, a single clone was picked from the naive library (before selection). A phage stock and a soluble scFv, designated N14, were prepared from this clone. Sequence analysis indicates that it belongs to the V_H4 -DP65 gene family. The sequence of the 11-mer V_H -CDR3 encoded by this clone, designated N14 CDR3, is as follows (SEQ ID NO:28):

Phe Leu Thr Tyr Asn Ser Tyr Glu Val Pro Thr

[296.] **3.2.2 C181 CDR3 sequence:** An additional negative clone, C181, was used in the binding analysis experiments. Clone C181 (reactive to recombinant hepatitis B virus [HBV] particles) belongs to the V_H3-DP35 family, and the sequence of the 9-mer V_H-CDR3 encoded by this clone, designated C181 CDR3, is as follows (SEQ ID NO:29):

Thr Asn Trp Tyr Leu Arg Pro Leu Asn

EXAMPLE 4:

[297.] **4. Production, purification, labeling and characterization of scFv clones**

[298.] **4.1 Production of soluble scFv:** pHEN1, a vector used to construct the original phagemid library, was designed with an amber stop codon encoded at the junction of the scFv gene and the pII1 gene. Therefore, when the vectors of selected clones are introduced by phagemid infection into *E. coli* HB215 1, which is a non-suppressor strain, this system enables production and secretion of soluble scFv into the bacterial periplasm (Harrison *et al.*, *Methods in Enzymology*, 267, 83-109 (1996)). The scFv is then readily retrievable from the culture broth. Soluble scFvs are produced under the control of the *lacZ* promoter (Gilbert and Muller-Hill, *PNAS (US)*, 58, 2415 (1967)), which is induced with IPTG.

[299.] A sequence encoding c-myc tag (10 amino acids - **Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu**; SEQ ID NO: 123.) is contained in the vector upstream to the amber mutation. The C-terminus of the expressed scFv should carry the c-myc tag, which can be detected using mouse anti-myc tag antibodies (derived from the European Collection of Cell Culture (ECACC) 9E10-hybridoma).

[300.] **4.2 Purification of scFv on Protein-A beads affinity column:** The scFvs of selected clones and of the control clone C181 all belong to the V_H3 family, allowing purification on a Protein -A affinity column. Periplasmic fractions (100-250 ml) from induced cultures of each clone were prepared and incubated with

Protein-A Sepharose beads. The bound scFvs were recovered from the column by acid elution (0.1 M glycine, pH 3.0), followed by eluate neutralization with Tris, pH.8.0. The concentration of the recovered protein was determined by A₂₈₀ measurement, followed by PBS buffer exchange by dialysis or on a G-25 Sepharose column.

[301.] **4.3 Purification of N14-scFv on a Sephacryl S-200 column:** The scFv of the negative clone N14 belongs to the V_H4 gene family and cannot, therefore, be purified on a Protein-A affinity column. For scFv-N14 purification, total protein in the periplasmic fraction of a 200ml induced culture was precipitated by 60% ammonium sulfate. The pellet was resuspended in 2ml 0.1xPBS, 5mM EDTA, 5mM PMSF and loaded on a Sephacryl S-200 column (1.5 x 90cm) pre-equilibrated with the running buffer (0.1 xPBS, 5mM EDTA). Proteins were fractionated, and fractions containing the N14-scFv (as detected by SDS-PAGE and Western analysis) were pooled, lyophilized, and suspended in 1/10 volume H₂O. The N14-scFv (unlabeled and FITC-labeled) was then used as a negative control in FACS analysis experiments.

[302.] **4.4. Labeling of purified scFvs with FITC:** Approximately one milligram of purified scFv from each preparation was resuspended in PBS and coupled to FITC using a Fluoro-Tag FITC conjugation commercial kit (Sigma cat. #FITC-1), according to the manufacturer's instructions.

[303.] **4.5 Quality Analysis of the purified and labeled scFv**

[304.] **4.5.1 Following purification and FITC labeling,** the profile of each preparation (labeled and unlabeled) was analyzed by SDS-PAGE, Western blotting, HPLC using a Superdex-75 column (A₂₈₀ and A₄₉₅) and fluorometry. The analysis indicated 80% purity of the N 14 scFv, and 90% purity for the V_H3 clones, with approximately 2 molecules of FITC conjugated to each scFv molecule (F/P ratio of 2:1).

[305.] **4.5.2 Binding activity following FITC labeling** was assessed to verify retention of scFv specificity (see Example 5).

[306.] **4.6 Biochemical characterization of phagemid clones:** Several types of analysis were used to evaluate the structure and assess the purity of the various scFv preparations (see Example 8) including SDS-PAGE, mass spectroscopy (for Y1 and Y17 scFvs only), and HPLC. Western analysis and EIA were used for identifying the scFv; and FACS was used to characterize scFv binding.

EXAMPLE 5:

[307.] **5. Binding assays**

[308.] The binding of the selected clones to cells was evaluated at two levels, the phagemid level and the soluble scFv level.

[309.] **5.1 Binding at the phagemid level**

[310.] To this end, a phagemid stock was prepared individually from each of the selected clones.

[311.] **5.1.1 Colony test:** In one set of experiments, a mixture of 10^9 specific phagemids, derived from the biopanning protocol, which render infected *E. coli* ampicillin resistant, and 10^{11} wild-type M13 phage, which do not carry ampicillin resistance and serve as a "blocker", was incubated with 10^5 cells, chosen from a panel of cell types. Following incubation and washing, the bound phage were eluted with trypsin, and an aliquot was used to infect *E. coli* TG-I. The *E. coli* were then plated on 2xTY/AMP plates and incubated overnight at 30°C. The number of colonies obtained for each clone was calculated and compared. The results give a measure of the binding affinity and specificity of the phagemids.

[312.] **5.1.2 White/Blue colony test:** In this test, in which each experiment includes an internal control, the specific phagemid was mixed at the same ratio as in Section 5.1.1 above, i.e., 1/100, with another control phagemid designated pGEM7 (Promega Corp., Madison, Wisconsin, USA). This pGEM7 phagemid carries resistance to ampicillin; however, it does not express any recombinant polypeptide at the N-terminus of its pIII gene. Following TG-I infection and incubation on ampicillin plates containing 1mM X-gal, colonies were enumerated. The colonies

obtained containing pGEM7 are blue, whereas the colonies obtained from the specific phagemids are white. The enrichment factor, derived from the ratio of input/output of the white/blue colonies (grown on the same plate) for each test tube, was then calculated.

[313.] **5.1.3 EIA of phagemids**

[314.] **5.1.3.1 Phagemid binding to selected cells:** Approximately 5×10^5 of the selected cells were fixed with acetone:methanol (1:1) on the surface of 24 well plates. The binding test required 10^9 phagemids. Binding was carried out at 37°C for 1hr, followed by an extensive wash with PBS/Tween (0.05%). After extensive washing with PBS, the plates were incubated with rabbit anti-M13, anti-rabbit IgG-HRP and substrate. The intensity of the color produced was read by an ELISA plate reader, at A_{405} , and was proportional to the level of bound phagemids.

[315.] **5.1.3.2 Phagemid binding to fixed platelets:** Polystyrene microtiter plates were coated with 10^8 fixed platelets and were incubated overnight, at 4°C . Approximately 10^{10} phagemids were used for evaluating binding. Washing and incubation of plates and determination of binding level were carried out as described in 5.1.3.1 above.

[316.] **5.1.4 Binding assays to specific proteins, selected from the group** consisting of human growth hormone (hGH), fibrinogen, fibronectin, BSA, SM (skim milk) and glyocalicin (proteolytic fragment of GPIb), were performed. Binding was assayed in the following manner. Polystyrene microtiter plate wells were coated with one of the proteins to be tested, at $2 \mu\text{g}/\text{well}$. Coating was allowed to proceed during overnight incubation, at 4°C . Approximately 10^{10} phagemids were added to test binding. After extensive washing with PBS, the plates were incubated with rabbit anti-M13, anti-rabbit BRP, and substrate. The level of binding was measured by the intensity of color produced. The optical density was measured at A_{405} . Each sample was assayed in duplicate, and the average was calculated.

[317.] **5.2 Binding Tests at the scFv level:** Binding of the scFvs produced in the periplasm of HB2151 was compared in several cell types by two different assays, by EIA and by FACS analysis.

[318.] **5.2.1 EIA of soluble scFv:** Approximately 5×10^5 AML cells were incubated with 5-10 μ g total protein. Binding was carried out at 4°C for 1hr, followed by EIA, using mouse anti-myc antibodies, anti-mouse HRP, and a substrate. Excess unbound antibodies were removed after each step by washing cells three times with PBS. The intensity of the color produced is read by an ELISA plate reader (O.D.₄₀₅). As above, the color intensity is proportional to the level of binding.

[319.] **5.2.2 FACS analysis of cells**

[320.] **5.2.2.1 Analysis of cells stained by the "three-step staining"**
procedure: FACS analysis was performed to test and confirm the specificity of the selected clones. Initially, a "three step staining" procedure was established, using crude extracts or purified unlabeled scFv, followed by mouse anti-myc antibodies and, finally, FITC- or PE-conjugated anti-mouse antibodies.

[321.] FACS analysis requires $5-8 \times 10^5$ cells, which have been Ficoll-purified and resuspended in PBS+1% BSA. Binding was carried out for 1hr at 4°C. After each step, cells were washed and resuspended in PBS+1% BSA. After the final staining step, cells were fixed by resuspending in PBS, 1% BSA, 2% formaldehyde, then read by FACS (Becton-Dickinson).

[322.] **5.2.2.2 Staining of cells with FITC-labeled scFv, in a single staining step:** FITC-labeled scFv was incubated with $5-8 \times 10^5$ Ficoll-purified cells in PBS+1% BSA. Binding was carried out for 1hr at 4°C. Cells were then washed and fixed as in section 5.2.2.1 above, and read by FACS.

EXAMPLE 6: Panning and Sequencing Results**[323.] 6.1 Results of AM Protocol**

[324.] 6.1.1 Panning Results for AM Protocol: The estimated number of phagemids used for panning (input), and the estimated number of bound phagemids eluted in the AM protocol (output) are summarized in the following table (Table 1):

Table 1. Panning results derived from protocol AM

Input stock	Cell source	Elution	Output	Amplified stock
Nissim library- 2×10^{11}	Membranes of AML	Bacterial TG-1	3×10^4	T16M1
T16M1 - 10^{11}	Membranes of AML	Bacterial TG- I	6.4×10^5	T16M2
T16M2- 10^{11}	Membranes of AML	Bacterial TG-1	10^6	T16M3
T16M3 - 10^{10}	AML cells	Trypsin	2×10^6	T16M3.1

[325.] Note the enrichment in the yield (output) obtained with each successive panning. In addition, there is no drop in the output when T16M3 was used to pan AML whole cells, suggesting that the bound phagemids are possibly specific for components on the external cell surface or that this specific system may contain a relatively high number of non-specific bound phagemids.

[326.] 6.1.2 Clone Sequence Results for AM Protocol: Although clones were picked and sequenced from T16M1, T16M2 and T16M3 output stocks, the results presented below are mainly of those clones that were derived from the **T16M3.1** output stock (AML intact cell panning). Clones AM10, AM11 and AM12 were identified in the T16M3 stock, but not in the subsequent output.

[327.] The amino acid sequences displayed in the V_H -CDR3 and their frequency in the tested clone output are summarized in Table 2.

Table 2. Selected clones following AM biopanning protocol, from the T16M3 and T16M3.1 outputs.

Clone #	V _H -CDR3 size	V _H -CDR3 sequence						Germline	Frequency in T16M3 output	Frequency in T16M3.1 output
AM1	8	Pro	Trp Pro 1 5	Asp Pro 2 6	Asp 3 7	Val 4 8	Thr	V _H 3- DP47	5/31	8/51
AM2	12		Gly Thr Glu 1 6 11	Phe Pro Ile 2 7 12	Pro Pro 3 8	Arg Ser 4 9	Ile Ala 5 10	V _H 3- DP46	11/31	20/51
AM3	5		Gly 1	Phe 2	Pro 3	Met 4	Pro 5	V _H 3- DP46	1/31	2/51
AM6	10		Gly Ser Arg 1 6	Phe Ser 2 7	Pro Val 3 8	His Ser 4 9	Ser 5 10	V _H 3- DP46	4/31	6/51
AM7	11		Arg Arg Asn 1 6 11	Phe His Tyr 2 7	Pro Glu 3 8	Met Lys 4 9	Thr 5 10	V _H 3- DP46	3/31	4/51
AMS	8		Arg Ala 1 6	Phe Thr 2 7	Pro Ile 3 8	Pro 4 5	Thr	V _H 3- DP46	6/31	8/51
AM9	7		Thr Asp 1 6	Gln Leu 2 7	Arg Gly 3	Arg 4 5		V _H 3- DP87	0/31	2/51
AM10	11		Lys Gly Gly 1 6 11	Phe Thr Leu 2 7	Pro Val Lys 3 8	Gly Arg 4 9	5 10	V _H 3- DP46	0/31	1/31
AM11	12		Gly Val Gln 1 6 11	Phe Glu Ser 2 7 12	Pro Gln Thr 3 8	Val Arg 4 9	Ile 5 10	V _H 3- DP49	0/31	1/31
AM12	10		Arg Arg Arg 1 6	Phe Val Val 2 7	Pro Asp 3 8	Gln Asn 4 9	5 10	V _H 3- DP46	0/31	1/31

[328.] The amino acid sequence of ^{Arg}/_{Gly}**PhePro** is present in seven of the ten isolated clones presented in Table 2, and represents a motif therein. In addition, note that the identified motif represents the N-terminal three amino acids of the CDR3 region in each case. Accordingly, this motif may be an effective anchor or binding

site on its own or in combination with other amino acid residues extending beyond either one or both ends of the CDR3 region or as part of a larger peptide or polypeptide or Fv molecule.

[329.] Other CDR3 regions with high affinity for binding to AML cells may be constructed based on the core sequence ^{Arg}/_{Gly}**PhePro**. They may be constructed by varying any of the above 5-12-mers by additions, deletions or mutations, while maintaining the ^{Arg}/_{Gly}**PhePro** core sequence.

[330.] CDR3 regions of the invention have the amino acid sequence **R1-^{Arg}/_{Gly}PhePro-R2**, where **R1** comprises 0-15 amino acids, preferably 0-9, most preferably 0-1 amino acid and **R2** comprises an amino acid sequence from 1-15 amino acids, most preferably 1-9 amino acids. **R1** and **R2** are amino acid sequences that do not adversely affect the specific binding of the ^{Arg}/_{Gly}**PhePro** sequence to AML cells.

[331.] The CDR3 region of the light chain of the above clones is identical and is recited in SEQ ID NO: 125.

[332.] 6.2 Results of YPR and YPNR Protocols

[333.] 6.2.1 Panning Results for YPR and YPNR Protocols: The estimated number of phagmids used for panning (input) and the estimated number of bound phagemids eluted (output) are summarized in the following tables (Tables 3, 4).

Table 3. Panning results derived from the YPR protocol.

Input stock	Elution	Output	Amplified stock
Nissim librarY10 ¹¹	Acid Trypsin	10 ⁷ 4x10 ⁷	R1(a) R1(t)
Pooled [R1(a) -10 ¹² , + R1(t)-10 ¹²)	Acid	5x10 ⁵	R2
R2 -10 ¹²	Acid	3x10 ⁸	R3

[334.] Table 3 demonstrates that trypsin elution yields a 4-fold greater output as compared to acid elution in the first round.

[335.] Re-panning according to the YPNR protocol without the amplification step minimized the possibility of preferentially amplifying phagemid infection or bacterial infection. The resulting output is depicted in Table 4.

Table 4. Panning results derived from the YPNR protocol.

Input stock	Elution	Output	Elution stock
Nissim library 10^{11}	Acid	3×10^7	YPNR1
YPNR1- 3×10^7	Acid	4×10^5	YPNR2
YPNR2- 4×10^5	Acid	10^3	YPNR3

[336.] As expected, the results presented in Table 4 show a decrease in phage yield after each round of panning. This protocol was used in order to prevent bias due to amplification of nonspecific phage.

[337.] 6.2.2 Clone Sequence Results for YPR and YPNR Protocols:

Several clones from the third panning from both protocols were selected for sequencing. The amino acid sequences presented in Table 5 are those of the CDR3 regions of the heavy chain (V_H -CDR3). The germline and the frequency with which the sequences appeared in the R3 output are also indicated in this table.

Table 5: Selected Y-series clones following the YPR biopanning protocol with the R3 output.

Clone #	V _H -CDR3 Size	V _H -CDR3 sequence				Germline	Frequency
Y1	6	Met Val 1 5	Arg Ile 2 6	Ala 3	Pro 4	VM-DP32	14/30
Y16	6	Thr Ile 1 5	Gly Lys 2 6	Gln Arg 3 7	Ser Ser 4 8	V _H 3-DP26	1/30
Y17	6	Leu Tyr 1 5	Thr Phe 2 6	His 3	Pro 4	V _H 3-DP32	7/30
Y-27	6	Leu Glu 1 5	Arg Ser 2 6	Pro 3	Pro 4	V _H 3-DPS2	3/30
Y-44	11	Thr Ser Ser Arg 1 5 9	Lys Ser His 2 6 10	Asn Ser 3 7 11	Thr Lys 4 8	V _H 3-DP32	2/30
Y-45	12	Arg Tyr Ser Thr 1 5 9	Tyr Ser Val 2 6 10	Cys Asp Ser 3 7 11	Arg Cys 4 8 12	V _H 3-DP49	1/30
Y-52	10	Phe Gln Ala 1 5 9	Arg Thr Pro 2 6 10	Arg Val 3 7	Met Pro 4 8	V _H 3-DP49	1/30

[338.] The majority of isolated clones from the YPNR protocol were Y1 as well.

[339.] The CDR3 region of the light chain of the above clones is identical and is recited in SEQ ID NO: 125.

EXAMPLE 7:

[340.] 7. Results of Binding Evaluation

[341.] **7.1 Binding of selected phagemid clones to AML cells (AM clone series):** The binding assay for assessing phagemid binding to cells, the White/Blue colony test as described in Example 5, was performed with the AM clones. With the exception of clone AM7, no preferential binding to the tested cells

was detected. Significant, but non-selective, binding of clone AM7 to all target cells, either as a phagemid or purified scFv, was observed. Results demonstrate no enrichment for the AM clone series.

[342.] **7.2 Binding of Y Clone Series**

[343.] **7.2.1 Phagemid binding - EIA using fixed platelets:** After three rounds of panning using two different protocols, phage clones were tested by EIA for binding to fixed platelets. Phagemid stock was prepared from each of the selected clones, and these clones were tested in two sets of EIA. Each sample was assayed in duplicate, and the average, was calculated. The results are summarized in Figure I and indicate that six of the nine Y-series clones show a positive EIA reaction. The highest degree of binding was associated with clones Y1, Y16, Y17, and Y-27. Phage stocks M13 (wild-type bacteriophage) and E6 (selected on CLL leukemia cells) were used as negative controls. The dominant clone, phage Y1, showed the highest binding to fixed platelets and, together with Y17, showed significantly higher binding than M 13 or E6 phage clones.

EXAMPLE 8:

[344.] **8. Detailed characterization of scFvs and clone binding**

[345.] **8.1 Structure and identification of scFv:** The native structure of Y-I was assessed by HPLC analysis with a Superdex 75 column and by mass spectroscopy. Results of the former method indicate the presence of monomers, dimers, and tetramers in the preparation. Mass spectroscopy was sufficiently sensitive to identify the expected molecular weight of 26.5 kD and, in cases in which the c-myc tag was cleaved, a molecular weight of 24 kD was obtained.

[346.] Results of SDS-PAGE, however, indicate that the intact, non-cleaved molecule has an apparent molecular weight of 30 kD, despite the expected molecular weight is 26.5 kD, according to the nucleic acid sequence and to the mass spectroscopy results above. Western analysis using c-myc-specific antibodies confirmed the SDS-PAGE 30 kD results and supported the implication that the c-myc

tag is present on the end of the intact molecule. The discrepancy between the results of the two procedures is due to the level of precision of the methods as well as the running conditions of SDS-PAGE that can alter the apparent molecular weight of the tested protein.

[347.] **8.2 Binding of platelet-selected clones to leukemic cells:** As noted in the introduction, platelet cell surface markers may be expressed on premature hematopoietic cells. The binding of platelet-selected clones was tested by FACS analysis. FACS analysis was performed after staining whole blood, followed by RBC lysis, or on Iso-prep- (Ficoll cushion) purified mononuclear cells. ScFvs were prepared from each clone, purified on protein-A, and FITC labeled (as described in Sections 4.1-4.4). In order to enable production of intact scFv in the non-suppressor *E. coli* strain HB2151, the amber codon (TAG) found in the V_H-CDR3 of the Y-27 clone was mutated by DNA site-directed mutagenesis to code for glutamic acid (GAG). The target cells for such studies were cells isolated from fresh blood samples of various patients with leukemia. The samples were obtained from three Medical Centers in Israel.

[348.] Clones Y1 and Y17 showed preferential binding to the leukemia cells tested whereas all the other Y-series clones gave binding at background levels only. Table 6 presents the binding of FITC-labeled Y- I and Y- 17 to a variety of leukemic cells.

Table 6. Y- I binding specificity for leukemia cells.

B cell lineage

ScFv	B cell lineage						
	AML	CML	B-CLL	B-ALL	Multiple myeloma	T lineage leukemia	Normal lymphocytes
N14/C181	0/68	0/6	0/6	0/6	0/5	0/3	0/18
Y1	54/68	2/6	1/6	3/6	4/5	2/3	0/15
Y17	3/3	N.D.*	1/1	2/2	N.D.*	N.D.*	11/11

*Not determined

[349.] The results, presented as fractions in Table 6, represent the fraction of patients, the cells of who were identified by FACS analysis as positively reacting with each tested antibody. The numerator represents the number of positive patients, with the denominator density the total number of patients tested for a given scFv/cell type combination. Y- 17 bound strongly to all tested cells; this binding was thus considered to be non-cell selective. However, Y1 binding was found to be highly selective for several specimens of leukemic cells, especially those in the acute phase. Y1-scFv binding was further analyzed as described below.

[350.] Representative results of Y1 binding to three AML samples are presented in Figure 3. In each case, a large proportion of the cell population fluoresces at a significantly higher intensity than that of the background fluorescence obtained by staining with the negative control scFv. These results indicate that, for each patient, Y1 binds to a different fraction of the total cell population. The right-hand Y-I peak in each graph is believed to represent the minimum number of Y1-binding cells in the population, with the proportion of the total cells under this peak most likely representing the minimum proportion of Y1 -binding cells in each sample.

[351.] **8.3 Binding of Y-I to normal blood cells:** Y1 binding to Ficoll purified normal blood cells was analyzed according to the different blood cell types. Although no binding to normal lymphocytes was detected, Y1 bound to Ficoll purified monocytes from 9/28 subjects, platelets from 5/8 subjects, and red blood cells (RBC) from 1/4 subjects. However, CD14-specific antibodies bound to cells in all of the monocyte preparations and in many of the neutrophil preparations. A summary of this analysis is presented in Table 7.

Table 7. FACS analysis of scFv binding to Ficoll-purified normal blood cells.

Antibody	Lymphocytes	Monocytes	Neutrophils	Platelets	RBCs
N14	0/18	0/4	0/4	0/3	0/4
Y1	0/28	9/28	0/4	5/8	1/4
CD14	0/15	14/14	8/14	0/5	0/4

[352.] These binding results represent the fraction of normal blood samples that were identified by FACS analysis as positively reacting with each tested antibody. Note that, although selected on fixed platelets, FITC-Y1 scFv shows relatively low binding affinity to platelets.

[353.] Figure 4 demonstrates the binding of Y1 to Ficoll-purified platelets (4a) and to monocyte-gated cells (4b). The shift on the monocyte cell population is greater than that observed on platelets, with a calculated mean fluorescence 30-fold and 5-fold greater, respectively, than the negative control. This observation is most probably due to the characteristic of platelets to adhere in multiples to Ficoll-purified monocytes. Subsequent experiments showed that, when assayed in whole blood samples, no Y1 binding was observed in any of the normal monocytes, granulocytes, platelets or RBC tested. Similarly, no Y1 binding to platelets was observed when derived from platelet-rich plasma (PRP). Under the same binding conditions (in whole blood, followed by RBC lysis with FACS lysing solution [Becton Dickinson]), Y1 bound to leukemia cells in a manner similar to that obtained after Ficoll purification. We may therefore conclude that, under natural conditions, the Y- I epitope on platelets or monocytes is hidden. During the Ficoll purification procedure the epitope is exposed, making it accessible for recognition by Y1, whereas for leukemic cells the epitope is exposed under both purified and non-purified conditions

[354.] In addition to normal hematopoietic cell progenitors of the lymphatic and myeloid lineages, Y1 binding to hematopoietic stem cells (CD34+ cells) in cord blood was tested. Figure 5 presents the binding results of FITC-labeled scFv clones to cord-blood CD34+ stem cells; Figure 5a presents the results of binding of CD34+ gated cells to the FITC-labeled negative control scFv, and Figure 5b presents the same analysis for binding of CD34+ gated cells to FITC-labeled scFv clone Y1. Figure 5c presents a FSC and SSC dot plot analysis of the same FITC-labeled scFv clone Y- I sample as in 5b. Results of this analysis indicated the presence of two CD34+ stem cell sub-populations derived from cord blood, with differences in forward scatter (FSC) an indication of cell size. Y1 binds to the smaller sized cells of the two populations. The circled areas in Figures 5b and 5c delineate the sub-population of

CD34+ cells that bind the clone Y1 scFv. Further analysis indicated that the smaller sized cells are dead cells that are present in the cell population, and Y1 binding may possibly indicate the presence of an intracellular ligand recognized by Y1.

[355.] The experiment was performed on peripheral blood cells of GM-CSF pre-treated healthy donors (GM-CSF treatment mobilizes stem cell release into the bloodstream) as well. Results similar to those presented in Figure 5 were obtained.

[356.] **8.4 Binding specificity of Y1 scFv compared to various cell markers on AML cells:** Y1 staining of Ficoll-purified peripheral cells and bone marrow cells from AML patients was compared to staining of those cells by a panel of other antibodies. Results of such FACS analyses, for samples obtained from 14 patients, are summarized in Table 8. Note that there is significant variability in the frequency of stained cells in preparations from various individuals for all of the markers tested, including Y1. Lack of correlation between the binding of various markers and that of Y1 suggests that Y1 does not bind to any of the ligands that are bound by the other tested markers, and that the Y- I ligand does not constitute any of the tested cell surface markers.

Table 8. Comparison of Y1 scFv binding with binding of antibodies to various cell markers

AML patient	Y1	CD13	CD14	CD33	CD34	BN/PB**
1	0	ND	2.5	47	4	PB
2	34	88	0	80	83	PB
3	66	100	20	87	9	BM
4	86	83	2	73	3	BM
5	100	100	0	100	0	BM
6	0	72	0	49	1	BM
7	59	20	93	100	0	BM
8	40	86	40	48	6.5	BM
9	70	75	67	75	1	PB
10	25	24	55	82	5	PB
11	26	76	17	83	52	PB
12	60	40	60	94	ND	PB
13	17	ND	13	75	15	PB
14	0	24	27	70	0	BM

**BM/PB- bone marrow/peripheral blood

[357.] The results are expressed as the percentage of cells in Ficoll-purified samples of a given patient, which was identified by FACS analysis as positively reacting with each individual antibody.

[358.] In light of the concentration of Y1 ($\sim 1\mu\text{g}/5 \times 10^5$) required for binding detection, the results indicate that Y1 scFv has a relatively high binding affinity to the specific ligand on AML cells.

[359.] In addition to the results presented in Table 8, which show binding of Y1 to AML cells, we have shown above (Table 6) that Y1 can also bind to most other types of leukemia cells tested, including B-ALL cells, although the sample size for these other leukemia specimens was limited. Figure 6 presents a FACS analysis of Y1 scFv binding to pre-B-ALL cells obtained from two patients. A double staining procedure, using either a commercially available PE-labeled CD 19 (a marker for normal peripheral B-cells; Figure 6a, 6c) or a PE-labeled CD34 (a marker for stem cells; Figure 6d) was employed, together with a FITC-labeled negative control scFv or FITC-labeled Y1 scFv. Figure 6b is a double negative control. Fluorescence intensity (x-axis) of cells bound by the FITC-labeled sample (scFv clone Y1), relative to the negative control-staining pattern, is presented (6e and 6f). The results of Figure

6 demonstrate that most of the leukemic, pre-B-ALL cells within each of the two samples tested are positive for Y1 cell staining due to Y- I binding.

[360.] **8.5 Binding of Y1-scFv to cell Lines:** Several cell lines derived from malignant hematopoietic lineages were screened for their ability to be recognized by Y1. FACS analysis indicates that Y1 binds to many of the tested cells (Table 9). Note that only one human B-cell line and one mouse myeloid cell line was tested. Importantly, this binding was restricted to exponentially growing cells. Cells in stationary phase generally did not bind to Y1, indicating that Y1 ligand expression is regulated during the life cycle of the cells. Additionally, binding strength differs among the reacting cells. This observation implies that there are differences in expression levels or in affinity of the ligand in different cells.

Table 9. Binding Of Y1 To Hematopoietic Cell Lines

Type	High Reactive	Medium Reactive	Low Reactive
Human Myeloid	KG-1; THP-1; U937; Tf-1; MEG	HL-60; HEL; K-562; MC1010	NB-4
Human B-cell			Namalwa; Daudi; UMUC3,RAJI
Human T-cell	Jurkat; Hs-602	CCRF-CEM; Molt-4; Hut-78;	
Mouse Myeloid			M1; P388D1; PU5-1.8; WEHI-274.1

[361.] **8.6 Binding of Y1 purified in the presence of DTT:** Once the Y1 clone was selected, the process for producing the scFv was further developed. Results of FTLC analysis of the Y1 batches indicated that the protein may multimerize with mainly monomers and tetramers forming, the ratio between the two forms differing from one preparation to the next. In order to obtain homogeneous material, 5mM DTT was added during the affinity purification on Protein-A sepharose column, followed by removal by PBS buffer exchange. Indeed, after DTT treatment, most (>90%) of the material was found in the monomeric fraction. No significant difference was found between the binding of the monomeric form of Y1 (purified in the presence of DTT and analyzed on HPLC) and the binding of the mixture of Y1 forms.

[362.] **8.7 Y1 is a specific clone to leukemia cells:** The Y1 cassette belongs to the V_H-DP32 germline. Several other clones, originating from the same germline, were isolated and are detailed in Example 6. These clones include Y17, Y-27, and Y-44. The primary sequences (i.e., germline cassette) of all these clones differ in their CDR3 regions only. However, only Y1 shows selectivity to leukemic cells. The CD3 sequences of these clones are summarized in Table 10, and the binding profiles of the clones are summarized in Table 11.

Table 10: The CDR3 sequence of V_H3-DP32 isolated clones

Clone #	V _H -CDR3 sequence						Germline
Y1	Met	Arg	Ali	Pro	Val	Ile	V _H 3-DP32
		1	2	3	4	5	
		6					
Y17	Leu	Thr	His	Pro	Tyr	Phe	V _H 3-DP32
		1	2	3	4	5	
		6					
Y-27	Leu	Arg	Pro	Pro	Glu	Ser	V _H 3-DP32
		1	2	3	4	5	
		6					
Y-44	Thr	Ser	Lys	Asn	Thr	Ser	V _H 3-DP32
		Ser	Ser	Lys	Arg	His	
		1	2	3	4	5	
		6	7	8	9	10	
		11					

Table 11: Binding profile of V_H3-DP32 isolated clones

Clone #	Binding specificity
Y1	Binds to many leukemia cells.
Y17	Binds to all tested hematopoietic cells, including normal lymphocytes.
Y-27	Does not bind to any of the hematopoietic cells tested.
Y-44	Does not bind to any of the hematopoietic cells tested.

[363.] Tables 10 and 11 indicate that, although the primary sequences are identical among the four clones with the exception of the V_H-CDR3 region, the binding profiles differ significantly from one clone to another. This observation reinforces the concept that the sequence of the V_H-CDR3 region plays an important

role in the specificity of the binding site to the antigen. Note that neither the length of the CDR3 sequence nor the specific germline cassette in which it is placed appears to be a primary determinant of binding specificity. Y17 and Y-27 each comprises a 6-mer CDR3, as does Y1, and heavy chains of all three clones are derived from the identical germline. In the case of Y17 and Y-27, selective binding to hematopoietic cells has not been demonstrated.

EXAMPLE 9

[364.] **9.1 Construction of triabodies:** The vector pHEN-Y1, encoding the original Y1, was amplified using PCR for both the V_L and the V_H regions, individually. The sense oligonucleotide 5'-AACTCGAGTGAGCTGACACAGGACCCT, and the anti-sense oligonucleotide 5'-TTTGTCTGACTCATTCTTTTTTGC GGCCGCACC were used for the V_L PCR reaction. The cDNA product of the expected size of ~350 bp was purified, sequenced, and digested with XhoI and NotI restriction enzymes.

[365.] The same procedure was employed to amplify the V_H region (using the sense oligonucleotide 5'-ATGAAATACCTATTGCCTACGG and anti-sense oligonucleotide 5'-AACTCGAGACGGTGACCAGGGTACC). The V_H PCR product was digested with NcoI and XhoI restriction enzymes. A triple ligation procedure into the pHEN vector, pre-digested with NcoI-NotI, was employed. The final vector was designated pTria-Y1.

[366.] Following *E. coli* transformation, several clones were picked for further analysis, which included DNA sequencing, protein expression, and extraction from the periplasmic space of the bacteria. SDS-PAGE under reducing conditions and Western blot analysis were performed to confirm the size of the Y1 triabodies.

[367.] **9.2 Construction of diabodies**

[368.] The pTria-Y1 vector from above was linearized with XhoI restriction enzyme, and synthetic complimentary double stranded oligonucleotides (5'-TCGAGAGGTGGAGGCGGT and 5' TCGAACCGCCTCCACCTC) were

pre-annealed and ligated into the XhoI site, between the Y1-heavy and Y1-light chains. This new vector was designated pDia-Y1. As described for the triabodies, the DNA sequence and protein expression was confirmed.

[369.] **9.3 Expression and purification of diabodies and triabodies**

[370.] Expression in *E.coli* was essentially as described above for the scFv-Y1. However, the purification of Y1 diabodies and triabodies from the periplasm of the transformed *E.coli* cells was different. The scFv Y1 monomer form can be purified on an affinity column of Protein-A Sepharose beads. Multimeric forms of Y1 are, however, ineffectually purified by this procedure. Therefore, periplasmic proteins extracted from the bacteria were precipitated over-night with 60% ammonium sulfate, resuspended in H₂O, and loaded onto a Sephacryl-200 (Pharmacia) size exclusion column pre-equilibrated with 0.1.xPBS. Fractions were collected and analyzed by HPLC, and separate fractions containing either the dimer or timer forms were collected for FITC labeling and FACS analysis.

[371.] **9.4 Binding of Y1 diabodies and triabodies to cells**

[372.] FACS analysis was performed on Jurkat cells using a "three step staining procedure." First, crude extracts or purified unlabeled scFv are stained, then mouse anti-myc antibodies, and finally, FITC- or PE-conjugated anti-mouse antibodies. FACS analysis requires 5-8x10⁵ cells, which have been Ficoll-purified and resuspended in PBS+1 % BSA. Binding was carried out for 1 hour at 4°C. After each step, cells were washed and resuspended in PBS+1% BSA. After the final staining step, cells were fixed by re-suspending in PBS, 1 % BSA, 2 % formaldehyde, and then read by FACS (Becton-Dickinson).

[373.] The binding of Y1-scFv was compared to that of diabodies and triabodies. In this analysis (Figure 7, the binding profile of all three forms was very similar, indicating that the above modifications in the molecule did not alter, conceal or destroy the apparent binding affinity of Y1 to its ligand.

[374.] **9.5 Production of Y1-cys-kak (cysteine dimer)**

[375.] One liter of λ pL-y1-cys-kak bacterial culture was induced at 42°C for 2-3 hrs. This culture was centrifuged at 5000 RPM for 30 minutes. The pellet was resuspended in 180 mls of TE (50mM Tris-HCl pH 7.4, 20mM EDTA). 8 mls of lysozyme (from a 5 mg/ml stock) was added and incubated for 1 hr. 20 mls of 5M NaCl and 25 mls of 25% Triton was added and incubated for another hour. This mixture was centrifuged at 13000 RPM for 60 minutes at 4° C. The supernatant was discarded. The pellet was resuspended in TE with the aid of a tissuemiser (or homogenizer). This process was repeated 3-4 times until the inclusion bodies (pellet) were gray/light brown in color. The inclusion bodies were solublized in 6M Guanidine-HCl, 0.1M Tris pH 7.4, 2 mM EDTA (1.5 grams of inclusion bodies in 10 mls solubilization buffer provided ~10 mg/ml soluble protein). This was incubated for at least 4 hrs. The protein concentration was measured and brought to a concentration of 10 mg/ml. DTE was added to a final concentration of 65 mM and incubated overnight at room temperature. Re-folding was initiated by dilution of 10 mls of protein (drop by drop) to a solution containing 0.5 M Arginine, 0.1 M Tris pH 8, 2 mM EDTA, 0.9 mM GSSG. The re-folding solution was incubated at ~10° C for 48 hrs. The re-folding solution containing the protein was dialyzed in a buffer containing 25 mM Phosphate buffer pH 6, 100 mM Urea, and concentrated to 500 mls. The concentrated/dialyzed solution was bound to an SP-sepharose column, and the protein was eluted by a gradient of NaCl (up to 1M).

[376.] **9.6 A Study of the Affinity of the S-S Y1-Dimer in Comparison to CONY1 and Y1-IgG, using a Radioreceptor Binding Assay (RRA) with KG-1 Cells**

[377.] The assay system involved the use of radioactive ligands that were prepared by iodination with ^{125}I using chloramine T on the Y1-IgG construct or the Bolton-Hunter reagent on the CONY1 (the Y1 scFv) construct. The assay tubes contained 5×10^6 KG-1 cells per 0.2 ml and a labeled tracer with varying amounts of unlabeled competitor, in PBS + 0.1 % BSA, pH 7.4. After 1-hour incubation with

agitation at 4°C, the cells were thoroughly washed with cold buffer and taken for radioactivity counting.

[378.] In the RRA study using labeled Y1-IgG, a 2 ng/tube of ^{125}I -Y1-IgG was used, and competition was performed with each of the three molecules. The results are provided in Figure 8. This results presented in this figure demonstrate that the affinity of the S-S Y1 dimer was 30 times higher than that of CONY1. A rough estimate of the affinity of the Y1-IgG in this experiment is 2×10^{-8} M. The corresponding affinity of the dimer is, therefore, 4×10^{-8} M.

[379.] In a second RRA using labeled CONY1, a 100 ng/tube of ^{125}I -Y1-IgG was used, and competition was performed with each of the three molecules. The results are provided in Figure 9. This figure shows that the affinity of the S-S dimer was 20 times higher than that of CONY1. A rough estimate of the affinity of CONY1 in this experiment is 10^{-6} M. The corresponding affinity of the dimer is, therefore, 5×10^{-8} M.

[380.] **9.7 ELISA to GC (glycocalicin)**

[381.] 100 μl of purified glycocalicin was incubated in a 96 flat well maxisorp plates, overnight at 4°C. The plate was washed with PBST (PBS+0.05% tween) 3 times, then 200 ml of PBST-milk (PBST + 2% Non fat milk), for 1 hr at room temperature. The plate was washed with PBST, and the monomer or dimer (100 μl) was added in PBST-milk at different concentrations for 1hr at room temp. Then the plate was washed and anti- V_L polyclonal (derived from immunized rabbits with V_L derived from Y1) (1:100 diluted in PBST-milk) was added for an hour. The plate was washed and anti-rabbit HRP was added for an additional hour. The plate was washed 5 times and 100 μl TMB substrate was added for approximately 15 minutes then 100 μl of 0.5 H_2SO_4 was added to stop the reaction. The optical density of the plate was measured at 450nm in an ELISA reader.

[382.] **9.8 Y1 reactivity with recombinant glycosialicin (GC) expressed in Prokaryotic (*E. coli*) system**

[383.] The DNA fragment encoding the N-terminal soluble part of human platelet GPIb - glycosialicin (GC, amino acid 1 to amino acid 493) was cloned into an IPTG inducible prokaryotic vector cassette. *E. coli* (BL21 DE3) cells harboring the newly constructed plasmid were grown at 37°C to O.D. 0.7-0.8, then at 37°C for 3 hours for in the presence of IPTG for induction. SDS-polyacrylamide gel loaded either with natural semi-purified human platelet derived GC or with *E. coli* cell lysates (total protein content) derived from induced and non-induced cells were analyzed. Western blot analysis was performed with scFv Y1-biotinylated, polyclonal rabbit anti-human GC antibody, commercially available mouse anti human CD42 monoclonal antibody (SZ2 Immunotech, PM640 Serotec, HIP1 Pharmigen, AN51 DAKO) and polyclonal antibody against the N-terminus of gpIba (Sc-7071, Santa Cruz). The two polyclonal antibodies recognized both the recombinant bacterial derived GC as well as the natural human platelet derived GC. The scFv Y1 and the commercially available antibodies recognized only the natural human derived GC, but not the bacterial derived recombinant platelet GC.

[384.] Post-translational modification, such as glycosilation and sulfation is essential for scFv and commercially available antibodies binding to GC. The prokaryotic (*E.coli*) system lacks post-translation modification mechanisms, such as glycosilation and sulfation.

[385.] **9.9 Making tetramers of Y1**

[386.] A construct was designed where the following sequence, LNDIFEAQKIEWHE, was added at the C-terminus of the Y1 by PCR and cloning into a IPTG inducible expression system. The clone was named Y1-biotag. This sequence is a substrate for the enzyme BirA, that in the presence of free biotin, the enzyme is capable of covalently connecting biotin to the lysine (K) residue (Phenotypic analysis of antigen-specific T lymphocytes. Science. 1996 Oct 4;274(5284):94-6, Altman JD et al). This construct was produced as inclusion bodies in BL21 bacterial cells. Refolding was performed as described previously. Inclusion

bodies were solubilized in guanidine-DTE. Refolding was done by dilution in a buffer containing arginine-tris-EDTA. Dialysis and concentration was performed followed by HiTrapQ ionic exchange purification.

[387.] The purified Y1-biotag scFv was incubated with BirA enzyme (purchased from Avidity) and biotin as recommended by the provider. The biotinylated Y1-biotag was analyzed by HABA test (that estimates the amount of biotin per molecule) and demonstrated that there was around >0.8 biotin residues/molecule.

[388.] The Y1-biotag biotinylated was incubated with Streptavidin-PE (Phycoerythrin) to form complexes and used in FACS experiments using KG-1 cells (positive for Y1). Streptavidin can bind up to 4 biotinylated Y-1-biotag molecules. The sensitivity of the binding was increased at least 100 fold due to the increase in avidity.

[389.] The sequence of Y1-biotag is as follows:

```

1    MEVQLVESGG GVVRPGGSLR LSCAASGFTF DDYGMSWVRQ
41   APGKGLEWVS GINWNGGSTG YADSVKGRFT ISRDNAKNSL
81   YLQMNSLRAE DTAVYYCARM RAPVIWGQGT LTVTSRGGGG
121  SGGGGSGGGG SSELTDQPAV SVALGQTVRI TCQGDSLRSY
161  YASWYQQKPG QAPVLVIYGK NNRPSGIPDR FSGSSSGNTA
201  SLTITGAQAE DEADYYCNSR DSSGNNVVFG GGTKLTVLGG
241  GGLNDIFEAQ KIEWHE

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[390.] **EXAMPLE 10: Construction of full sized Y1-IgG1**

[391.] Whole IgG molecules have several advantages over the Fv forms, including a longer half-life *in vivo* and the potential for inducing an *in vivo* cellular response, such as those mediated by ADCC or CDC (complement dependent cytotoxicity; Tomlinson, *Current Opinions of Immunology*, 5, 83-89(1993)). By a molecular cloning approach that is described below, we have converted the Y1 Fv

regions into full sized IgG1 molecules. Y1-IgG1 construction was accomplished by joining fragments of cDNA to each other in the following order:

[392.] **10.1 A leader sequence compatible for a mammalian expression**

system: An exchangeable system was designed to allow convenient insertion of elements required for a full IgG molecule. The following complimentary double stranded oligonucleotides encoding a putative leader sequence were synthesized, annealed, and ligated into the XhoI site of mammalian expression vector (under the SR α 5 promoter).

5'-

TCGACCTCATCACCATGGCCTGGGCTCTGCTGCTCCTCACCTCCTCACTC
AGGACACAGGGTCCTGGGCCGAT

and

5'-

GATCGATTGCACCAGCTGGATATCGGCCCAGGACCCTGTGTCCTGAGTGA
G GAGGGTGAGGAGCAGCAGCCCAGGCCATGGTGATGAGG. Upstream of the initiation ATG codon, two Kozak elements were included. In addition, an internal EcoRV site was introduced between the putative cleavage site of the leader sequence and the XhoI site to allow subcloning of the variable regions. This modified vector was designated pBJ-3.

[393.] **10.2 The V_L encoding sequence** derived from the Y1 scFv cDNA

sequence was inserted between the leader and the constant light region-encoding sequence. Similarly, the V_H encoding sequence derived from the Y1 scFv cDNA sequence was inserted between the leader and the constant heavy region-encoding sequence. This was accomplished by PCR amplification of the vector pHEN-Y1, encoding for the original Y1, to obtain the V_L and the V_H regions, individually.

[394.] **10.3 The oligonucleotides**

5'-TTTGATATCCAGCTGGTGGAGTCTGGGGGA (sense) and
5'-GCTGACCTAGGACGGTCAGCTTGGT (anti-sense) were used for the V_L PCR reaction. The cDNA product of the expected size of ~350 bp was purified, sequenced and digested with EcoRV and AvrII restriction enzymes. The same procedure was

employed to amplify and purify the V_H cDNA region, using the sense and the anti-sense oligonucleotides

5'-GGGATATCCAGCTG(C/G)(A/T)GGAGTCGGGC

and

5'-GGAAGTCGAGACGGTGACCAGGGTACCTTG, respectively.

[395.] **10.4 Constant regions:** The constant $\lambda 3$ (CL- $\lambda 3$) region and the constant heavy regions CH1-CH3 derived for IgG1 cDNA were individually synthesized as follows:

[396.] **10.4.1 For the constant CL- $\lambda 3$ region,** RT-PCR was performed on mRNA extracted from a pool of normal peripheral B-cells (CD 19+ cells) in combination with the sense 5'-CCGTCCTAGGTCAGCCCAAGGCTGC and the anti-sense 5'-TTTGCGGCCGCTCATGAACATTCTGTAGGGGCCACTGT oligonucleotides. The PCR product of the expected size (~400 bp) was purified, sequenced, and digested with AvrII and NotI restriction enzymes.

[397.] **10.4.2 For the constant IgG1 regions (γ chain),** a human B cell clone (CMV - clone #40), immortalized at BTG, was selected for PCR amplification. This clone was shown to secrete IgG1 against human CMV and was also shown to induce ADCC response in *in-vitro* assays. For the CH1-CH3 cDNA, oligonucleotides 5'-CCGCTCGAGTGC(T/C)TCCACCAAGGGCCCATC(G/C)GTCTTC (sense) and 5'-TTTGCGGCCGCTCATTTACCC(A/G)GAGACAGGGAGAGGCT (anti-sense) were synthesized and used for PCR amplification. As described for the CL cDNA encoding sequence, the PCR product of expected size (~1500 bp) was purified, sequenced, and digested with AvrII and NotI restriction enzymes.

[398.] **10.5 For the final expression vectors,** a triple ligation procedure was carried out using the EcoRV-NotI pre-digested vector, EcoRV-AvrII variable cDNAs and AvrII-NotI constant regions. The final vectors for heavy chain and light chain expression were designated Y-I-HC and Y1-LC, respectively.

[399.] **10.6 An additional vector, pBJ-Y1-LP**, was constructed based on the Y1-LC to allow double selection based on the puromycin resistant gene (PAC). In this vector the neomycin-resistant gene of the Y1-LC plasmid was replaced with a fragment of ~1600bp encoding for the PAC gene (from the pMCC-ZP vector).

[400.] **10.7 The open reading frame (ORF)** of both the Y-l-IgG-HC and Y1-IgG-LC and their encoded amino acid sequences are presented below:

[401.] **10.7.1 The ORF of Y1-IgG-HC (V_H C_{H1} C_{H2} C_{H3})**

```

1      ATGGCCTGGGCTCTGCTGCTCCTOACCCTCCTCACTCAGGACACAGGGTCCTGGGCCGAT
1      M A W A L L L L T L L T Q D T G S W A D
61     ATCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCCCTGAGACTCTCC
21     I Q L V E S G G G V V R P G G S L R L S
121    TGTGCAGCCTCTGGATTACCTTTGATGATTATGGCATGAGCTGGGTCCGCCAAGCTCCA
41     C A A S G F T F D D Y G M S W V R Q A P
181    GGAAGGGGCTGGAGTGGGTCTCTGGTATTAATTGGAATGGTGGTAGCACAGGTTATGCA
61     G K G L E W V S G I N W N G G S T G Y A
241    GACTCTGTGAAGGGCCGATTACCATCTCTAGAGACAACGCCAAGAACTCCCTGTATCTG
81     D S V K G R F T I S R D N A K N S L Y L
301    CAAATGAACAGTCTGAGAGCCGAGGACACGGCCGTGTATTACTGTGCAAGAATGAGGGCT
101    Q M N S L R A E D T A V Y Y C A R M R A
361    CCTGTGATTTGGGGCCAAGGTACCCTGGTCACCGTCTCGAGTGCTTCCACCAAGGGCCCA
121    P V I W G Q G T L V T V S S A S T K G P
421    TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGC
141    S V F P L A P S S K S T S G G T A A L G
481    TGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAGTACAGGCGCCCTG
161    C L V K D Y F P E P V T V S W N S G A L
541    ACCAGCGGCGTGACACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGC
181    T S G V H T F P A V L Q S S G L Y S L S
601    AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAAT
201    S V V T V P S S S L G T Q T Y I C N V N
661    CACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTGACAAAAGT
221    H K P S N T K V D K R V E P K S C D K T
721    CACACATGCCCACCGTGCCAGCACCTGAACTCCTGGGGGGACTGTGAGTCTTCOTCTTC
241    H T C P P C P A P E L L G G P S V F L F
781    CCCCCAAAACCCAAGGACACCCTCATGATCTCCCGACCCCTGAGGTACATGCGTGGTG
261    P P K P K D T L M I S R T P E V T C V V
841    GTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAG
281    V D V S H E D P E V K F N W Y V D G V E
901    GTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTC
301    V H N A K T K P R E E Q Y N S T Y R V V
961    AGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTC

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321      S V L T V L H Q D W L N G K E Y K C K V
1021    TCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCC
341      S N K A L P A P I E K T I S K A K G Q P
1081    OGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTC
361      R E P Q V Y T L P P S R E E M T K N Q V
1141    AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
381      S L T C L V K G F Y P S D I A V E W E S
1201    AATGGGCAGCCGGAGAACAACACTACAAGACCACGTCTCCCGTGCTGGACTCCGACGGCTCC
401      N G Q P E N N Y K T T S P V L D S D G S
1261    TTCTTCCTCTATAGCAAGCTCACCGTGCAACAAGAGCAGGTGGCAGCAGGGGAACGTCTTC
421      F F L Y S K L T V D K S R W Q Q G N V F
1321    TCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTG
441      S C S V M H E A L H N H Y T Q K S L S L
1381    TCTCTGGGTAAATGA
461      S L G K *

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[402.] **10.7.2 The ORF of Y1-IgG-LC (V_L C_L)**

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1      ATGGCCTGGGCTCTGCTGCTCCTCACCTCCTCACTCAGGACACAGGGTCTGGGCCGAT
1      M A W A L L L L T L L T Q D T G S W A D
61     GCAGAGCTGACTCAGGACCCTGCTGTGTCTGTGGCCTTGGGACAGACAGTCAGGATCACA
21     A E L T Q D P A V S V A L G Q T V R I T
1212   TGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAGCAGAAGCCAGGACAG
41     C Q G D S L R S Y Y A S W Y Q Q K P G Q
181    GCCCCTGTACTTGTCTATCTATGGTAAAAACAACCGGCCCTCAGGGATCCAGACCGATTCC
161    A P V L V I Y G K N N R P S G I P D R F
241    TCTGGCTCCAGCTCAGGAAACACAGCTTCCTTGACCATCACTGGGGCTCAGGCGGAAGAT
81     S G S S S G N T A S L T I T G A Q A E D
301    GAGGCTGACTATTACTGTAACCTCCCGGACAGCAGTGGAACCATGTGGTATTTCGGCGGA
101    E A D Y Y C N S R D S S G N H V V F G G
361    GGGACCAAGCTGACCGTCCTAGGTGAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCCCG
121    G T K L T V L G Q P K A A P S V T L F P
421    CCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGTCTCATAAGTGACTTC
141    P S S E E L Q A N K A T L V C L I S D F
481    TACCCGGGAGCCGTGACAGTGGCCTGGAAGGCAGATAGCAGCCCCGTCAAGGCGGGAGTG
161    Y P G A V T V A W K A D S S P V K A G V
541    GAGACCACCACACCCTCCAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTACCTGAGC
181    E T T T P S K Q S N N K Y A A S S Y L S
601    CTGACGCCTGAGCAGTGAAGTCCCACAAAAGCTACAGCTGCCAGGTCACGCATGAAGGG
201    L T P E Q W K S H K S Y S C Q V T H E G
661    AGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATGTTTCATGA
221    S T V E K T V A P T E C S *

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[403.] The leader sequence is underlined. The V_H and V_L regions are each encoded by amino acid sequences that are bolded, followed by either the IgG1 (for the heavy chain) or the λ 3 (for the light chain) constant region sequences.

[404.] **10.8 Expression of Y1 heavy and light chain in CHO cells.**

Vectors Y1-HC and Y1-LC were used individually for the transfection and selection of stable cells expressing the heavy or light chains. Following selection on G418 and cell growth, the secreted protein in the supernatant was analyzed for IgG1 expression by the capture EIA assay and by Western blot analysis, as described below.

[405.] **10.8.1 Capture EIA assay:** The wells of 96 well plates were pre-coated with mouse anti-human IgG1 Fc (Sigma). The supernatant from above was added to the wells, and the presence of heavy chain IgG1 was detected with biotinylated goat anti- γ chain specific antibody (Sigma), streptavidin-HRP and substrate. An ELISA plate reader monitored development of the color at A₄₀₅.

[406.] **10.8.2 Western blot analysis:** The supernatant for the above cells was run on 12.5% SDS-PAGE. Expression of each chain was detected with (a) goat anti-human IgG-HRP (H+L; Sigma Cat #A8667) for heavy chain detection and (b) biotinylated goat anti-human λ 3 chain (Southern Biotechnology Association, Cat #2070-08) for light chain detection.

Expression of both chains was confirmed by the above assays, and co-transfection was carried out to obtain full size Y1-IgG1.

[407.] **10.9 Expression and Purification of Y1-IgG**

[408.] **10.9.1 Cell Culture and Transfection:** CHO cells were cultivated in F-12 medium with 10% fetal calf serum and 40 μ g/ml gentaMycin at 37°C in 5% CO₂ atmosphere. One day before transfection 0.8 x 10⁶ cells were seeded on 90mm dishes. The cultures were co-transfected with 10 μ g of light and heavy chains DNA by the FuGene (Roche) transfection reagent technique. After 2 days of growth in nonselective medium, the cells were cultured for 10-12 days in F-12 medium containing 550 μ g/ml neomycin and 3 μ g/ml puromycin. The cells were trypsinized

and cloned by limiting dilution of 0.5 cell/well in Costar 96-well plates. Individual colonies were picked, grown in six-well dishes and transferred to flasks.

[409.] **10.9.2 Determination of heavy and light chain secretion:** A sandwich ELISA assay was used to determine the concentration of the antibody secreted into the supernatant of transfected CHO cells. In order to determine the concentration of the antibody, the following reagents were used: monoclonal anti human IgG1(Fc) (Sigma) as the coated antibody, goat anti-human IgG (γ -chain specific) biotin conjugate as the detector (Sigma), and pure human IgG1, lambda (Sigma) as standard. Based on this ELISA assay the production rate varied between 3-4 $\mu\text{g/ml}$.

[410.] **10.9.3 Production and Purification of MAb from the cells:** Cells were grown in roller bottles to a final concentration of $1-2 \times 10^8$ cells per bottle in F-12 medium with 10% fetal calf serum, supplemented with neomycin and puromycin. For the production, cells were cultured in the same medium, but with 2% of fetal calf serum for an additional two days. The secreted antibody was purified on a protein G-Sepharose column (Pharmacia). Binding was in 20mM sodium phosphate buffer, pH 7.0; elution was performed in 0.1M glycine buffer, pH 2.5-3.0. The quantity of the purified antibody was determined by UV absorbance; purity was analyzed by SDS-PAGE. Under non-denaturing conditions the full IgG antibody has its expected molecular weight of 160kD. In denaturing gels both heavy and light chains have the expected molecular size of 55 and 28 kD, respectively.

[411.] **10.9.4 Binding of full size Y1-IgG molecule:** Binding experiments were performed to determine the level of binding of the Y1-IgG molecule compared to the binding level of the scFv-Y1 molecule. A two-step staining procedure was employed, wherein 5 ng of Y1-IgG were reacted with both RAJI cells (negative control, Figure 7a) and Jurkat cells (Y1 positive cells, Figure 7b). For detection, PE-labeled goat anti-human IgG was used. Similarly, 1 μg of scFv-Y1 was reacted with Jurkat cells (Figure 7c), and PE-labeled rabbit anti-scFv was used for detection. Results indicate that both Y1-IgG and scFv-Y1 bind to the Jurkat cells, with

approximately 10^3 -fold more scFv-Y1 molecules needed to obtain a level of detection similar to that of the Y1-IgG.

Brief Description of the Tables

[412.] **Table 1: Panning results derived from protocol AM.** The estimated number of phagemids used for panning (input), and the estimated number of bound phagemids eluted (output) are summarized for the four consecutive steps of the AM biopanning protocol. The cell source and elution medium for each output result is listed, as well as the term used to distinguish each separate stock.

[413.] **Table 2: Selected clones following the AM biopanning protocol.** The number of amino acid residues in the CDR3 region (V_H -CDR3 size) and the CDR3 amino acid sequences for the different clone types isolated are summarized. In addition, the frequency of each of the clone types in the two AM biopanning outputs, the T16M3 and T16M3.1 outputs, are presented.

[414.] **Table 3: Panning results derived from the YPR protocol.** The estimated number of phagemids used for panning (input), and the estimated number of bound phagemids eluted (output) are summarized. The elution medium for each output result is listed, as well as the term used to distinguish each separate stock.

[415.] **Table 4: Panning results derived from the YPNR protocol.** The estimated number of phagemids used for panning (input), and the estimated number of bound phagemids eluted (output) are summarized for the three consecutive steps of the YPNR biopanning protocol. The elution medium for each output result is listed, as well as the term used to distinguish each separate stock.

[416.] **Table 5: Selected Y-series clones following the YPR biopanning protocol with the R3 output.** Several different clones were identified in the R3 output stock. The number of amino acid residues comprising, and the amino acid sequences of, the V_H -CDR3 regions of the identified clones, as well as germline designations, are detailed.

[417.] **Table 6: Y1 binding specificity for leukemia cells.** The results of binding experiments of three different scFv clones, each reacted with mixtures of cells containing primarily each of seven different leukemic cell types, as determined by FACS analysis, are presented. The results represent the fraction of patients, the cells of whom were identified by FACS analysis as positively reacting with each tested antibody. The numerator represents the number of positive patients, with the denominator denoting the total number of patients tested for a given scFv/leukemic cell type combination

[418.] **Table 7: FACS analysis of scFv binding to Ficoll-purified normal blood cells.** Three scFv clones are each analyzed for binding to five different Ficoll-purified normal blood cell types. These binding results represent the fraction of normal blood samples that were identified by FACS analysis as positively reacting with each tested antibody.

[419.] **Table 8: Comparison of Y1 scFv binding with binding of antibodies to various cell markers.** Results of FACS analysis of staining by Y1 and by a panel of other antibodies are presented. Ficoll-purified peripheral and bone marrow cells from ANE patients were prepared and the binding specificity of Y1 scFv compared to various cell markers on AML cells was studied. The results are expressed as the percentage of cells in the Ficoll-purified samples of a given patient, which was identified by FACS analysis as positively reacting with each Fv. Four other antibodies were run for comparison: (1) CD13 - a marker for granulocytes and monocytes; (2) CD14 - a marker for monocytes and neutrophils; (3) CD33 - a marker for normal myeloid cells and leukemic myeloid cells; and (4) CD34 - a marker for stem cells.

[420.] **Table 9: Binding of Y1 to hematopoietic cell lines.** FACS analysis was performed to determine the binding of Y1 scFv to three different categories of human leukemia cell lines, and to one murine cell line. Cell lines to which Y1 was positively bound (reactive) or not (non-reactive) are listed.

[421.] **Table 10: The CDR3 sequence of V_H3-DP32 isolated clones.** Following different biopanning and selection procedures several clones based on the

DP32 germline were isolated. Clones Y1, Y17, Y-27 and Y-44 were identified during the biopanning selection on platelets (YPR and YPNR protocols). The sequence of the V_H-CDR3 region of each of these clones is presented.

[422.] **Table 11: Binding profile of V_H3-DP32 isolated clones.** The binding specificity of DP32-derived clones to several hematopoietic cells was tested by FACS analysis.

[423.] The invention has been described with reference to specific examples, materials and data. As one skilled in the art will appreciate, alternate means for using or preparing the various aspects of the invention may be available. Such alternate means are to be construed as included within the intent and spirit of the present invention as defined by the following claims:

Claims:

1. A peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment having enhanced binding characteristics so as to bind selectively and/or specifically to a target cell in favor of other cells, wherein the binding selectivity or specificity is primarily determined by a first hypervariable region, and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags.
2. The peptide or polypeptide of claim 1, wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24.
3. The peptide or polypeptide of claim 1 wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24, and wherein the binding selectivity or specificity is secondarily influenced by a second hypervariable region, by a third hypervariable region, and/or by one or more of the upstream or downstream region flanking the first, the second and/or the third hypervariable regions.
4. The peptide or polypeptide of claim 2 wherein the peptide or polypeptide is a scFv having SEQ ID NO: 25 in which the first hypervariable region is a CDR3 region which is identical to SEQ ID NO: 8.
5. The peptide or polypeptide of claim 1 wherein the scFv molecule comprises a straight or branched chain spacer of 20 or fewer amino acid residues.
6. The peptide or polypeptide of claim 5 wherein the spacer comprises SEQ ID NO: 123 or SEQ ID NO: 124.
7. The peptide or polypeptide of claim 1 wherein the target cell is an activated, excited, modified, changed, disturbed, abnormal or diseased cell.
8. The peptide or polypeptide of claim 7, wherein the diseased cell is a cancer cell.

9. The peptide or polypeptide of claim 7 wherein the cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma cells.
10. The peptide or polypeptide of claim 9 wherein the cell is a leukemia or myeloma cell.
11. The peptide or polypeptide of claim 9 wherein the leukemia or myeloma cell is a B-cell malignancy.
12. The peptide or polypeptide of claim 10, wherein the leukemia cell is an acute myeloid leukemia cell or a B-cell malignancy.
13. The peptide or polypeptide of claim 2 further comprising a cassette of consecutive amino acids having an amino acid sequence selected from the group consisting of SEQ ID NOs:30-113, or having at least 90% amino acid similarity therewith, or fragment thereof, wherein the cassette or fragment provides a framework into which is built, inserted, attached coupled, combined, or fused a CDR3 region having an amino, acid sequence selected from the group consisting of SEQ ID NOs:8-24.
14. The peptide or polypeptide of claim 13 wherein the cassette has an amino acid sequence selected from the group consisting of SEQ ID NOs:30-32,33, 37-39,41, 43, 45, 46, 48, 51, 54, 57, 59-68, 70, 71, 76-85, 87, 89-92, 94, 97, 99, 103, 106, 112, and 113, or having at least 90% amino acid similarity therewith.
15. The peptide or polypeptide of claim 13 wherein the cassette has the amino acid sequence of SEQ ID NO: 61, or has at least 90% amino acid similarity therewith.
16. The peptide or polypeptide of claim 15, wherein the cassette has the amino acid sequence of SEQ ID NO: 61, or has at least 90% amino acid similarity therewith.

17. The peptide or polypeptide of claim 15, wherein the seven carboxy-terminal amino acid residues of SEQ ID NO: 61 are replaced by the seven amino acid residues of SEQ ID NO: 122.
18. The peptide or polypeptide of claim 3, wherein the second and third hypervariable regions are a CDR2 and a CDR1 hypervariable region, respectively.
19. The peptide or polypeptide of claim 2, wherein the CDR3 region has the amino acid sequence SEQ ID NO: 8.
20. The peptide or polypeptide of claim 18 wherein the CDR2 and CDR1 regions have the amino acid sequences SEQ ID NO: 115 and SEQ ID NO: 114, respectively.
21. The peptide or polypeptide of claim 3, wherein the second and third hypervariable regions are a CDR2 and CDR1 hypervariable region respectively and wherein the CDR3, CDR2 and CDR1 regions have the amino acid sequences SEQ ID NOs:8, 115 and 114, respectively.
22. The peptide or polypeptide of claim 3, wherein the upstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 117, and wherein the downstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 116.
23. The peptide or polypeptide of claim 3, wherein the second hypervariable region is a CDR2 hypervariable region and wherein the upstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 119, and wherein the downstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 118.
24. The peptide or polypeptide of claim 3 wherein the third hypervariable region is a CDR1 hypervariable region and wherein the upstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 121, and wherein

the downstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 120.

25. The peptide or polypeptide of claim 18 wherein the CDR2 and CDR1 regions of a cassette of consecutive amino acids selected from the group consisting of SEQ ID NOs:30-113 or a fragment thereof are replaced by SEQ ID NOs:115 and 114, respectively.
26. The peptide or polypeptide of claim 18, wherein the CDR2 and CDR1 regions of a cassette of consecutive amino acids selected from the group consisting of SEQ ID NOs:30-32, 35, 37-39, 41, 43, 45, 46, 48, 51, 54, 57, 59-68, 70, 71, 76-85, 87, 89-92, 94, 97, 99, 103, 106, 112, and 113 or a fragment thereof are replaced by SEQ ID NOs:115 and 114, respectively.
27. The peptide or polypeptide of claim 3 wherein
 - (a) the second and third hypervariable regions are a CDR2 and a CDR1 hypervariable region, respectively,
 - (b) the CDR3 amino acid sequence is SEQ ID NO: 8,
 - (c) the CDR2 amino acid sequence is SEQ ID NO: 115,
 - (d) the CDR1 amino acid sequence is SEQ ID NO: 114,
 - (e) the upstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 117,
 - (f) the downstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 116,
 - (g) the upstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 119,
 - (h) the downstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 118,

- (i) the upstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 121, and
 - (j) the downstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 120.
28. The peptide or polypeptide of claim 1 wherein the Fv is an scFv obtainable from a phage display library.
29. The peptide or polypeptide of claim 28, wherein the phage display library was constructed from peripheral blood lymphocytes of a non-immunized human, and wherein the scFv peptide is selected against previously uncharacterized and unpurified antigens on the surface of a target cell.
30. A method for selecting or identifying the peptide or polypeptide of claim 28 comprising biopanning, wherein the biopanning comprises binding phage to a target, removing non-bound phage, eluting bound phage, and propagating and amplifying eluted phage.
31. A peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment, having enhanced binding characteristics so as to bind selectively and/or specifically to a substantially exposed and/or over-expressed binding site on or in a target cell, wherein the binding to the target cell occurs in favor of other cells on or in which the binding site is not substantially available and/or expressed, wherein the binding selectivity or specificity is primarily determined by a first hypervariable region, wherein the Fv is a scFv or a dsFv, and wherein the FV optionally has one or more tags.
32. The peptide or polypeptide of claim 31, wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24.
33. The peptide or polypeptide of claim 31 wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group

consisting of SEQ ID NOs:8-24, and wherein the binding selectivity or specificity is secondarily influenced by a second hypervariable region, by a third hypervariable region, and/or by one or more upstream or downstream region flanking the first, the second and/or the third hypervariable regions, and wherein the second and third hypervariable regions are a CDR2 and a CDR1 region, respectively.

34. A peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment having enhanced binding characteristics so as to bind selectively and/or specifically to a target cell in favor of other cells, wherein the Fv molecule comprises a first chain having a first, a second and a third hypervariable region and a second chain having a first, a second and a third hypervariable region, wherein one of the hypervariable regions of the first chain has a sequence selected from the group consisting of SEQ ID NOs:8-24, and wherein one of the hypervariable regions of the second chain has a sequence selected from the group consisting of SEQ ID NOs:1-6 and 125-202, and wherein the first, second and third hypervariable regions are a CDR3, CDR2 and CDR1 region, respectively, wherein the Fv is a scFv or a dsFv, and wherein the Fv optionally has one or more tags.
35. The peptide or polypeptide of claim 34 wherein
- (a) the first chain and the second chain each comprises a first hypervariable region selected from the group consisting of SEQ ID NOs:8-24; or
 - (b) the first hypervariable region of the first chain and the first hypervariable region of the second chain are identical and are selected from the group consisting of SEQ ID NOs:8-24; or
 - (c) the first hypervariable region of the first chain is selected from the group consisting of SEQ ID NOs:8-24, and the first hypervariable region of the second chain is selected from the group consisting of SEQ ID NOs:1-6 and 125-202; or

- (d) the first hypervariable region of the first chain is selected from the group consisting of SEQ ID NOs:1-6 and 125-202, and the first hypervariable region of the second chain is selected from the group consisting of SEQ ID NOs:8-24.
36. The peptide or polypeptide of claim 34, wherein the second and third hypervariable regions of the first chain are SEQ ID NOs:114 and 115, respectively.
37. A peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either or a construct of a fragment that
- (a) binds to an unknown ligand on a first cell having a first and a second state, wherein the binding is effective in the second state but is not substantially effective in the first state, and
 - (b) by virtue of immuno-cross-reactivity, binds specifically or selectively to a ligand on a second cell, and wherein the Fv is a scFv or a dsFv and wherein the Fv optionally has one or more tags.
38. The peptide or polypeptide of claim 37, wherein the first cell is a normal cell.
39. The peptide or polypeptide of claim 37, wherein the first state is a non-activated state and the second state is an activated, excited, modified, changed or disturbed state.
40. The peptide or polypeptide of claim 37, wherein the second cell is a diseased cell.
41. The peptide or polypeptide of claim 40, wherein the diseased cell is a cancer cell.
42. The peptide or polypeptide of claim 40 wherein the diseased cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma cells.

43. The peptide or polypeptide of claim 42, wherein the diseased cell is a leukemia cell.
44. The peptide or polypeptide of claim 43, wherein the leukemia cell is an acute myeloid leukemia cell.
45. The peptide or polypeptide of claim 37, wherein the selective and/or specific binding of the peptide or polypeptide to the ligand of the second cell is determined primarily by a first hypervariable region.
46. The peptide or polypeptide of claim 45, wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8-24.
47. The peptide or polypeptide of claim 46 wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8-24, and wherein the binding selectivity or specificity is secondarily influenced by a second hypervariable region, by a third hypervariable region, and/or by one or more of the upstream or downstream region flanking the first, the second and the third hypervariable regions, respectively.
48. A ligand that is expressed by the second cell and that is capable of being bound by the peptide or polypeptide of claim 37.
49. A molecule that recognizes and binds the ligand of claim 48.
50. A nucleic acid molecule encoding the peptide or polypeptide according to any one of claims 1, 31, 34, or 37.
51. The nucleic acid molecule of claim 50, wherein the nucleic acid is DNA.
52. The peptide or polypeptide of claim 37 wherein the first and second states of the first cell are the same, and wherein the first cell is derived from a cell line.

53. The peptide or polypeptide of claim 52, wherein the cell line is selected from the group consisting of Jurkat, Molt-4, HS-602, U937, TF-1, THP-1, KG-1, ML-2, and HUT-78.
54. A method for identifying a targeting molecule, which binds to unknown immuno-cross-reactive binding sites on first and second cells, comprising
- (a) performing one or more biopanning on a first target cell that, in a second state but not in a first state, substantially exposes or displays a binding site comprising at least one unknown ligand, thereby producing a first population of recognition molecules;
 - (b) performing subsequent biopanning and/or selection steps, commencing with the first population of recognition molecules of step (a), that are performed on a second cell that displays a binding site comprising at least one unknown ligand having immuno-cross-reactivity to the unknown ligand of the first cell so as to produce a second population of recognition molecules;
 - (c) amplification and purification of the second population of recognition molecules of step (b); and
 - (d) construction from the recognition sites of the purified recognition molecules of step (c) peptides or polypeptides that comprise targeting molecules that are selective and/or specific for unknown ligands on the second cell.
55. The method of claim 54, wherein the first cell is a normal cell and wherein the first state is a non-activated state and the second state is an activated, excited, modified, changed, or disturbed state.
56. The method of claim 54 wherein the second cell is a diseased cell.
57. The method of claim 56, wherein the diseased cell is a cancer cell.

58. The method of claim 56, wherein the cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma cells.
59. The method of claim 58, wherein the cell is a leukemia cell.
60. The method of claim 59, wherein the leukemia cell is an acute myeloid leukemia cell.
61. The use of the peptide or polypeptide of claim 1 or claim 37, optionally in association with or attached, coupled, combined, linked or fused to a pharmaceutical agent, in the manufacture of a medicament.
62. The use of claim 61, wherein the medicament has activity against a diseased cell.
63. The use of claim 62 wherein the diseased cell is a cancer cell.
64. The use of claim 62, wherein the cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma.
65. The use of claim 64 wherein the cell is a leukemia cell.
66. The use of claim 65, wherein the leukemia cell is an acute myeloid leukemia cell.
67. The peptide or polypeptide of claim 1 or claim 37, optionally in association with or attached, coupled, combined, linked or fused to a pharmaceutical agent, for use as a medicament.
68. The peptide or polypeptide of claim 67 wherein the medicament has activity against a diseased cell.
69. The peptide or polypeptide of claim 68 wherein the diseased cell is a cancer cell.

70. The peptide or polypeptide of claim 68, wherein the cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma cells.
71. The peptide or polypeptide of claim 70 wherein the cell is a leukemia cell.
72. The peptide or polypeptide of claim 71, wherein the leukemia cell is an acute myeloid leukemia cell.
73. The use of the peptide or polypeptide of claim 1 or claim 37 for preparing a composition for use in inhibiting the growth of a diseased or cancer cell.
74. The use of the peptide or polypeptide of claim 73, wherein the cell is a leukemia cell.
75. The use of the peptide or polypeptide of claim 74 wherein the leukemia cell is an acute myeloid leukemia cell.
76. The use of the peptide or polypeptide of claim 1 or claim 37 for preparing a composition for use in inhibiting the growth of a cancer cell, said composition comprising at least one compound having a pharmaceutical ligand selective and/or specific for the cancer cell.
77. A composition comprising at least one peptide of claim 1 or claim 37, in association with, or attached, coupled, combined, linked, or fused to a pharmaceutical agent in a pharmaceutically effective amount and, optionally, a pharmaceutically effective carrier.
78. The composition of claim 77 wherein the peptide or polypeptide and the pharmaceutical agent are linked via a linker compound.
79. The composition of claim 78, wherein the linker compound is selected from the group consisting of a dicarboxylic acid, a maleimido hydrazide, PDPH, a carboxylic acid hydrazide, and a small peptide.

80. The composition of claim 79, wherein the small peptide is selected from a group consisting of AU1, AU5, BTag, c-myc, FLAG, Glu-Glu, HA, His6, HSV, HTTPHH, IRS, KT3, Protein C, S•Tag[®], T7, V5, VSV-G, and KAK-Tag.
81. The peptide or polypeptide according to any one of claims 1, 31, 34 and 37 wherein the tag is selected from a group consisting of: AU1, AU5, BTag, c-myc, FLAG, Glu-Glu, HA, His6, HSV, HTTPHH, IRS, KT3, Protein C, S•Tag[®], T7, V5, VSV-G, and KAK-Tag.
82. The composition of claim 77, wherein the pharmaceutical agent is selected from the group consisting of radioisotope, toxin, oligonucleotide, recombinant protein, antibody fragment, and anti-cancer agent.
83. The composition of claim 82 wherein the radioisotope is selected from a group consisting of indium, ¹¹¹indium, ¹¹³indium, ^{99m}rhenium, ¹⁰⁵rhenium, ¹⁰¹rhenium, ^{99m}technetium, ^{121m}tellurium, ^{122m}tellurium, ^{125m}tellurium, ¹⁶⁵thulium, ¹⁶⁷thulium, ¹⁶⁸thulium, ¹²³iodine, ¹²⁶iodine, ¹³¹iodine, ¹³³iodine, ^{81m}krypton, ³³xenon, ⁹⁰yttrium, ²¹³bismuth, ⁷⁷bromine, ¹⁸fluorine, ⁹⁵ruthenium, ⁹⁷ruthenium, ¹⁰³ruthenium, ¹⁰⁵ruthenium, ¹⁰⁷mercury, ²⁰³mercury, ⁶⁷gallium and ⁶⁸gallium.
84. The composition of claim 82, wherein the toxin is selected from the group consisting of gelonin, Pseudomonas exotoxin (PE), PE40, PE38, diphtheria toxin, ricin, and modifications and derivatives thereof.
85. The composition of claim 82, wherein the anti-cancer agent is selected from the group consisting of doxorubicin, morpholino-doxorubicin (MDOX), adriamycin, cis-platinum, taxol, calicheamicin, vincristine, cytarabine (Ara-C), cyclophosphazdde, prednisone, daunorubicin, idarubicin, fludarabine, chlorambucil, interferon alpha, hydroxyurea, temozolomide, thalidomide, bleomycin, and derivatives thereof.
86. A method of inhibiting the growth of a cell which comprises contacting the cell with an amount of the peptide or polypeptide of claim 1 or claim 37.

87. The method of claim 86 wherein the cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma.
88. The method of claim 87, wherein the cell is a leukemia cell.
89. The method of claim 88, wherein the leukemia cell is an acute myeloid leukemia cell.
90. A pharmaceutical composition comprising at least one peptide of claim 1 or claim 37 attached, coupled, combined, linked, or fused to an imaging agent for use in the diagnostic localization and imaging of a tumor.
91. A method of treating a patient suffering from a disease or cancer, which comprises administering to the patient an amount of the peptide or polypeptide of claim 1 or claim 37 effective to treat the disease or cancer.
92. The method of claim 91 wherein the disease or cancer is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma.
93. The method of claim 92 wherein the disease is a leukemia.
94. The method of claim 93, wherein the leukemia is an acute myeloid leukemia.
95. The peptide or polypeptide of claim 1 or claim 37 wherein the Fv specifically or selectively binds to acute myeloid leukemia (AML) cells.
96. A ligand presented on AML cells bound by the peptide or polypeptide of claim 95.
97. A peptide or polypeptide that binds the ligand of claim 96.
98. A diagnostic kit for in vitro analysis of treatment efficacy before, during, or after treatment, comprising the peptide or polypeptide of claim 1 or claim 37 attached, coupled, combined, linked or fused to an indicative marker molecule.

99. The kit of claim 98 wherein the indicative marker molecule is a fluorescent marker.
100. The kit of claim 99, wherein the fluorescent marker is selected from the group consisting of fluorescein, rhodamine, phycoerythrin, and modifications and conjugates thereof.
101. The kit of claim 98, wherein the kit is used for diagnosis of a disease or cancer.
102. The peptide or polypeptide of claim 1 or claim 37, wherein the construct is an Ig polypeptide.
103. A method for producing the peptide or polypeptide of claim 102, wherein the Ig polypeptide is expressed as a recombinant polypeptide and is produced in a eukaryotic cell system.
104. The method of claim 103 wherein the eukaryotic system is a mammalian cell system.
105. The peptide or polypeptide of claim 102, wherein the Ig polypeptide is an IgG polypeptide.
106. The peptide or polypeptide of claim 105, wherein the IgG polypeptide comprises a CDR3, CDR2 and a CDR1 region having SEQ ID NOs:8, 115 and 114, respectively.
107. The IgG polypeptide of claim 106, wherein the CDR3, CDR2 and CDR1 regions are of the heavy chain.
108. The IgG polypeptide of claim 106 wherein the CDR3, CDR2 and CDR1 regions are of the light chain.
109. The IgG polypeptide of claim 102, wherein the IgG has a heavy chain comprising SEQ ID NO:26 and a light chain comprising SEQ ID NO:27 or chains having at least 90% amino acid similarity therewith.

110. A method for producing the peptide or polypeptide of claim 1 or claim 37 wherein the peptide or polypeptide is produced in a prokaryotic cell system or in a eukaryotic cell system.
111. The method of claim 110, wherein the prokaryotic system comprises *E. coli*, said *E. coli* comprising an expression vector and the eukaryotic system is a mammalian cell system.
112. The method of claim 111, wherein the expression vector of the prokaryotic system comprises a promoter selected from the group consisting of *osmB*, *deo*, β -lac-U5, λP_L , SR α 5, and CMV.
113. A peptide or polypeptide that comprises a binding motif which comprises an amino acid sequence of R₁-X Phe Pro-R₂ wherein R₁ and R₂, wherein each sequence comprises 0-15 amino acid residues and wherein X is either Arg, Gly, or Lys.
114. The peptide or polypeptide of any of claims 2, 34, or 46, wherein the CDR3 comprises the amino acid sequence of R₁-X Phe Pro-R₂, wherein R₁ and R₂ each comprises 0- 15 amino acid residues, and wherein X is either Arg, Gly, or Lys.
115. The peptide or polypeptide of claim 1 or claim 37, wherein said peptide or polypeptide includes at least one non-naturally occurring modification.
116. The peptide or polypeptide of claim 115, wherein said non-naturally occurring modification renders the peptide or polypeptide more immunogenic or more stable.
117. The peptide or polypeptide of claim 116, wherein said at least one modification is selected from the group consisting of peptoid modification, semipeptoid modification, cyclic peptide modification, N-terminus modification, C terminus modification, peptide bond modification, backbone modification, and residue modification.

118. The peptide or polypeptide of any of claims 1, 31, 34, 37 or 67, for *ex vivo* purging of autologous bone marrow to remove abnormal cells.
119. A method of production of a targeting agent comprising the following steps:
- a) isolating and selecting one or more targeting molecules comprising a primary recognition site by a biopanning procedure directly on a target cell or by a biopanning procedure indirectly on a first target cell in a second but not in a first state and subsequently by a biopanning procedure directly on a second target cell to produce one or more said targeting molecules;
 - b) amplification, purification and identification of the one or more targeting molecules; and
 - c) construction of a targeting agent from the one or more targeting molecules or wherein the targeting agent can be a peptide, polypeptide, antibody or antibody fragment, or a multimer thereof.
120. The method of claim 119 wherein the targeting agent is coupled, attached, combined, linked, fused to, or in association with a pharmaceutical agent.
121. The method of claims 119 and 120 wherein the targeting agent is an anti-disease or anti-cancer agent.
122. The method of claim 120 wherein the pharmaceutical agent is selected from the group consisting of radioisotope, toxin, oligonucleotide, recombinant protein, antibody fragment, and anti-cancer agent.
123. The method of claim 122 wherein the radioisotope is selected from a group consisting of ¹¹¹indium, ¹¹³indium, ^{99m}rhenium, ¹⁰⁵rhenium, ¹⁰¹rhenium, ^{99m}technetium, ^{121m}tellurium, ^{122m}tellurium, ^{125m}tellurium, ¹⁶⁵thulium, ¹⁶⁷thulium, ¹⁶⁸thulium, ¹²³iodine, ¹²⁶iodine, ¹³¹iodine, ¹³³iodine, ^{81m}krypton, ³³xenon, ⁹⁰yttrium, ²¹³bismuth, ⁷⁷bromine, ¹⁸fluorine, ⁹⁵ruthenium, ⁹⁷ruthenium, ¹⁰³ruthenium, ¹⁰⁵ruthenium, ¹⁰⁷mercury, ²⁰³mercury, ⁶⁷gallium and ⁶⁸gallium.

124. The method of claim 122 wherein the toxin is selected from the group consisting of gelonin, *Pseudomonas exotoxin* (PE), PE40, PE38, Diphtheria, ricin, and modifications and derivatives thereof.
125. The method of claim 122 wherein the anti-cancer agent is selected from the group vincristine, cytarabine, (Ara-C), cyclophosphamide, prednisone, daunorubicin, idarubicin, fludarabine, chlorambucil, interferon alpha, hydroxyurea, temozolomide, thalidomide, bleomycin, and derivatives thereof.
126. A peptide or polypeptide having the formula or structure:
- $$A - X - B$$
- wherein X is a hypervariable CDR3 region of 3 to 30 amino acids; and A and B can each be amino acid chains from 1 to 1000 amino acids in length wherein A is the amino end and B is the carboxy end.
127. The peptide, of claim 126 wherein A is 150-250 amino acid residues and wherein B is 350-500 amino acid residues.
128. The peptide of claim 126 wherein the CDR3 region is 5-13 amino acid residues.
129. The peptide or polypeptide of claim 126 wherein X is an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24.
130. The peptide or polypeptide of claim 127 which is part of a larger or full antibody or a multimer.
131. A dimeric molecule comprising two peptides or polypeptides one of which is the peptide or polypeptide of claim 126.
132. A dimeric molecule comprising two peptides or polypeptides of claim 126 which are identical.
133. The dimeric molecule of claim 131 or claim 132 wherein X is an amino acid sequence selected from the group consisting of SEQ ID NOs:8-24.

134. A nucleic acid molecule encoding the peptide or polypeptide of claim 126 or the dimeric molecule of claim 130.
135. The method of claim 104, wherein the mammalian cell system comprises the SR α 5 promoter.
136. The method of claim 104, wherein the mammalian cell system comprises the CMV promoter.
137. A peptide or polypeptide substantially as described herein.
138. A peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment having enhanced binding characteristics so as to bind selectively and/or specifically to a target cell in favor of other cells, wherein the binding selectivity or specificity is primarily determined by a first hypervariable region, wherein the first hypervariable region is a CDR3 region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos 8 or 20, and wherein the Fv is a scFv or a dsFv, and optionally having one or more tags.
139. The peptide or polypeptide of claim 138, wherein the binding selectivity or specificity is secondarily influenced by a second hypervariable region, by a third hypervariable region, and/or by one or more of the upstream or downstream region flanking the first, the second and/or the third hypervariable regions.
140. The peptide or polypeptide of claim 138 wherein the peptide or polypeptide is a scFv having SEQ ID NO: 25 in which the first hypervariable region is a CDR3 region which is identical to SEQ ID NO: 8.
141. The peptide or polypeptide of claim 138 wherein the peptide or polypeptide is a scFv having SEQ ID NO: 203 in which the first hypervariable region is a CDR3 region which is identical to SEQ ID NO: 20.

142. The peptide or polypeptide of claim 138 wherein the scFv molecule comprises a straight or branched chain spacer of 20 or fewer amino acid residues.
143. The peptide or polypeptide of claim 142 wherein the spacer comprises SEQ ID NO: 123 or SEQ ID NO: 124.
144. The peptide or polypeptide of claim 138 wherein the target cell is an activated, excited, modified, changed, disturbed, abnormal, or diseased cell.
145. The peptide or polypeptide of claim 144, wherein the diseased cell is a cancer cell.
146. The peptide or polypeptide of claim 144 wherein the cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma cells.
147. The peptide or polypeptide of claim 146 wherein the cell is a leukemia or myeloma cell.
148. The peptide or polypeptide of claim 146 wherein the leukemia or myeloma cell is a B-cell malignancy.
149. The peptide or polypeptide of claim 147, wherein the leukemia cell is an acute myeloid leukemia cell or a B-cell malignancy.
150. The peptide or polypeptide of claim 138 further comprising a cassette of consecutive amino acids having an amino acid sequence selected from the group consisting of SEQ ID NOs:30-113, or having at least 90% amino acid similarity therewith, or fragment thereof, wherein the cassette or fragment provides a framework into which is built, inserted, attached coupled, combined, or fused a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NO:8 or 20.
151. The peptide or polypeptide of claim 150 wherein the cassette has an amino acid sequence selected from the group consisting of SEQ ID NOs:30-32,33, 37-39,41, 43, 45, 46, 48, 51, 54, 57, 59-68, 70, 71, 76-85, 87, 89-92, 94, 97,

- 99, 103, 106, 112, and 113, or having at least 90% amino acid similarity therewith.
152. The peptide or polypeptide of claim 150 wherein the cassette has the amino acid sequence of SEQ ID NO: 61, or has at least 90% amino acid similarity therewith.
153. The peptide or polypeptide of claim 152, wherein the cassette has the amino acid sequence of SEQ ID NO: 61, or has at least 90% amino acid similarity therewith.
154. The peptide or polypeptide of claim 152, wherein the seven carboxy-terminal amino acid residues of SEQ ID NO: 61 are replaced by the seven amino acid residues of SEQ ID NO: 122.
155. The peptide or polypeptide of claim 139, wherein the second and third hypervariable regions are a CDR2 and a CDR1 hypervariable region, respectively.
156. The peptide or polypeptide of claim 138, wherein the CDR3 region has the amino acid sequence SEQ ID NO: 8.
157. The peptide or polypeptide of claim 138, wherein the CDR3 region has the amino acid sequence SEQ ID NO: 20.
158. The peptide or polypeptide of claim 155 wherein the CDR2 and CDR1 regions have the amino acid sequences SEQ ID NO: 115 and SEQ ID NO: 114, respectively.
159. The peptide or polypeptide of claim 140, wherein the second and third hypervariable regions are a CDR2 and CDR1 hypervariable region respectively and wherein the CDR3, CDR2 and CDR1 regions have the amino acid sequences SEQ ID NOs: 8, 115 and 114, respectively.
160. The peptide or polypeptide of claim 140, wherein the second and third hypervariable regions are a CDR2 and CDR1 hypervariable region

respectively and wherein the CDR3, CDR2 and CDR1 regions have the amino acid sequences SEQ ID NOs: 20, 115 and 114, respectively.

161. The peptide or polypeptide of claim 139, wherein the upstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 117, and wherein the downstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 116.
162. The peptide or polypeptide of claim 139, wherein the second hypervariable region is a CDR2 hypervariable region and wherein the upstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 119, and wherein the downstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 118.
163. The peptide or polypeptide of claim 139 wherein the third hypervariable region is a CDR1 hypervariable region and wherein the upstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 121, and wherein the downstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 120.
164. The peptide or polypeptide of claim 155 wherein the CDR2 and CDR1 regions of a cassette of consecutive amino acids selected from the group consisting of SEQ ID NOs:30-113 or a fragment thereof are replaced by SEQ ID NOs:115 and 114, respectively.
165. The peptide or polypeptide of claim 155, wherein the CDR2 and CDR1 regions of a cassette of consecutive amino acids selected from the group consisting of SEQ ID NOs:30-32, 35, 37-39, 41, 43, 45, 46, 48, 51, 54, 57, 59-68, 70, 71, 76-85, 87, 89-92, 94, 97, 99, 103, 106, 112, and 113 or a fragment thereof are replaced by SEQ ID NOs:115 and 114, respectively.
166. The peptide or polypeptide of claim 139 wherein
 - (a) the second and third hypervariable regions are a CDR2 and a CDR1 hypervariable region, respectively,

- (b) the CDR3 amino acid sequence is SEQ ID NO: 8,
- (c) the CDR2 amino acid sequence is SEQ ID NO: 115,
- (d) the CDR1 amino acid sequence is SEQ ID NO: 114,
- (e) the upstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 117,
- (f) the downstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 116,
- (g) the upstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 119,
- (h) the downstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 118,
- (i) the upstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 121, and
- (j) the downstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 120.

167. The peptide or polypeptide of claim 139 wherein

- (a) the second and third hypervariable regions are a CDR2 and a CDR1 hypervariable region, respectively,
- (b) the CDR3 amino acid sequence is SEQ ID NO: 20,
- (c) the CDR2 amino acid sequence is SEQ ID NO: 115,
- (d) the CDR1 amino acid sequence is SEQ ID NO: 114,
- (e) the upstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 117,

- (f) the downstream region flanking the CDR3 region has the amino acid sequence of SEQ ID NO: 116,
 - (g) the upstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 119,
 - (h) the downstream region flanking the CDR2 region has the amino acid sequence of SEQ ID NO: 118,
 - (i) the upstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 121, and
 - (j) the downstream region flanking the CDR1 region has the amino acid sequence of SEQ ID NO: 120.
168. The peptide or polypeptide of claim 138 wherein the Fv is an scFv obtainable from a phage display library.
169. The peptide or polypeptide of claim 165, wherein the phage display library was constructed from peripheral blood lymphocytes of a non-immunized human, and wherein the scFv peptide is selected against previously uncharacterized and unpurified antigens on the surface of a target cell.
170. A method for selecting or identifying the peptide or polypeptide of claim 165 comprising biopanning, wherein the biopanning comprises binding phage to a target, removing non-bound phage, eluting bound phage, and propagating and amplifying eluted phage.
171. A peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment, having enhanced binding characteristics so as to bind selectively and/or specifically to a substantially exposed and/or over-expressed binding site on or in a target cell, wherein the binding to the target cell occurs in favor of other cells on or in which the binding site is not substantially available and/or expressed, wherein the binding selectivity or specificity is primarily determined by a first hypervariable region, wherein the first hypervariable region is a CDR3 region

consisting of SEQ ID Nos: 8 or 20, wherein the Fv is a scFv or a dsFv, and wherein the FV optionally has one or more tags.

172. The peptide or polypeptide of claim 171, wherein the binding selectivity or specificity is secondarily influenced by a second hypervariable region, by a third hypervariable region, and/or by one or more upstream or downstream region flanking the first, the second and/or the third hypervariable regions, and wherein the second and third hypervariable regions are a CDR2 and a CDR1 region, respectively.
173. A peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either, or a construct of a fragment having enhanced binding characteristics so as to bind selectively and/or specifically to a target cell in favor of other cells, wherein the Fv molecule comprises a first chain having a first, a second and a third hypervariable region and a second chain having a first, a second and a third hypervariable region, wherein one of the hypervariable regions of the first chain comprises a sequence of SEQ ID NOs:8 or 20, and wherein one of the hypervariable regions of the second chain has a sequence selected from the group consisting of SEQ ID NOs:1-6 and 125-202, and wherein the first, second and third hypervariable regions are a CDR3, CDR2 and CDR1 region, respectively, wherein the Fv is a scFv or a dsFv, and wherein the FV optionally has one or more tags.
174. The peptide or polypeptide of claim 173 wherein
- (a) the first hypervariable region of the first chain and the first hypervariable region of the second chain are identical and are selected from the group consisting of SEQ ID NOs:8 or 20; or
- (b) the first hypervariable region of the first chain is selected from the group consisting of SEQ ID NOs:8 or 20, and the first hypervariable region of the second chain is selected from the group consisting of SEQ ID NOs:1-6 and 125-202; or

(d) the first hypervariable region of the first chain is selected from the group consisting of SEQ ID NOs:1-6 and 125-202, and the first hypervariable region of the second chain is selected from the group consisting of SEQ ID NOs:8 or 20.

175. The peptide or polypeptide of claim 173, wherein the second and third hypervariable regions of the first chain are SEQ ID NOs:114 and 115, respectively.
176. A peptide or polypeptide comprising an Fv molecule, a construct thereof, a fragment of either or a construct of a fragment that
- (a) binds to an unknown ligand on a first cell having a first and a second state, wherein the binding is effective in the second state but is not substantially effective in the first state, and,
 - (b) by virtue of immuno-cross-reactivity, binds specifically or selectively to a ligand on a second cell, and wherein the Fv is a scFv or a dsFv, and wherein the Fv optionally has one or more tags, and wherein the first hypervariable region is a CDR3 region having an amino acid sequence selected from the group consisting of SEQ ID NOs 8 or 20.
177. The peptide or polypeptide of claim 176, wherein the first cell is a normal cell.
178. The peptide or polypeptide of claim 176, wherein the first state is a non-activated state and the second state is an activated, excited, modified, changed or disturbed state.
179. The peptide or polypeptide of claim 176, wherein the second cell is a diseased cell.
180. The peptide or polypeptide of claim 179, wherein the diseased cell is a cancer cell.

181. The peptide or polypeptide of claim 179 wherein the diseased cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma cells.
182. The peptide or polypeptide of claim 181, wherein the diseased cell is a leukemia cell.
183. The peptide or polypeptide of claim 182, wherein the leukemia cell is an acute myeloid leukemia cell.
184. The peptide or polypeptide of claim 176, wherein the selective and/or specific binding of the peptide or polypeptide to the ligand of the second cell is determined primarily by a first hypervariable region.
185. The peptide or polypeptide of claim 176, wherein the binding selectivity or specificity is secondarily influenced by a second hypervariable region, by a third hypervariable region, and/or by one or more of the upstream or downstream region flanking the first, the second, and the third hypervariable regions, respectively.
186. A ligand that is expressed by the second cell and that is capable of being bound by the peptide or polypeptide of claim 176.
187. A molecule that recognizes and binds the ligand of claim 186.
188. A nucleic acid molecule encoding the peptide or polypeptide according to any one of claims 138, 171, 173 or 176.
189. The nucleic acid molecule of claim 188, wherein the nucleic acid is DNA.
190. The peptide or polypeptide of claim 176 wherein the first and second states of the first cell are the same, and wherein the first cell is derived from a cell line.
191. The peptide or polypeptide of claim 190, wherein the cell line is selected from the group consisting of Jurkat, Molt-4, HS-602, U937, TF-1, THP-1, KG-1, and HUT-78.

192. The use of the peptide or polypeptide of claim 138 or claim 176, optionally in association with or attached, coupled, combined, linked, or fused to a pharmaceutical agent, in the manufacture of a medicament.
193. The use of claim 192, wherein the medicament has activity against a diseased cell.
194. The use of claim 193 wherein the diseased cell is a cancer cell.
195. The use of claim 193, wherein the cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma.
196. The use of claim 195 wherein the cell is a leukemia cell.
197. The use of claim 196, wherein the leukemia cell is an acute myeloid leukemia cell.
198. The peptide or polypeptide of claim 138 or claim 176, optionally in association with or attached, coupled, combined, linked or fused to a pharmaceutical agent, for use as a medicament.
199. The peptide or polypeptide of claim 198 wherein the medicament has activity against a diseased cell.
200. The peptide or polypeptide of claim 199 wherein the diseased cell is a cancer cell.
201. The peptide or polypeptide of claim 199, wherein the cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma cells.
202. The peptide or polypeptide of claim 201 wherein the cell is a leukemia cell.
203. The peptide or polypeptide of claim 202, wherein the leukemia cell is an acute myeloid leukemia cell.

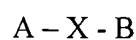
204. The use of the peptide or polypeptide of claim 138 or claim 176 for preparing a composition for use in inhibiting the growth of a diseased cell.
205. The use of the peptide or polypeptide of claim 204, wherein the cell is a leukemia cell.
206. The use of the peptide or polypeptide of claim 205 wherein the leukemia cell is an acute myeloid leukemia cell.
207. The use of the peptide or polypeptide of claim 138 or claim 176 for preparing a composition for use in inhibiting the growth of a cancer cell, said composition comprising at least one compound having a pharmaceutical ligand selective and/or specific for the cancer cell.
208. A composition comprising at least one peptide of claim 138 or claim 176, in association with, or attached, coupled, combined, linked, or fused to a pharmaceutical agent in a pharmaceutically effective amount and, optionally, a pharmaceutically effective carrier.
209. The composition of claim 208 wherein the peptide or polypeptide and the pharmaceutical agent are linked via a linker compound.
210. The composition of claim 209, wherein the linker compound is selected from the group consisting of a dicarboxylic acid, a maleimido hydrazide, PDPH, a carboxylic acid hydrazide, and a small peptide.
211. The composition of claim 210, wherein the small peptide is selected from a group consisting of AU1, AU5, BTag, c-myc, FLAG, Glu-Glu, HA, His6, HSV, HTTPHH, IRS, KT3, Protein C, S•Tag[®], T7, V5, and VSV-G.
212. The peptide or polypeptide according to any one of claims 138, 171, 173 and 176 wherein the tag is selected from a group consisting of: AU1, AU5, BTag, c-myc, FLAG, Glu-Glu, HA, His6, HSV, HTTPHH, IRS, KT3, Protein C, S•Tag[®], T7, V5, and VSV-G.

213. The composition of claim 208, wherein the pharmaceutical agent is selected from the group consisting of radioisotope, toxin, oligonucleotide, recombinant protein, antibody fragment, and anti-cancer agent.
214. The composition of claim 213 wherein the radioisotope is selected from a group consisting of indium, $^{111}\text{indium}$, $^{113}\text{indium}$, $^{99\text{m}}\text{rhenium}$, $^{105}\text{rhenium}$, $^{101}\text{rhenium}$, $^{99\text{m}}\text{technetium}$, $^{121\text{m}}\text{tellurium}$, $^{122\text{m}}\text{tellurium}$, $^{125\text{m}}\text{tellurium}$, $^{165}\text{thulium}$, $^{167}\text{thulium}$, $^{168}\text{thulium}$, $^{123}\text{iodine}$, $^{126}\text{iodine}$, $^{131}\text{iodine}$, $^{133}\text{iodine}$, $^{81\text{m}}\text{krypton}$, $^{33}\text{xenon}$, $^{90}\text{yttrium}$, $^{213}\text{bismuth}$, $^{77}\text{bromine}$, $^{18}\text{fluorine}$, $^{95}\text{ruthenium}$, $^{97}\text{ruthenium}$, $^{103}\text{ruthenium}$, $^{105}\text{ruthenium}$, $^{107}\text{mercury}$, $^{203}\text{mercury}$, $^{67}\text{gallium}$ and $^{68}\text{gallium}$.
215. The composition of claim 213, wherein the toxin is selected from the group consisting of gelonin, Pseudomonas exotoxin (PE), PE40, PE38, ricin, and modifications and derivatives thereof.
216. The composition of claim 213, wherein the anti-cancer agent is selected from the group consisting of doxorubicin, adriamycin, cis-platinum, taxol, calicheamicin, vincristine, cytarabine (Ara-C), cyclophosphazdde, prednisone, daunorubicin, idarubicin, fludarabine, chlorambucil, interferon alpha, hydroxyurea, temozolomide, thalidomide, bleomycin, and derivatives thereof.
217. A method of inhibiting the growth of a cell which comprises contacting the cell with an amount of the peptide or polypeptide of claim 138 or claim 176.
218. The method of claim 217 wherein the cell is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma.
219. The method of claim 218, wherein the cell is a leukemia cell.
220. The method of claim 219, wherein the leukemia cell is an acute myeloid leukemia cell.

221. A pharmaceutical composition comprising at least one peptide of claim 138 or claim 176 attached, coupled, combined, linked, or fused to an imaging agent for use in the diagnostic localization and imaging of a tumor.
222. A method of treating a patient suffering from a disease, which comprises administering to the patient an amount of the peptide or polypeptide of claim 138 or claim 176 effective to treat the disease.
223. The method of claim 222 wherein the disease is selected from the group consisting of carcinoma, sarcoma, leukemia, adenoma, lymphoma, myeloma, blastoma, seminoma, and melanoma.
224. The method of claim 223 wherein the disease is a leukemia.
225. The method of claim 224, wherein the leukemia is an acute myeloid leukemia.
226. The peptide or polypeptide of claim 138 or claim 176 wherein the Fv specifically or selectively binds to acute myeloid leukemia (AML) cells.
227. A ligand presented on AML cells bound by the peptide or polypeptide of claim 226.
228. A peptide or polypeptide that binds the ligand of claim 227.
229. A diagnostic kit for in vitro analysis of treatment efficacy before, during, or after treatment, comprising the peptide or polypeptide of claim 138 or claim 176 attached, coupled, combined, linked or fused to an indicative marker molecule.
230. The kit of claim 229 wherein the indicative marker molecule is a fluorescent marker.
231. The kit of claim 230, wherein the fluorescent marker is selected from the group consisting of fluorescein, rhodamine, phycoerythrin, and modifications and conjugates thereof.
232. The kit of claim 229 wherein the kit is used for diagnosis of cancer.

233. The peptide or polypeptide of claim 139 or claim 176, wherein the construct is an Ig polypeptide.
234. A method for producing the peptide or polypeptide of claim 233, wherein the Ig polypeptide is expressed as a recombinant polypeptide and is produced in a eukaryotic cell system.
235. The method of claim 234 wherein the eukaryotic system is a mammalian cell system.
236. The peptide or polypeptide of claim 233, wherein the Ig polypeptide is an IgG polypeptide.
237. The peptide or polypeptide of claim 236, wherein the IgG polypeptide comprises a CDR3, CDR2 and a CDR1 region having SEQ ID NOs:8, 115 and 114, respectively.
238. The peptide or polypeptide of claim 236, wherein the IgG polypeptide comprises a CDR3, CDR2 and a CDR1 region having SEQ ID Nos: 20, 115 and 114, respectively.
239. The IgG polypeptide of claim 237, wherein the CDR3, CDR2 and CDR1 regions are of the heavy chain.
240. The IgG polypeptide of claim 238, wherein the CDR3, CDR2 and CDR1 regions are of the heavy chain.
241. The IgG polypeptide of claim 237 wherein the CDR3, CDR2 and CDR1 regions are of the light chain.
242. The IgG polypeptide of claim 238, wherein the CDR3, CDR2 and CDR1 regions are of the light chain.
243. The IgG polypeptide of claim 233, wherein the IgG has a heavy chain comprising SEQ ID NO:26 and a light chain comprising SEQ ID NO:27 or chains having at least 90% amino acid similarity therewith.

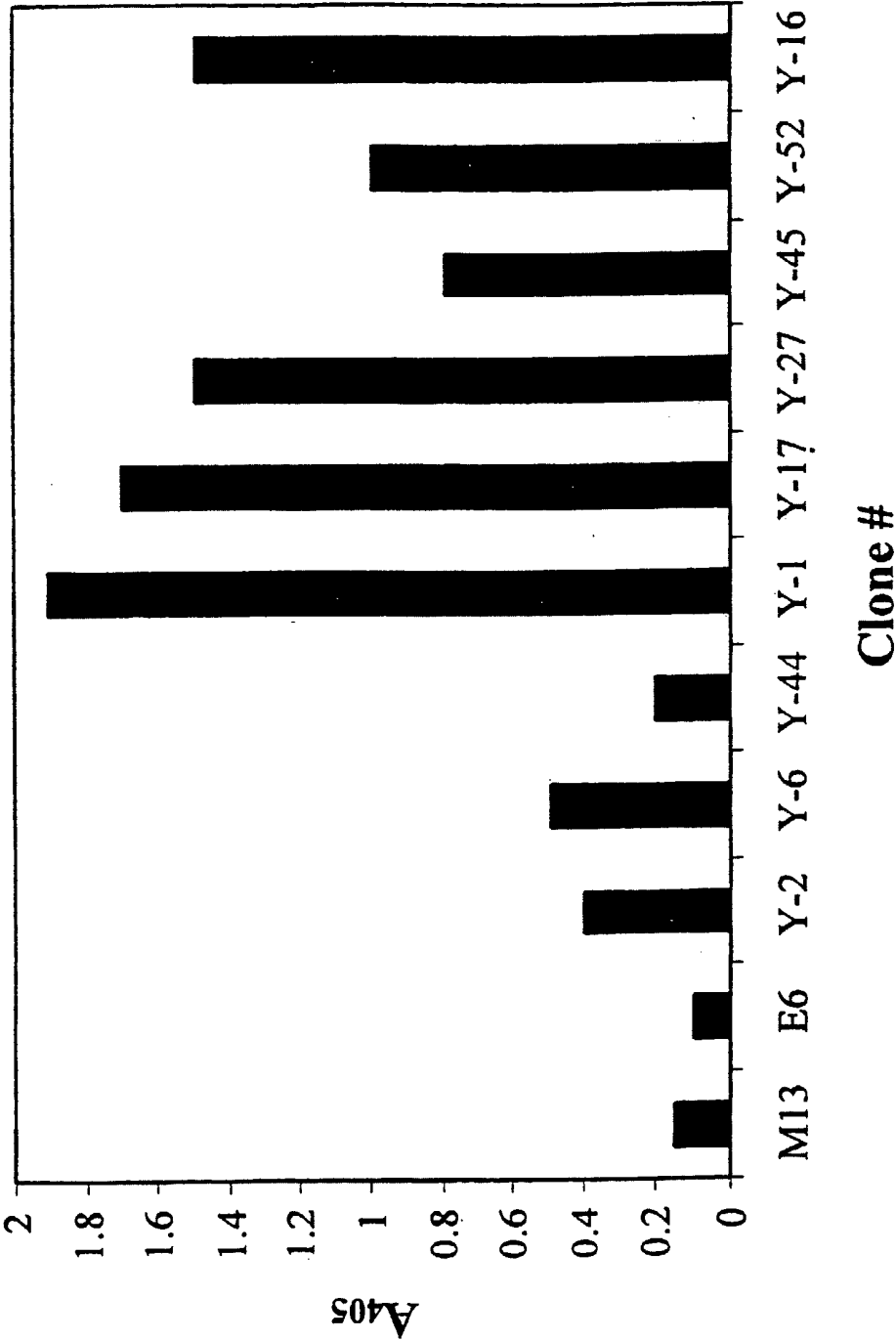
244. A method for producing the peptide or polypeptide of claim 139 or claim 176 wherein the peptide or polypeptide is produced in a prokaryotic cell system or in a eukaryotic cell system.
245. The method of claim 244, wherein the prokaryotic system comprises *E. coli*, said *E. coli* comprising an expression vector and the eukaryotic system is a mammalian cell system.
246. The method of claim 245, wherein the expression vector of the prokaryotic system comprises a promoter selected from the group consisting of *osmB*, *deo*, β -lac-U5, λP_L and CMV.
247. The peptide or polypeptide of any of claims 138 or 176, wherein the CDR3 comprises the amino acid sequence of R_1 -X Phe Pro- R_2 , wherein R_1 and R_2 each comprises 0- 15 amino acid residues, and wherein X is either Arg, Gly, or Lys.
248. The peptide or polypeptide of claim 138 or claim 176, wherein said peptide or polypeptide includes at least one non-naturally occurring modification.
249. The peptide or polypeptide of claim 248, wherein said non-naturally occurring modification renders the peptide or polypeptide more immunogenic or more stable.
250. The peptide or polypeptide of claim 249, wherein said at least one modification is selected from the group consisting of peptoid modification, semipeptoid modification, cyclic peptide modification, N-terminus modification, C terminus modification, peptide bond modification, backbone modification, and residue modification.
251. The peptide or polypeptide of any of claims 138, 171, 173, 176 or 198, for *ex vivo* purging of autologous bone marrow to remove abnormal cells.
252. A peptide or polypeptide having the formula or structure:



wherein X is a hypervariable CDR3 region comprising SEQ ID NO. 8 or 20; and A and B can each be amino acid chains from 1 to 1000 amino acids in length wherein A is the amino end and B is the carboxy end.

253. The peptide, of claim 252 wherein A is 150-250 amino acid residues and wherein B is 350-500 amino acid residues.
254. The peptide or polypeptide of claim 253 which is part of a larger or full antibody or a multimer.
255. A dimeric molecule comprising two peptides or polypeptides, one of which is the peptide or polypeptide of claim 252.
256. A dimeric molecule comprising two peptides or polypeptides of claim 252 which are identical.
257. A nucleic acid molecule encoding the peptide or polypeptide of claim 252 or the dimeric molecule of claim 254.
258. The method of claim 235, wherein the mammalian cell system comprises the SR α 5 promoter.
259. The method of claim 235, wherein the mammalian cell system comprises the CMV promoter.

Figure 1.



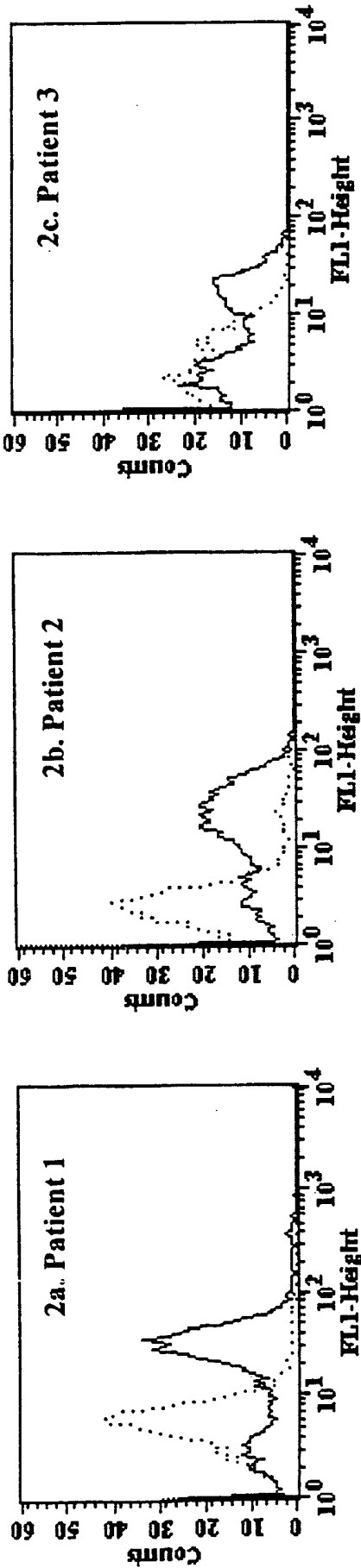
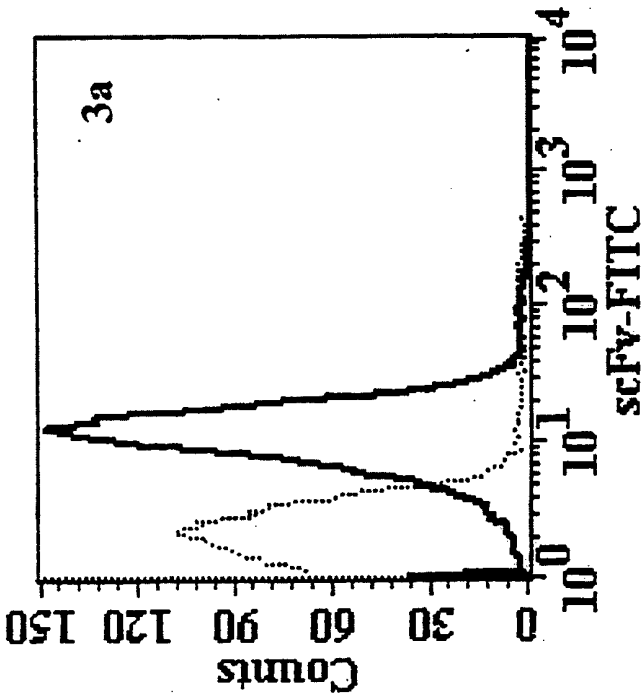


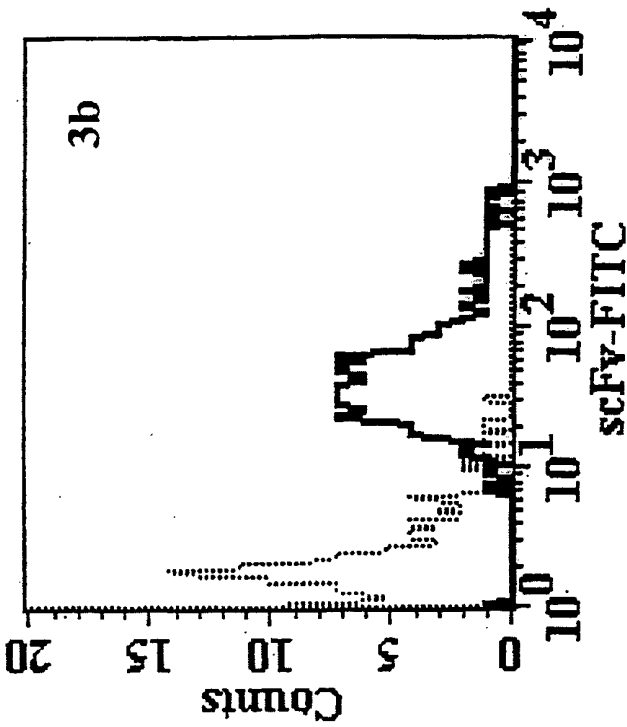
Figure 2.

Figure 3.

Platelets



Monocytes



— Y-1 scFv

..... Control scFv

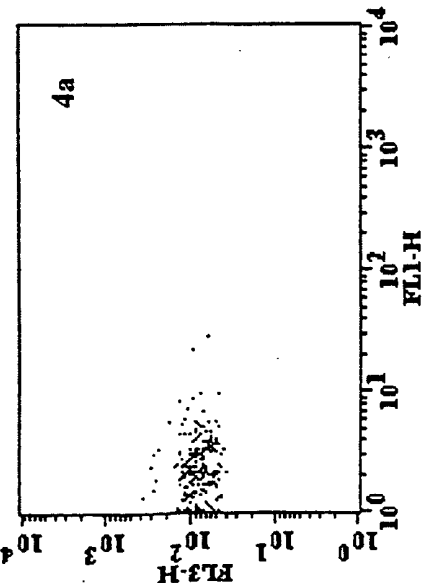
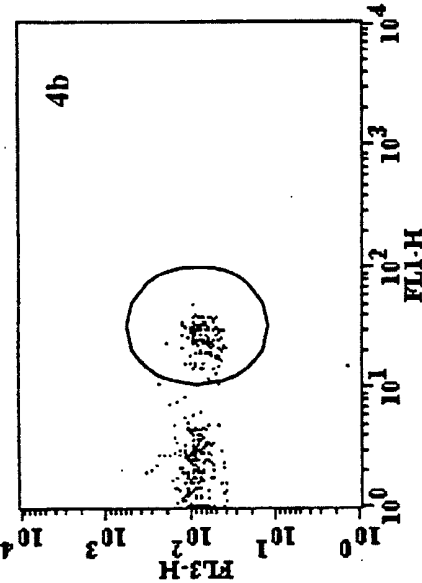
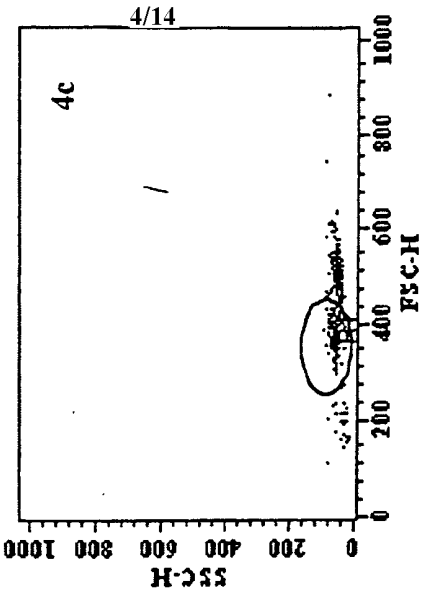
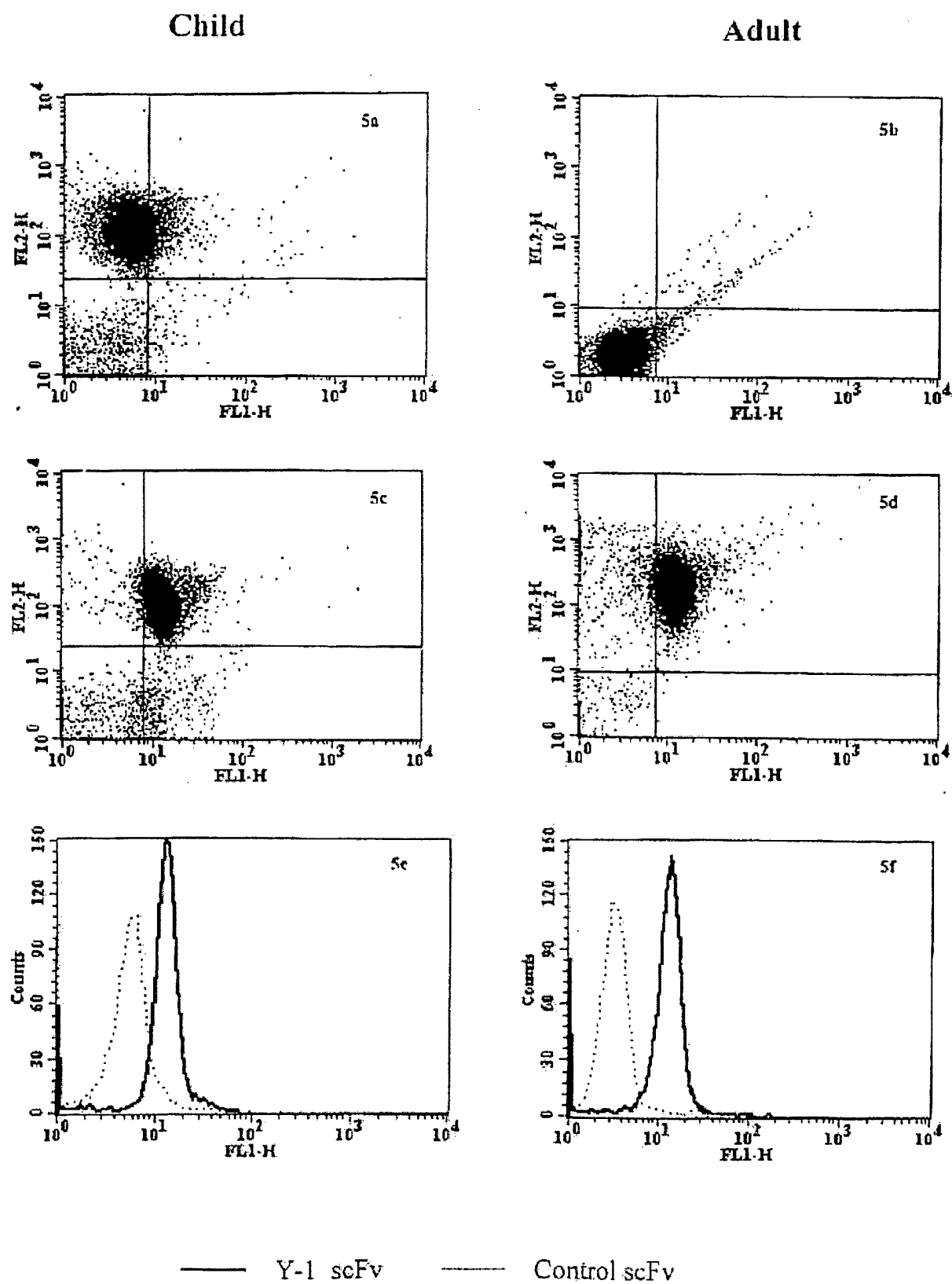


Figure 4.

Figure 5.



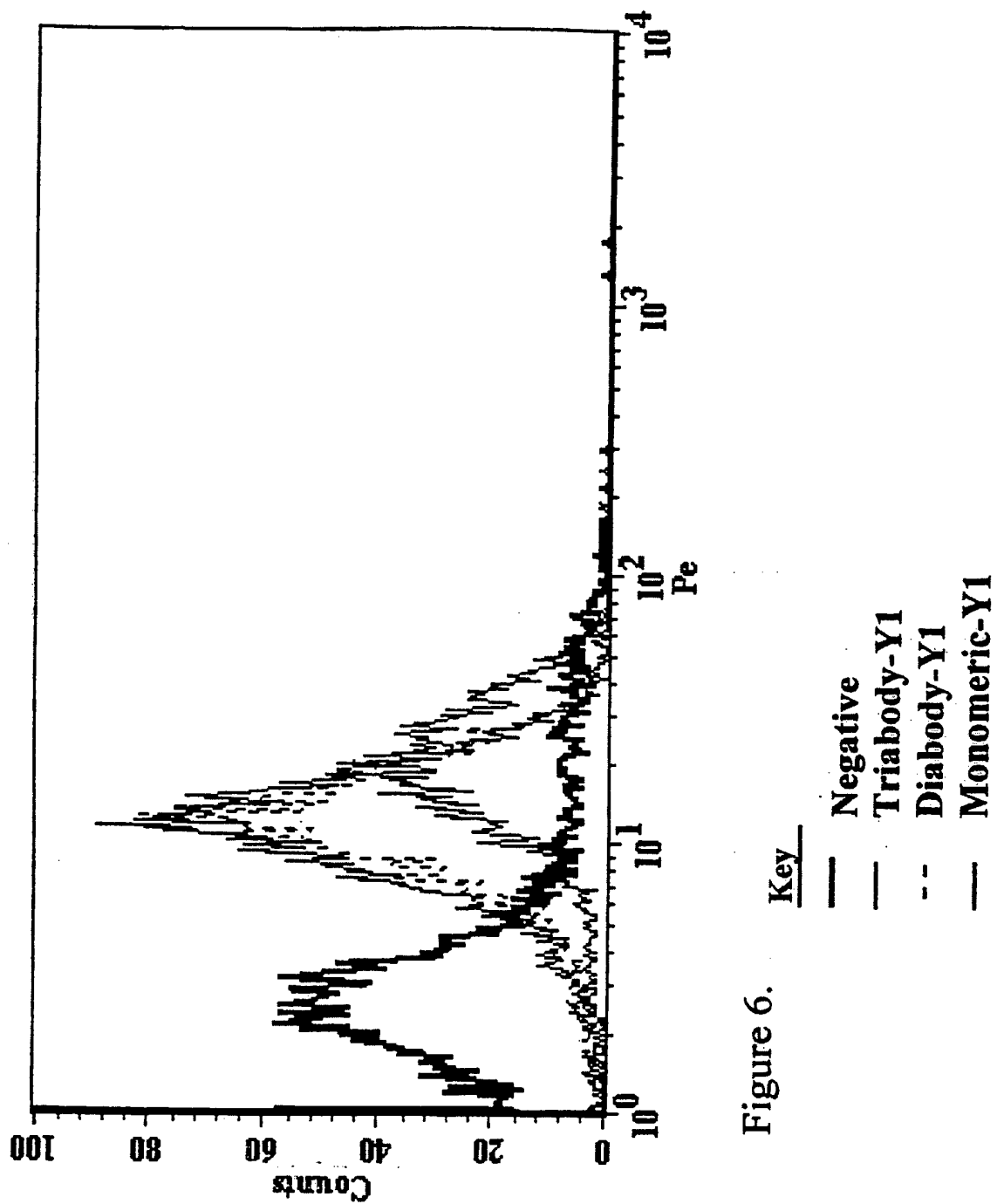


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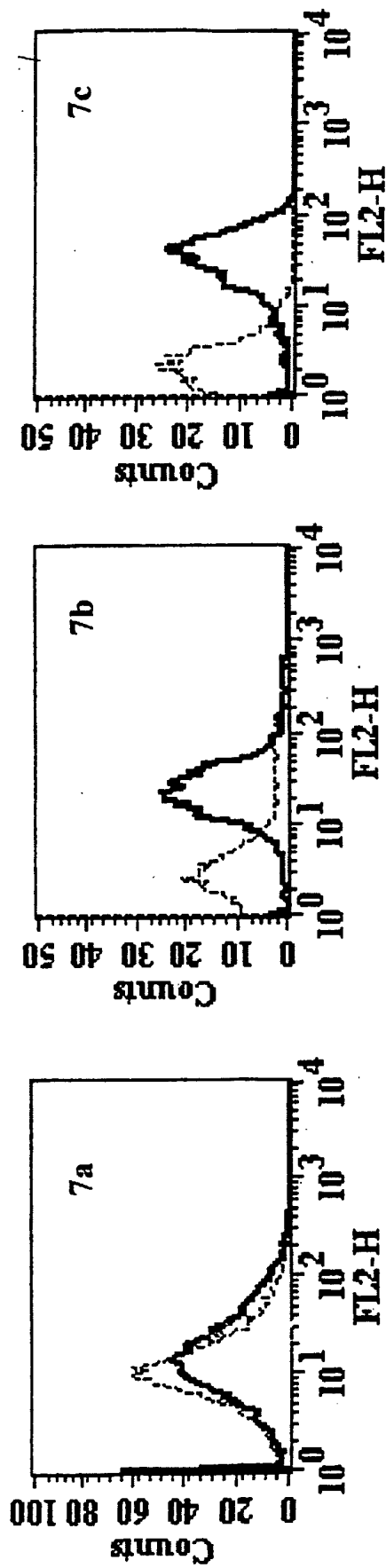


Figure 7.

Y-1 antibody

Control antibody

Figure 8

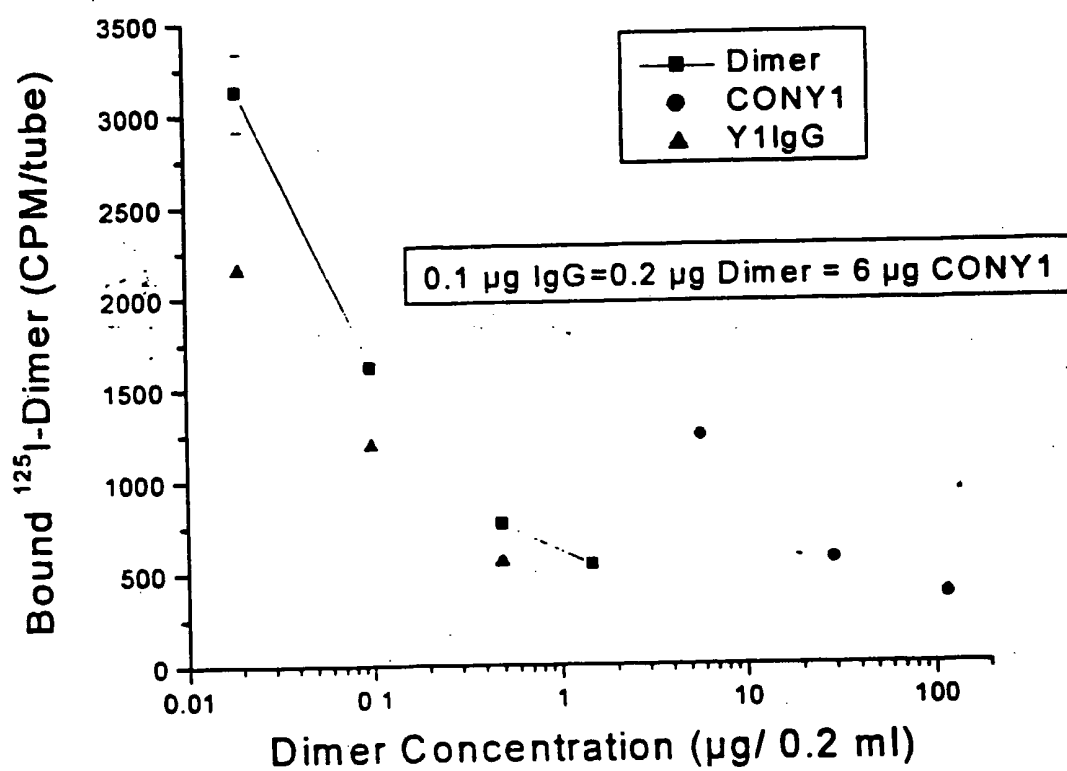
Fig. 1

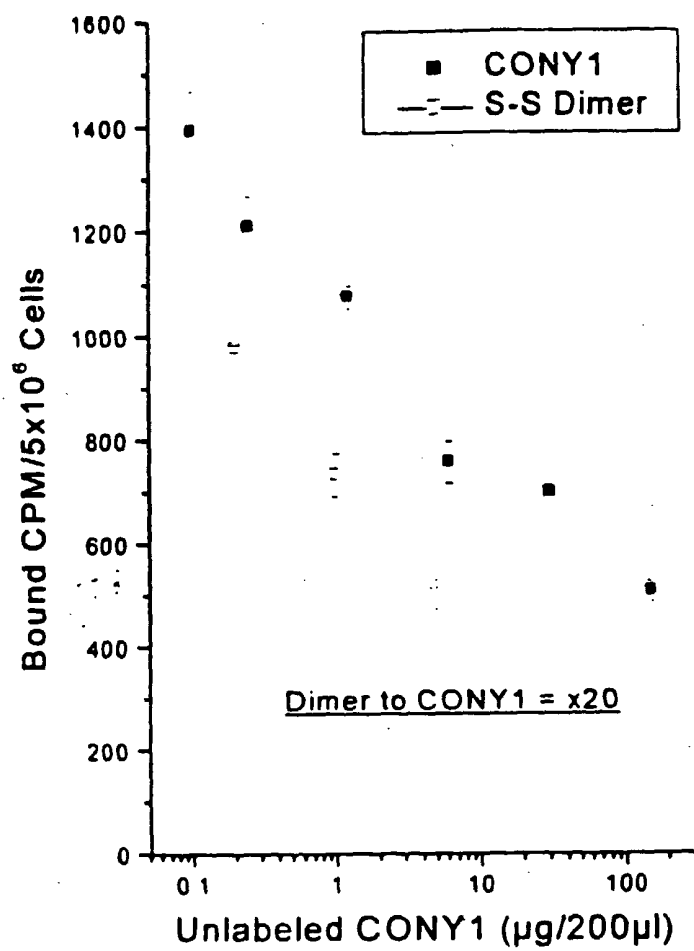
Figure 9

Figure 10

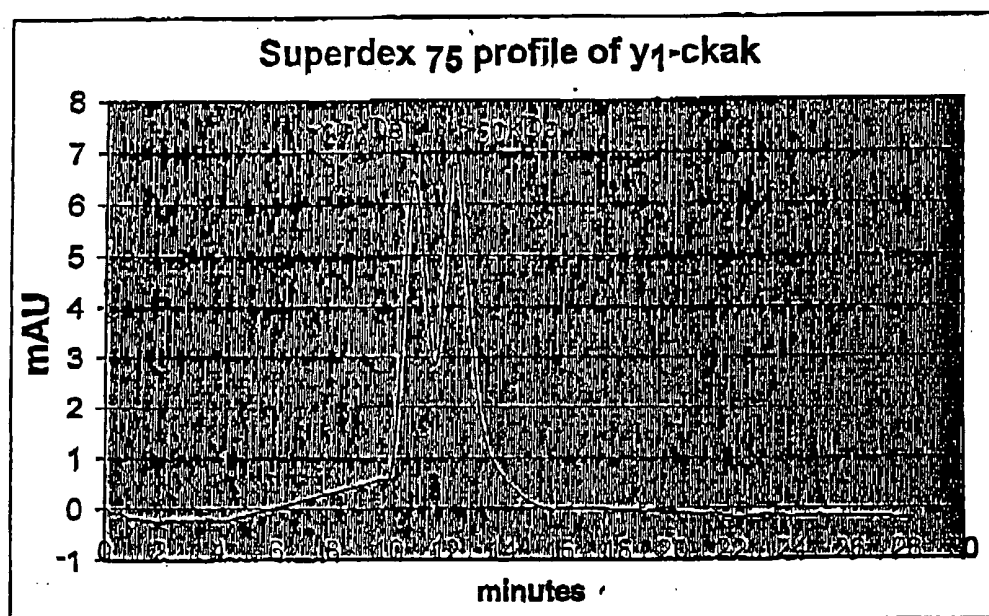


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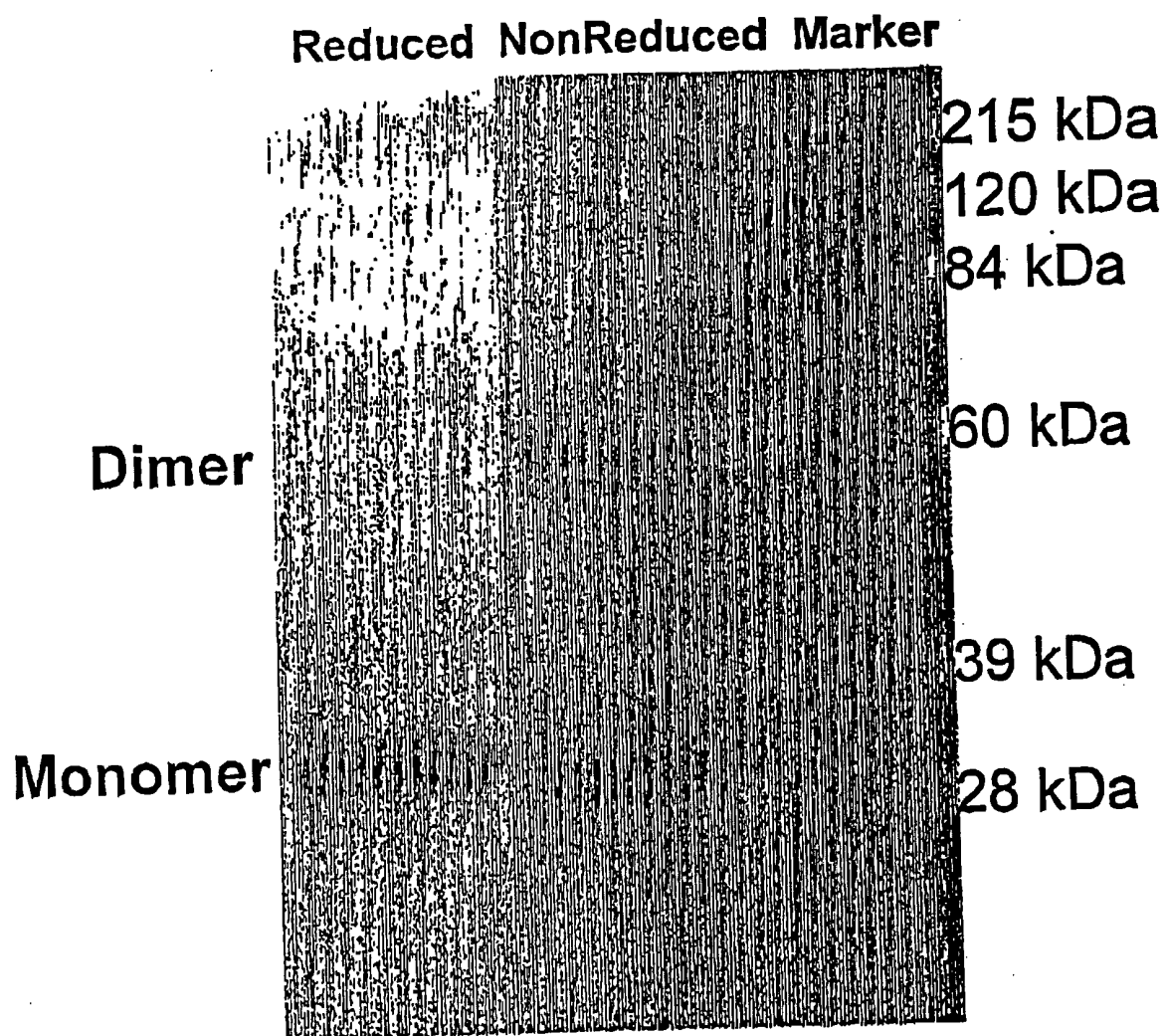
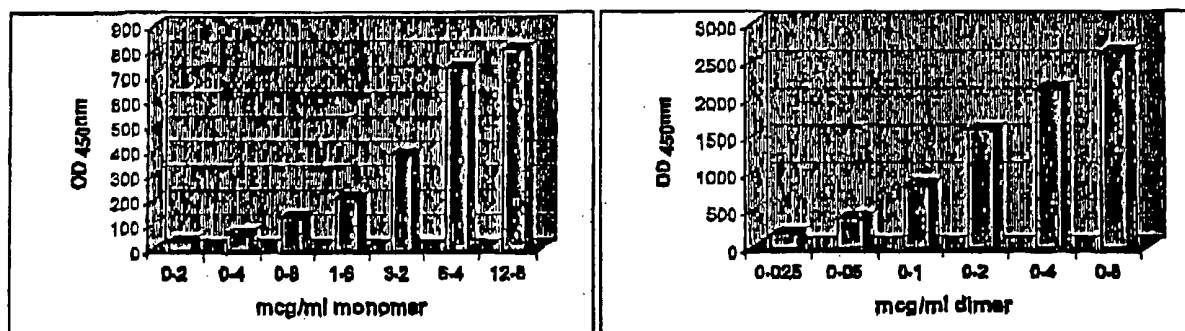


Figure 12



Epitopes of anti-GPIIb α antibodies

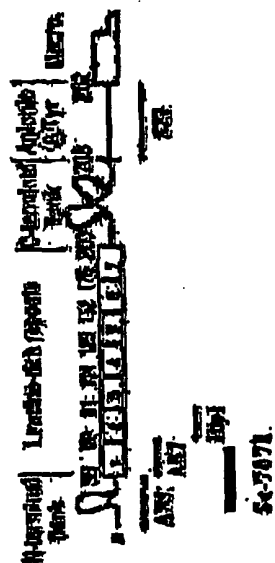
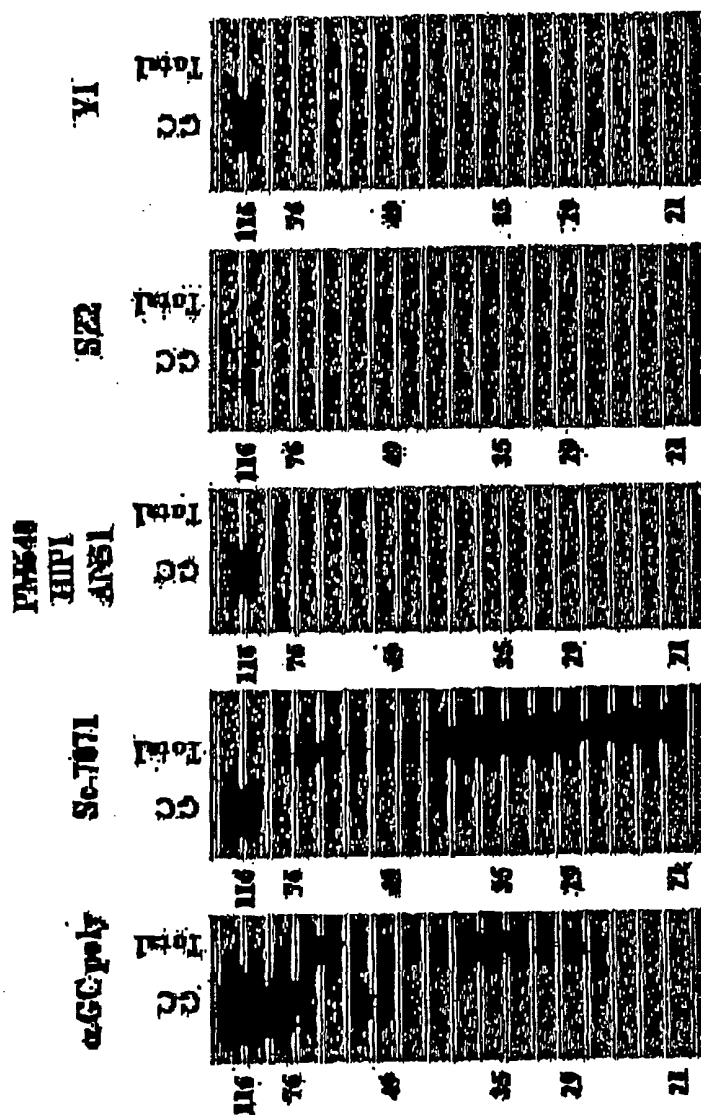


Figure 13

FIG. 14**Y17 scFv**

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<213> Homo sapiens

<400> 203

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Ala Gln Pro Ala Met Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly
20      25      30
Val Val Arg Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
35      40      45
Phe Thr Phe Asp Asp Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly
50      55      60
Lys Gly Leu Glu Trp Val Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr
65      70      75      80
Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
85      90      95
Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
100     105     110
Thr Ala Val Tyr Tyr Cys Ala Arg Leu Thr His Pro Tyr Phe Trp Gly
115     120     125
Gln Gly Thr Leu Val Thr Val Ser Arg Gly Gly Gly Gly Ser Gly Gly
130     135     140
Gly Gly Ser Gly Gly Gly Gly Ser Ser Glu Leu Thr Gln Asp Pro Ala
145     150     155     160
Val Ser Val Ala Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly Asp
165     170     175
Ser Leu Arg Ser Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln
180     185     190
Ala Pro Val Leu Val Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile
195     200     205
Pro Asp Arg Phe Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr
210     215     220
Ile Thr Gly Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser
225     230     235     240
Arg Asp Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu
245     250     255
Thr Val Leu Gly Ala Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp
260     265     270
Leu Asn Gly Ala Ala
275

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<213> Homo sapiens

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<213> Homo sapiens

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1				5				10	

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<213> Homo sapiens

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1 5

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

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Met Arg Ala Pro Val Ile
 1 5

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Pro Trp Asp Asp Val Thr Pro Pro
 1 5

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Arg Phe Pro Met Arg His Glu Lys Thr Asn Tyr
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1 5

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<400> 16

Lys Phe Pro Gly Gly Thr Val Arg Gly Leu Lys
1 5 10

<210> 17

<211> 12

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<400> 17

Gly	Phe	Pro	Val	Ile	Val	Glu	Glu	Arg	Gln	Ser	Thr
1				5					10		

<210> 18

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Arg	Phe	Pro	Gln	Arg	Val	Asp	Asn	Arg	Val
1				5					10

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Thr	Gly	Gln	Ser	Ile	Lys	Arg	Ser
1				5			

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Leu Thr His Pro Tyr Phe
1 5

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1 5

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1 5 10

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Arg Tyr Tyr Cys Arg Ser Ser Asp Cys Thr Val Ser
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 20 25 30

Val Val Arg Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
 35 40 45

Phe Thr Phe Asp Asp Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly
 50 55 60

Lys Gly Leu Glu Trp Val Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr
 65 70 75 80

Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
 85 90 95

Ala Lys Asn Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp
 100 105 110

Thr Ala Val Tyr Tyr Cys Ala Arg Met Arg Ala Pro Val Ile Trp Gly
 115 120 125

Gln Gly Thr Leu Val Thr Val Ser Arg Gly Gly Gly Gly Ser Gly Gly
 130 135 140

Gly Gly Ser Gly Gly Gly Gly Ser Ser Glu Leu Thr Gln Asp Pro Ala
 145 150 155 160

Val Ser Val Ala Leu Gly Gln Thr Val Arg Ile Thr Cys Gln Gly Asp
 165 170 175

Ser Leu Arg Ser Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln
 180 185 190
 Ala Pro Val Leu Val Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile
 195 200 205
 Pro Asp Arg Phe Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr
 210 215 220
 Ile Thr Gly Ala Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser
 225 230 235 240
 Arg Asp Ser Ser Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu
 245 250 255
 Thr Val Leu Gly Ala Ala Ala Glu Gln Lys Leu Ile Ser Glu Glu Asp
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 Leu Asn Gly Ala Ala
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<213> Homo sapiens

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

Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro
 130 135 140
 Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly
 145 150 155 160
 Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn
 165 170 175
 Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln
 180 185 190
 Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser
 195 200 205
 Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser
 210 215 220
 Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr
 225 230 235 240
 His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser
 245 250 255
 Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
 260 265 270
 Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro
 275 280 285
 Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala
 290 295 300
 Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val
 305 310 315 320
 Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr
 325 330 335
 Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr
 340 345 350
 Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu
 355 360 365
 Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys
 370 375 380
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser
 385 390 395 400
 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Ser Pro Val Leu Asp
 405 410 415
 Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser
 420 425 430
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala

435	440	445
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser	Leu Ser Leu Gly Lys	
450	455	460

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35	45
Tyr Tyr Ala Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu	
50	60
Val Ile Tyr Gly Lys Asn Asn Arg Pro Ser Gly Ile Pro Asp Arg Phe	
65	80
Ser Gly Ser Ser Ser Gly Asn Thr Ala Ser Leu Thr Ile Thr Gly Ala	
85	95
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Asn Ser Arg Asp Ser Ser	
100	110
Gly Asn His Val Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly	
115	125
Gln Pro Lys Ala Ala Pro Ser Val Thr Leu Phe Pro Pro Ser Ser Glu	
130	140
Glu Leu Gln Ala Asn Lys Ala Thr Leu Val Cys Leu Ile Ser Asp Phe	
145	160
Tyr Pro Gly Ala Val Thr Val Ala Trp Lys Ala Asp Ser Ser Pro Val	
165	175
Lys Ala Gly Val Glu Thr Thr Thr Pro Ser Lys Gln Ser Asn Asn Lys	
180	190
Tyr Ala Ala Ser Ser Tyr Leu Ser Leu Thr Pro Glu Gln Trp Lys Ser	
195	205
His Lys Ser Tyr Ser Cys Gln Val Thr His Glu Gly Ser Thr Val Glu	

210 215 220
 Lys Thr Val Ala Pro Thr Glu Cys Ser
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Phe Leu Thr Tyr Asn Ser Tyr Glu Val Pro Thr
 1 5 10

<210> 29

<211> 9

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<213> Homo sapiens

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Thr Asn Trp Tyr Leu Arg Pro Leu Asn
 1 5

<210> 30

<211> 98

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<213> Homo sapiens

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

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

Tyr Met His Trp Val Gln Gln Ala Pro Gly Lys Gly Leu Glu Trp Met
 35 40 45
 Gly Leu Val Asp Pro Glu Asp Gly Glu Thr Ile Tyr Ala Glu Lys Phe
 50 55 60
 Gln Gly Arg Val Thr Ile Thr Ala Asp Thr Ser Thr Asp Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Thr

<210> 31

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<213> Homo sapiens

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

<210> 32

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

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

Ala Arg

<210> 34

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<213> Homo sapiens

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

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

Tyr	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
		35					40					45			

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

Gln	Gly	Arg	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser	Ile	Ser	Thr	Ala	Tyr
65					70					75					80

Met	Glu	Leu	Ser	Arg	Leu	Arg	Ser	Asp	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Ala Arg

<210> 35

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<213> Homo sapiens

<400> 35

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

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

Tyr	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

35							40				45					
Gly	Trp	Ile	Asn	Pro	Asn	Ser	Gly	Gly	Thr	Asn	Tyr	Ala	Gln	Lys	Phe	
	50					55					60					
Gln	Gly	Trp	Val	Thr	Met	Thr	Arg	Asp	Thr	Ser	Ile	Ser	Thr	Ala	Tyr	
65					70					75					80	
Met	Glu	Leu	Ser	Arg	Leu	Arg	Ser	Asp	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	
				85					90					95		
Ala	Arg															

<210> 36

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<223> Xaa

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 20 25 30

Cys Met His Trp Val Arg Gln Val His Ala Gln Gly Leu Glu Trp Met
 35 40 45

Gly Leu Val Cys Pro Ser Asp Gly Ser Thr Ser Tyr Ala Gln Lys Phe
 50 55 60

Gln Ala Arg Val Thr Ile Thr Arg Asp Thr Ser Met Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

Val Arg

<210> 38

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<213> Homo sapiens

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 1 5 10 15

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 20 25 30

Ala Val Gln Trp Val Arg Gln Ala Arg Gly Gln Arg Leu Glu Trp Ile
 35 40 45

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

Gln Glu Arg Val Thr Ile Thr Arg Asp Met Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Ala

<210> 39

<211> 98

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<213> Homo sapiens

<400> 39

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

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

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35 40 45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 40

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<213> Homo sapiens

<400> 40

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

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

<210> 41

<211> 98

<212> PRT

<213> Homo sapiens

<400> 41

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

<210> 42

<211> 98

<212> PRT

<213> Homo sapiens

<400> 42

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

<210> 43

<211> 98

<212> PRT

<213> Homo sapiens

<400> 43

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

85

90

95

Ala Arg

<210> 44

<211> 98

<212> PRT

<213> Homo sapiens

<400> 44

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

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

Ala Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

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

Thr Gly Arg Phe Val Phe Ser Leu Asp Thr Ser Val Ser Thr Ala Tyr
 65 70 75 80

Leu Gln Ile Ser Ser Leu Lys Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 45

<211> 98

<212> PRT

<213> Homo sapiens

<400> 45

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

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

Asp Ile Asn Trp Val Arg Gln Ala Thr Gly Gln Gly Leu Glu Trp Met

35 40 45
 Gly Trp Met Asn Pro Asn Ser Gly Asn Thr Gly Tyr Ala Gln Lys Phe
 50 55 60
 Gln Gly Arg Val Thr Met Thr Arg Asn Thr Ser Ile Ser Thr Ala Tyr
 65 70 75 80
 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 46

<211> 98

<212> PRT

<213> Homo sapiens

<400> 46

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

<210> 47

<211> 92

<212> PRT

<213> Homo sapiens

<400> 47

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

<210> 48

<211> 98

<212> PRT

<213> Homo sapiens

<400> 48

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

Ala Arg

<210> 49

<211> 98

<212> PRT

<213> Homo sapiens

<400> 49

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

<210> 50

<211> 98

<212> PRT

<213> Homo sapiens

<400> 50

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

85

90

95

Ala Arg

<210> 51

<211> 98

<212> PRT

<213> Homo sapiens

<400> 51

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

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

Tyr Leu His Trp Val Arg Gln Ala Pro Gly Gln Ala Leu Glu Trp Met
 35 40 45

Gly Trp Ile Thr Pro Phe Asn Gly Asn Thr Asn Tyr Ala Gln Lys Phe
 50 55 60

Gln Asp Arg Val Thr Ile Thr Arg Asp Arg Ser Met Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 52

<211> 96

<212> PRT

<213> Homo sapiens

<400> 52

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

Thr Leu Thr Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Ser Asn Ala
 20 25 30

Arg Met Gly Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
 35 40 45

Trp Leu Ala His Ile Phe Ser Asn Asp Glu Lys Ser Tyr Ser Thr Ser
 50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Ser Gln Val
 65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
 85 90 95

<210> 53

<211> 99

<212> PRT

<213> Homo sapiens

<400> 53

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

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30

Glu Trp Cys Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu Trp
 35 40 45

Leu Ala Leu Ile Tyr Trp Asn Asp Asp Lys Arg Tyr Ser Pro Ser Leu
 50 55 60

Lys Ser Arg Leu Thr Ile Thr Lys Asp Thr Ser Lys Asn Gln Val Val
 65 70 75 80

Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr Cys
 85 90 95

Ala His Arg

<210> 54

<211> 96

<212> PRT

<213> Homo sapiens

<400> 54

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
 1 5 10 15

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

<210> 55

<211> 96

<212> PRT

<213> Homo sapiens

<400> 55

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

<210> 56

<211> 100

<212> PRT

<213> Homo sapiens

<400> 56

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

<210> 57

<211> 100

<212> PRT

<213> Homo sapiens

<400> 57

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

<210> 58

<211> 100

<212> PRT

<213> Homo sapiens

<400> 58

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

<210> 59

<211> 100

<212> PRT

<213> Homo sapiens

<400> 59

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

85

90

95

Tyr Cys Ala Arg
100

<210> 60

<211> 98

<212> PRT

<213> Homo sapiens

<400> 60

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45

Ser Gly Ile Ser Trp Asn Ser Gly Ser Ile Gly Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

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

Ala Lys

<210> 61

<211> 98

<212> PRT

<213> Homo sapiens

<400> 61

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Arg Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp Asp Tyr
20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr His Cys
 85 90 95
 Ala Arg

<210> 62

<211> 98

<212> PRT

<213> Homo sapiens

<400> 62

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

<210> 63

<211> 98

<212> PRT

<213> Homo sapiens

<400> 63

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

<210> 64

<211> 100

<212> PRT

<213> Homo sapiens

<400> 64

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

Tyr Cys Thr Thr
100

<210> 65

<211> 98

<212> PRT

<213> Homo sapiens

<400> 65

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

<210> 66

<211> 98

<212> PRT

<213> Homo sapiens

<400> 66

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn His
20 25 30
Tyr Thr Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val

35						40					45				
Ser	Tyr	Ser	Ser	Gly	Asn	Ser	Gly	Tyr	Thr	Asn	Tyr	Ala	Asp	Ser	Val
	50					55					60				
Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn	Ser	Leu	Tyr
65					70					75					80
Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	
Val	Lys														

<210> 67

<211> 98

<212> PRT

<213> Homo sapiens

<400> 67

[illegible]

<210> 68

<211> 97

<212> PRT

<213> Homo sapiens

<400> 68

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

Arg

<210> 69

<211> 97

<212> PRT

<213> Homo sapiens

<400> 69

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

Arg

<210> 70

<211> 97

<212> PRT

<213> Homo sapiens

<400> 70

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

Arg

<210> 71

<211> 97

<212> PRT

<213> Homo sapiens

<400> 71

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

Ser Ala Ile Gly Thr Gly Gly Gly Thr Tyr Tyr Ala Asp Ser Val Lys
50 55 60

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
65 70 75 80

Gln Met Asn Ser Leu Arg Ala Glu Asp Met Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

<210> 72

<211> 98

<212> PRT

<213> Homo sapiens

<400> 72

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

Ala Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Tyr Val
35 40 45

Ser Ala Ile Ser Ser Asn Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

Val Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Val Arg

<210> 73

<211> 35

<212> PRT

<213> Homo sapiens

<400> 73

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

<210> 74

<211> 98

<212> PRT

<213> Homo sapiens

<400> 74

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

<210> 75

<211> 98

<212> PRT

<213> Homo sapiens

<400> 75

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

<210> 76

<211> 98

<212> PRT

<213> Homo sapiens

<400> 76

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

<210> 77

<211> 98

<212> PRT

<213> Homo sapiens

<400> 77

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

<210> 78

<211> 97

<212> PRT

<213> Homo sapiens

<400> 78

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

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

Gln Met Asn Ser Leu Arg Ala Gly Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg

<210> 79

<211> 98

<212> PRT

<213> Homo sapiens

<400> 79

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30

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

Ser Tyr Ile Ser Ser Ser Gly Ser Thr Ile Tyr Tyr Ala Asp Ser Val
50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg

<210> 80

<211> 98

<212> PRT

<213> Homo sapiens

<400> 80

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg

<210>	81
<211>	98
<212>	PRT
<213>	Homo sapiens

<210> 82

<211> 98

<212> PRT

<213> Homo sapiens

<400> 82

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

<210> 83

<211> 97

<212> PRT

<213> Homo sapiens

<400> 83

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

Gln Met Asn Ser Leu Ile Ala Glu Asp Met Ala Val Tyr Tyr Cys Ala
 85 90 95

Arg

<210> 84

<211> 98

<212> PRT

<213> Homo sapiens

<400> 84

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Trp Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Val Trp Val
 35 40 45

Ser Arg Ile Asn Ser Asp Gly Ser Ser Thr Thr Tyr Ala Asp Ser Val
 50 55 60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg

<210> 85

<211> 98

<212> PRT

<213> Homo sapiens

<400> 85

Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

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

<210> 86

<211> 97

<212> PRT

<213> Homo sapiens

<400> 86

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

<210> 87

<211> 97

<212> PRT

<213> Homo sapiens

<400> 87

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

Arg

<210> 88

<211> 97

<212> PRT

<213> Homo sapiens

<400> 88

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

85

90

95

Arg

<210> 89

<211> 99

<212> PRT

<213> Homo sapiens

<400> 89

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

Thr Leu Ser Leu Thr Cys Ala Val Ser Gly Gly Ser Ile Ser Ser Gly
 20 25 30

Gly Tyr Ser Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
 35 40 45

Trp Ile Gly Tyr Ile Tyr His Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
 50 55 60

Leu Lys Ser Arg Val Thr Ile Ser Val Asp Arg Ser Lys Asn Gln Phe
 65 70 75 80

Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
 85 90 95

Cys Ala Arg

<210> 90

<211> 99

<212> PRT

<213> Homo sapiens

<400> 90

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

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
 20 25 30

Gly Tyr Tyr Trp Ser Trp Ile Arg Gln His Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
 65 70 75 80
 Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg

<210> 91

<211> 99

<212> PRT

<213> Homo sapiens

<400> 91

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

<210> 92

<211> 98

<212> PRT

<400> 92

[illegible]

<210> 93

<211> 98

<212> PRT

<213> Homo sapiens

<400> 93

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

Ala Arg

<210> 94

<211> 98

<212> PRT

<213> Homo sapiens

<400> 94

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

Thr	Leu	Ser	Leu	Thr	Cys	Ala	Val	Ser	Gly	Tyr	Ser	Ile	Ser	Ser	Ser
			20					25					30		

Asn	Trp	Trp	Gly	Trp	Ile	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp
		35					40					45			

Ile	Gly	Tyr	Ile	Tyr	Tyr	Ser	Gly	Ser	Thr	Tyr	Tyr	Asn	Pro	Ser	Leu
	50					55					60				

Lys	Ser	Arg	Val	Thr	Met	Ser	Val	Asp	Thr	Ser	Lys	Asn	Gln	Phe	Ser
65					70					75					80

Leu	Lys	Leu	Ser	Ser	Val	Thr	Ala	Val	Asp	Thr	Ala	Val	Tyr	Tyr	Cys
				85					90					95	

Ala Arg

<210> 95

<211> 98

<212> PRT

<213> Homo sapiens

<400> 95

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

Thr	Leu	Ser	Leu	Thr	Cys	Ala	Val	Ser	Gly	Tyr	Ser	Ile	Ser	Ser	Ser
			20					25					30		

Asn	Trp	Trp	Gly	Trp	Ile	Arg	Gln	Pro	Pro	Gly	Lys	Gly	Leu	Glu	Trp
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

35 40 45
 Ile Gly Tyr Ile Tyr Tyr Ser Gly Ser Ile Tyr Tyr Asn Pro Ser Leu
 50 55 60
 Lys Ser Arg Val Thr Met Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
 65 70 75 80
 Leu Lys Leu Ser Ser Val Thr Ala Val Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 Ala Arg

<210> 96

<211> 98

<212> PRT

<213> Homo sapiens

<400> 96

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

<210> 97

<211> 98

<212> PRT

<213> Homo sapiens

<400> 97

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

<210> 98

<211> 98

<212> PRT

<213> Homo sapiens

<400> 98

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

<210> 99

<211> 98

<212> PRT

<213> Homo sapiens

<400> 99

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

<210> 100

<211> 99

<212> PRT

<213> Homo sapiens

<400> 100

Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
 20 25 30
 Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
 35 40 45

Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe
 65 70 75 80
 Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg

<210> 101
 <211> 99
 <212> PRT
 <213> Homo sapiens

<400> 101
 Gln Leu Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
 1 5 10 15
 Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser
 20 25 30
 Ser Tyr Tyr Trp Gly Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu
 35 40 45
 Trp Ile Gly Ser Ile Tyr Tyr Ser Gly Ser Thr Tyr Tyr Asn Pro Ser
 50 55 60
 Leu Lys Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn His Phe
 65 70 75 80
 Ser Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg

<210> 102
 <211> 97
 <212> PRT
 <213> Homo sapiens

<400> 102

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

Arg

<210> 103

<211> 97

<212> PRT

<213> Homo sapiens

<400> 103

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

Arg

<210> 104

<211> 97

<212> PRT

<213> Homo sapiens

<400> 104

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

Arg

<210> 105

<211> 97

<212> PRT

<213> Homo sapiens

<400> 105

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

Arg

<210> 106

<211> 98

<212> PRT

<213> Homo sapiens

<400> 106

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

Ala Arg

<210> 107

<211> 98

<212> PRT

<213> Homo sapiens

<400> 107

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

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Pro Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg

<210> 108

<211> 98

<212> PRT

<213> Homo sapiens

<400> 108

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

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
20 25 30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg

<210> 109

<211> 98

<212> PRT

<213> Homo sapiens

<400> 109

Glu Val Gln Leu Leu Gln Ser Ala Ala Glu Val Lys Arg Pro Gly Glu
1 5 10 15

Ser Leu Arg Ile Ser Cys Lys Thr Ser Gly Tyr Ser Phe Thr Ser Tyr

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

<210> 110

<211> 98

<212> PRT

<213> Homo sapiens

<400> 110

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

Ala Arg

<210> 111

<211> 98

<212> PRT

<213> Homo sapiens

<400> 111

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

Ser Leu Arg Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
20 25 30

Trp Ile Ser Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Arg Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Ser Pro Ser Phe
50 55 60

Gln Gly His Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg

<210> 112

<211> 101

<212> PRT

<213> Homo sapiens

<400> 112

Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Ser Val Ser Ser Asn
20 25 30

Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Ser Arg Gly Leu Glu
35 40 45

Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Asn Asp Tyr Ala
50 55 60

Val Ser Val Lys Ser Arg Ile Thr Ile Asn Pro Asp Thr Ser Lys Asn
65 70 75 80

Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val
85 90 95

Tyr Tyr Cys Ala Arg
100

<210> 113

<211> 87

<212> PRT

<213> Homo sapiens

<400> 113

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

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<210> 114

<211> 5

<212> PRT

<213> Homo sapiens

<400> 114

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Asp Tyr Gly Met Ser
1           5

```

<210> 115

<211> 17

<212> PRT

<213> Homo sapiens

<400> 115

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Gly Ile Asn Trp Asn Gly Gly Ser Thr Gly Tyr Ala Asp Ser Val Lys
1           5           10           15

```

Gly

<210> 116

<211> 11

<212> PRT

<213> Homo sapiens

<400> 116

Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Arg
1				5					10	

<210> 117

<211> 11

<212> PRT

<213> Homo sapiens

<400> 117

Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala	Arg
1				5					10	

<210> 118

<211> 11

<212> PRT

<213> Homo sapiens

<400> 118

Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Lys	Asn
1				5					10	

<210> 119

<211> 8

<212> PRT

<213> Homo sapiens

<400> 119

Gly Lys Gly Leu Glu Trp Val Ser
1 5

<210> 120

<211> 6

<212> PRT

<213> Homo sapiens

<400> 120

Trp Val Arg Gln Ala Pro
1 5

<210> 121

<211> 11

<212> PRT

<213> Homo sapiens

<400> 121

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Asp
1 5 10

<210> 122

<211> 7

<212> PRT

<213> Homo sapiens

<400> 122

Ala Val Tyr Tyr Cys Ala Arg
1 5

<210> 123

<211> 20

<212> PRT

<213> Homo sapiens

<400> 123

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

Gly Gly Gly Ser
20

<210> 124

<211> 15

<212> PRT

<213> Homo sapiens

<400> 124

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

<210> 125

<211> 9

<212> PRT

<213> Homo sapiens

<400> 125

Asn Ser Arg Asp Ser Ser Gly Asn His
1 5

<210> 126

<211> 8

<212> PRT

<213> Homo sapiens

<400> 126

Ala Ala Trp Asp Asp Ser Leu Val
1 5

<210> 127

<211> 8

<212> PRT

<213> Homo sapiens

<400> 127

Met Gln Ser Ile Gln Leu Pro Thr
1 5

<210> 128

<211> 9

<212> PRT

<213> Homo sapiens

<400> 128

Met Gln Ser Ile Gln Leu Pro Ala Thr
1 5

<210> 129

<211> 10

<212> PRT

<213> Homo sapiens

<400> 129

Ala Ala Trp Asp Asp Gly Leu Ser Leu Val
1 5 10

<210> 130

<211> 10

<212> PRT

<213> Homo sapiens

<400> 130

Ala Ala Trp Asp Asp Ser Leu Ser Gly Val
1 5 10

<210> 131

<211> 11

<212> PRT

<213> Homo sapiens

<400> 131

Asn	Ser	Arg	Asp	Ser	Ser	Gly	Ser	Val	Arg	Val
1				5					10	

<210> 132

<211> 9

<212> PRT

<213> Homo sapiens

<400> 132

Leu	Leu	Tyr	Tyr	Gly	Gly	Ala	Tyr	Val
1				5				

<210> 133

<211> 11

<212> PRT

<213> Homo sapiens

<400> 133

Asn	Ser	Arg	Asp	Ser	Ser	Gly	Val	Ser	Arg	Val
1				5					10	

<210> 134

<211> 10

<212> PRT

<213> Homo sapiens

<400> 134

Ala	Ala	Trp	Asp	Asp	Ser	Leu	Pro	Tyr	Val
1				5					10

<210> 135

<211> 12

<212> PRT

<213> Homo sapiens

<400> 135

Ala	Ala	Trp	Asp	Asp	Ser	Leu	Cys	Pro	Glu	Phe	Val
1				5					10		

<210> 136

<211> 11

<212> PRT

<213> Homo sapiens

<400> 136

Ala	Ala	Trp	Asp	Asp	Ser	Leu	Ala	Trp	Phe	Val
1				5					10	

<210> 137

<211> 10

<212> PRT

<213> Homo sapiens

<400> 137

Leu	Ala	Trp	Asp	Thr	Ser	Pro	Arg	Trp	Val
1				5					10

<210> 138

<211> 10

<212> PRT

<213> Homo sapiens

<400> 138

Thr	Ala	Trp	Asp	Asp	Ser	Leu	Ala	Val	Val
1				5					10

<210> 139

<211> 11

<212> PRT

<213> Homo sapiens

<400> 139

Asn	Ser	Arg	Asp	Ser	Ser	Gly	Asn	His	Arg	Val
1				5					10	

<210> 140

<211> 9

<212> PRT

<213> Homo sapiens

<400> 140

Gln	Gln	Tyr	Gly	Ser	Ser	Gln	Arg	Thr
1				5				

<210> 141

<211> 10

<212> PRT

<213> Homo sapiens

<400> 141

Ala	Ala	Trp	Asp	Asp	Ser	Leu	Arg	Leu	Val
1				5					10

<210> 142

<211> 9

<212> PRT

<213> Homo sapiens

<400> 142

Met Gln Gly Thr His Trp Arg Pro Thr

1 5

<210> 143

<211> 9

<212> PRT

<213> Homo sapiens

<400> 143

Met Gln Gly Lys His Trp Pro Leu Thr
1 5

<210> 144

<211> 9

<212> PRT

<213> Homo sapiens

<400> 144

Ala Ala Trp Asp Asp Ser Leu Gly Phe
1 5

<210> 145

<211> 9

<212> PRT

<213> Homo sapiens

<400> 145

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Met Arg Gly Thr His Arg Arg Ala Thr
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Met Gln Gly Thr His Trp His Pro Thr
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Ala Ala Trp Asp Asp Ser Leu Arg Ser Val
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Ala Ala Trp Asp Asp Ser Leu Leu Val
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Ala Ala Trp Asp Asp Ser Leu Phe Tyr Pro Val
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Ala Ala Trp Asp Asp Ser Leu Phe Tyr Val
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Met Gln Ser Ile Gln Leu Pro Leu Thr
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Ala Ala Trp Asp Asp Ser Leu Leu Gly Ser Val
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Cys Ser Tyr Ala Gly Ser Ser Tyr Val
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Gln Gln Asp Tyr Asn Leu Leu Thr
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Val Leu Tyr Met Gly Ser Gly Ser Ala Val
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Met Gln Arg Ile Glu Phe Pro Asn Thr
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Gln Gln Ala Asn Ser Phe Arg Thr
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Ala Ala Trp Asp Asp Ser Leu Ser Arg Pro Val
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Ala Ala Trp Asp Asp Ser Leu Tyr Asn Val
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Ala Ala Trp Asp Asp Ser Leu Asn Arg Asn Val
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Met Gln Ala Leu Gln Thr Arg Thr
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Gln Gln Ser Tyr Ser Thr Arg Met
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Ala Ala Trp Asp Asp Ser Leu Gly Phe Val
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Ala Ala Trp Asp Asp Ser Leu Ser Ile Val
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Met Gln Gly Thr His Trp Pro Thr
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Met Gln Ala Leu His Thr Arg Thr
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Asn Ser Arg Asp Ser Ser Gly Ser Val
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Gln Gln Tyr Gly Ser Ser Pro Tyr Thr
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Gln Gln Ser Tyr Ser Thr Arg Thr
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Gln Gln Ala Asn Ser Phe Ala Ala Thr
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Gln Gln Ala Asn Ser Phe Pro Ala Thr
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Val Leu Tyr Met Gly Ser Gly Val Tyr Val
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Ala Ala Trp Asp Asp Ser Leu Trp Ser Ala Val
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Ala Ala Trp Asp Asp Ser Leu Pro Arg Arg Leu Val
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Ala Ala Trp Asp Asp Ser Leu Pro Ser Gly Val
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Met Gln Ala Leu Gln Thr Leu Thr
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Ala Ala Trp Asp Asp Gly Leu Leu Arg Val
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Ala Ala Trp Asp Asp Ser Leu Ala Leu Val
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Asn Ser Arg Asp Ser Ser Gly Phe Gln Leu Val
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Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
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Ala Gln Pro Ala Met Ala Glu Val Gln Leu Val Glu Ser Gly Gly Gly
20 25 30

Val Val Arg Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
35 40 45

Phe Thr Phe Asp Asp Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly
50 55 60

Lys Gly Leu Glu Trp Val Ser Gly Ile Asn Trp Asn Gly Gly Ser Thr
65 70 75 80

Gly Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
85 90 95

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