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DESCRIPTION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application is a nonprovisional and claims the benefit of 61/420,291, filed December 6, 2010, and 61/446,990, filed February 25, 2011.

BACKGROUND

[0002] LIV-1 is a member of the LZT (LIV-1-ZIP Zinc Transporters) subfamily of zinc transporter proteins. Taylor et al., *Biochim. Biophys. Acta* 1611:16-30 (2003). Computer analysis of the LIV-1 protein reveals a potential metalloprotease motif, fitting the consensus sequence for the catalytic zinc-binding site motif of the zinc metalloprotease. LIV-1 mRNA is primarily expressed in breast, prostate, pituitary gland and brain tissue.

[0003] The LIV-1 protein has also been implicated in certain cancerous conditions, e.g. breast cancer and prostate cancer. The detection of LIV-1 is associated with estrogen receptor-positive breast cancer, McClelland et al., *Br. J. Cancer* 77:1653-1656 (1998), and the metastatic spread of these cancers to the regional lymph nodes. Manning et al., *Eur. J. Cancer* 30A:675-678 (1994).

[0004] Antibodies against Liv-1 are known from WO2004067564 and US2008171039.

SUMMARY

[0005] The subject-matter of the invention is defined by the appended claims.

[0006] The disclosure provides a humanized antibody comprising a mature heavy chain variable region having an amino acid sequence at least 90% identical to SEQ ID NO:53 provided that position H27 is occupied by L, position H29 is occupied by I, H30 by E and H94 by V and a mature light chain variable region at least 90% identical to SEQ ID NO:60 provided position L36 is occupied by Y and position L46 by P. Optionally, the humanized antibody comprises three CDRs of SEQ ID NO:53 and three CDRs of SEQ ID NO:60. Those CDRs are shown in Figure 16. Optionally, position H76 is occupied by N. Optionally, the humanized comprises a mature heavy chain variable region having an amino acid sequence at least 95% identical to SEQ ID NO:53 and a mature light chain variable region at least 95% identical to SEQ ID NO:60. Optionally, the mature heavy chain variable region is fused to a heavy chain constant region and the mature light chain constant region is fused to a light chain constant region. Optionally, the heavy chain constant region is a mutant form of natural human constant region which has reduced binding to an Fcγ receptor relative to the natural human

constant region. Optionally, the heavy chain constant region is of IgG1 isotype. Optionally, the heavy chain constant region has an amino acid sequence comprising SEQ ID NO:44 and the light chain constant region has an amino acid sequence comprising SEQ ID NO:42. Optionally, the heavy chain constant region has an amino acid sequence comprising SEQ ID NO:46 (S239C) and the light chain constant region has an amino acid sequence comprising SEQ ID NO:42. In some such humanized antibodies, any differences in CDRs of the mature heavy chain variable region and mature light variable region from SEQ ID NOS. 52 and 60 respectively reside in positions H60-H65. In some such humanized antibodies, the mature heavy chain variable region has an amino acid sequence designated SEQ ID NO:52 or 53 and the mature light chain variable region has an amino acid sequence designated SEQ ID NO: 59 or 60. In some such humanized antibodies, the mature heavy chain variable region has an amino acid sequence designated SEQ ID NO:53 and the mature light chain variable region has an amino acid sequence designated SEQ ID NO:60. Some such humanized antibodies are conjugated to a cytotoxic or cytostatic agent. Some such humanized antibodies have an association constant for human or cynomolgus monkey LIV-1 of 0.5 to $2 \times 10^9 \text{ M}^{-1}$.

[0007] The disclosure also provides a humanized antibody comprising a mature heavy chain variable region comprising the three Kabat CDRs of SEQ ID NO:52, wherein position H27 is occupied by L, position H29 is occupied by I, H30 by E, H76 by N, and H94 by V and a mature light chain variable region comprising the three Kabat CDRs of SEQ ID NO:60 provided position L36 is occupied by Y and position L46 by P. The disclosure also provides a nucleic acid encoding a mature heavy chain variable region and/or a mature light chain variable region of any of the above defined humanized antibodies.

[0008] The disclosure further provides a method of treating a patient having or at risk of cancer, comprising administering to the patient an effective regime of any of the above defined humanized antibodies. The cancer can be for example a breast cancer, cervical cancer, melanoma, or a prostate cancer.

[0009] The invention further provides a pharmaceutical composition comprising a humanized antibody of the invention.

[0010] The disclosure further provides methods of treating a subject afflicted with a melanoma that expresses the LIV-1 protein by administering to the subject a LIV-1 specific antibody or a LIV-1 antibody drug conjugate, in an amount sufficient to inhibit growth of the melanoma cancer cells.

[0011] The disclosure further provides methods of treating a subject afflicted with a cervical cancer that expresses the LIV-1 protein by administering to the subject a LIV-1 specific antibody or a LIV-1 antibody drug conjugate, in an amount sufficient to inhibit growth of the cervical cancer cells.

[0012] The disclosure further provides a humanized antibody comprising a mature heavy chain variable region having an amino acid sequence at least 90% identical to HB (SEQ ID NO:10)

and a mature light chain variable region at least 90% identical to LB (SEQ ID NO:15). Optionally, the antibody comprises a mature heavy chain variable region having an amino acid sequence at least 95% identical to HB and a mature light chain variable region at least 95% identical to LB. Optionally, in any such antibody, positions H29, H30 and H76 are occupied by I, E and N, and L36 is occupied by Y. Optionally, any difference in the variable region frameworks of the mature heavy chain variable region and SEQ ID NO:10 is/are selected from the group consisting of H27 occupied by F, H28 occupied by N, H48 occupied by I, H66 occupied by K, H67 occupied by A, H71 occupied by A, H76 occupied by N, H93 occupied by N, H94 occupied by V, L37 occupied by L, L39 occupied by K, L45 occupied by K, and L46 occupied by L. Optionally, the 3 CDRs of the mature heavy chain variable region are those of SEQ ID NO. 10 and the 3 CDRs of the mature light chain variable region are those of SEQ ID NO:15. The CDRs are shown in Fig. 1. Optionally, the mature heavy chain variable region is fused to a heavy chain constant region and the mature light chain constant region is fused to a light chain constant region. Optionally, the heavy chain constant region is a mutant form of natural human constant region which has reduced binding to an Fcγ receptor relative to the natural human constant region. Optionally, the heavy chain constant region is of IgG1 isotype. Optionally, the heavy chain constant region has an amino acid sequence comprising SEQ ID NO:6 and the light chain constant region has an amino acid sequence comprising SEQ ID NO:4. Optionally, the heavy chain constant region has an amino acid sequence comprising SEQ ID NO:8 (S239C) and the light chain constant region has an amino acid sequence comprising SEQ ID NO:4. Optionally, any differences in CDRs of the mature heavy chain variable region and mature light variable region from SEQ ID NOS. 10 and 15 respectively reside in positions H60-H65. Optionally, the mature heavy chain variable region has an amino acid sequence comprising SEQ ID NO: 10 and the mature light chain variable region has an amino acid sequence comprising SEQ ID NO:15. Optionally, the antibody is conjugated to a cytotoxic or cytostatic agent. Preferred humanized antibodies having greater affinity for LIV-1 than the antibody BR2-14a In another aspect, the humanized antibody has an association constant for human or cynomolgus monkey LIV-1 of 0.5 to $2 \times 10^9 \text{ M}^{-1}$.

[0013] The disclosure further provides a humanized antibody comprising a mature heavy chain variable region comprising the 3 CDRs of SEQ ID NO: 10 and wherein positions H29, H30 and H76 are occupied by I, E and N respectively, and a mature light chain variable region comprising the 3 CDRs of SEQ ID NO: 15, and wherein position L36 is occupied by Y.

[0014] The disclosure further provides a nucleic acid encoding a mature heavy chain variable region and/or a mature light chain variable region of any of the humanized antibodies described above.

[0015] The disclosure further provides a method of treating a patient having or at risk of cancer, comprising administering to the patient an effective regime of a humanized antibody as described above. Optionally, the cancer is breast cancer, cervical cancer, melanoma, or a prostate cancer.

[0016] The disclosure further provides a pharmaceutical composition comprising a humanized

antibody as described above.

[0017] The disclosure further provides a method of treating a patient having or at risk of triple negative breast cancer, comprising administering to the patient an effective regime of an antibody that specifically binds to LIV-1. Optionally, in such methods, the antibody is conjugated to a cytotoxic or cytostatic agent.

BRIEF DESCRIPTION OF THE FIGURES

[0018]

Figure 1 shows an alignment of the amino acid sequences of the parental murine mAb (referred to as BR2-14a) with the humanized LIV-1 heavy (upper two panels) and light chain variable (lower two panels) regions.

Figure 2 shows the binding curves for the humanized LIV-1 mAbs and the parental murine antibody (referred to as BR2-14a).

Figure 3 shows the results of competition binding studies of the humanized LIV-1 mAbs and the parental murine antibody (referred to as BR2-14a). The numbers in parentheses after each variant indicate the number of back mutations.

Figure 4 shows the results of saturation binding studies on MCF7 cells. BR2-14a-AF refers to AF-labeled parental murine antibody. hLIV-14 refers to AF-labeled HBLB antibody, a humanized antibody that specifically binds to LIV-1.

Figure 5 shows the results of competition binding studies on CHO cells expressing recombinant LIV-1 protein. BR2-14a refers to the parental murine antibody. hLIV-14 HBLB WT refers to the HBLB antibody. hLIV-14 HBLB S239C refers to the HBLB antibody having serine to cysteine substitutions at each position in the heavy chain.

Figure 6 shows an analysis of LIV-1 protein expression by IHC on post-hormone treated breast cancer patient samples.

Figure 7 shows an analysis of LIV-1 protein expression by IHC on hormone-refractory metastatic prostate cancer patient samples.

Figure 8 shows an analysis of LIV-1 protein expression by IHC on triple negative breast cancer patient samples.

Figure 9 shows the results of cytotoxicity assays on hLIV-14 antibody drug conjugates, i.e., the HBLB mAb conjugated to vcMMAE (1006) or mcMMAF (1269), as well as conjugates of control murine (mIgG) and human (hIgG) antibodies. hLIV-14-SEA-1006 refers to a non-fucosylated form of the HBLB mAb conjugated to vcMMAE (1006).

Figure 10 shows the results of an in vitro ADCC assay on MCF7 cells using human NK cells

(donor 1; V/V). hLIV-14 WT refers to the HBLB mAb. hLIV-14 SEA refers to the non-fucosylated form of the HBLB mAb. hLIV-14 mcMMAF refers to an antibody drug conjugate of the HBLB mAb conjugated to mcMMAF. hLIV-14 vcMMAE refers to an antibody drug conjugate of the HBLB mAb conjugated to vcMMAE. hLIV-14 SEA vcMMAE refers to a non-fucosylated form of the HBLB mAb-vcMMAE antibody drug conjugate.

Figure 11 shows the results of an in vitro ADCC assay on MCF7 cells using human NK cells (donor 2). hLIV-14 WT refers to the HBLB mAb. hLIV-14 SEA refers to the non-fucosylated form of the HBLB mAb. cLIV-14 SEA refers to the non-fucosylated form of the chimeric parental murine antibody. hLIV-14 mcF(4) refers to an antibody drug conjugate of the HBLB mAb with an average of 4 mcMMAF drug linker molecules per antibody. hLIV-14 vcE(4) refers to an antibody drug conjugate of the HBLB mAb with an average of 4 vcMMAE drug linker molecules per antibody. hLIV-14 vcE(4) SEA refers to a non-fucosylated form of the HBLB mAb-vcMMAE antibody drug conjugate having an average of four vcMMAE drug linker molecules per antibody. hIgG refers to control human IgG. H00-mcF(4) refers to a control antibody drug conjugate of a nonbinding antibody with an average of 4 mcMMAF drug linker molecules per antibody. H00-vcE(4) refers to a control antibody drug conjugate of a nonbinding antibody with an average of 4 vcMMAE drug linker molecules per antibody.

Figure 12 shows the results of a xenograft study of the MCF7 breast cancer line in nude mice. cLIV-14-mcMMAF(4) refers to an antibody drug conjugate of the chimeric form of the parental murine antibody having an average of 4 mcMMAF drug linker molecules per antibody. cLIV-14-vcMMAE(4) refers to an antibody drug conjugate of the chimeric form of the parent murine antibody having an average of 4 vcMMAE drug linker molecules per antibody. H00-mcMMAF(4) refers to an antibody drug conjugate of a nonbinding control antibody having an average of 4 mcMMAF drug linker molecules per antibody. H00-vcMMAE(4) refers to an antibody drug conjugate of a nonbinding control antibody having an average of 4 vcMMAE drug linker molecules per antibody. The dose and time of administration of indicated on the figure.

Figure 13 shows the results of a xenograft study of the PC3 prostate cancer line in male nude mice. cLIV-14-vcMMAE(4) refers to an antibody drug conjugate of the chimeric form of the parent murine antibody having an average of 4 vcMMAE drug linker molecules per antibody. hBU12-vcMMAE(4) refers to an antibody drug conjugate of an anti-CD 19 antibody having an average of 4 vcMMAE drug linker molecules per antibody. The dose and time of administration of indicated on the figure.

Figure 14 shows the results of a xenograft study of the MCF7 breast cancer line in nude mice. hLIV-14-vcMMAE (4) refers to an antibody drug conjugate of the HBLB antibody having an average of 4 vcMMAE drug linker molecules per antibody. hLIV-14d-vcMMAE (2) refers to an antibody drug conjugate of the HBLB antibody having an average of 2 vcMMAE drug linker molecules per antibody, each conjugated at the S239C position of each heavy chain. H00-vcMMAE(4) refers to an antibody drug conjugate of a nonbinding control antibody having an average of 4 vcMMAE drug linker molecules per antibody. The dose and time of administration of indicated on the figure.

Figure 15 shows the results of a xenograft study of the PC3 prostate cancer line in male nude mice. hLIV-14-vcMMAE (4) refers to an antibody drug conjugate of the HBLB antibody having an average of 4 vcMMAE drug linker molecules per antibody. hLIV-14-mcMMAF(4) refers to an antibody drug conjugate of the HBLB antibody having an average of 4 mcMMAF drug linker molecules per antibody. hLIV-14d-vcMMAE(2) refers to an antibody drug conjugate of the HBLB antibody having an average of 2 vcMMAE drug linker molecules per antibody, each conjugated at the S239C position of each heavy chain. hLIV-14d-mcMMAF(2) refers to an antibody drug conjugate of the HBLB antibody having an average of 2 mcMMAF drug linker molecules per antibody, each conjugated at the S239C position of each heavy chain. H00-vcMMAE(4) refers to an antibody drug conjugate of a nonbinding control antibody having an average of 4 vcMMAE drug linker molecules per antibody. H00-mcMMAF(4) refers to an antibody drug conjugate of a nonbinding control antibody having an average of 4 mcMMAF drug linker molecules per antibody. The dose and time of administration of indicated on the figure.

Figures 16A and 16B show alignments of humanized heavy chain (**Figure 16A**) and light chain (**Figure 16B**) mature variable regions with those of the mouse BR2-22a.

Figure 17 shows competition binding assays of different permutations of humanized heavy chains HA-HF and humanized light chains LA-LF derived from the murine monoclonal anti LIV-1 antibody BR2-22a. The total number of murine back mutations in each light or heavy chain is shown in parentheses. Only HELF showed sufficient retention of binding.

Figure 18 shows systematic variation of the HE and LF chains to test contribution of individual backmutations to antigen binding. Sites of potential somatic hypermutation are in parentheses. Mouse residues are underlined. The remaining residues are human germline residues.

Figure 19 shows competition binding of the LF variants on the top of the figure. The tested back mutations are shown in the bottom of the figure. Mouse residues are underlined. The remaining residues are human germline residues.

Figure 20 shows competition binding of the HE variants on the top of the figure. The tested back mutations are shown in the bottom of the figure. Mouse residues are underlined. The remaining residues are human germline residues.

Figure 21 shows competition binding of different permutations of HE, HF, HG and LF and LG.

Figure 22 shows saturation binding of humanized LIV14 antibody and humanized LIV22 antibody on human and cynomolgus LIV-1 expressed from CHO cells.

Figure 23 shows cytotoxic activity of humanized LIV22-vcMMAE on MCF-7 cells after 144 hr of treatment. h00-1006 is a control drug-conjugated antibody.

Figure 24 shows cytotoxic activity of hLIV22-mcMMAF on MCF-7 cells after 144 hr of treatment. h00-1269 is a control drug-conjugated antibody.

Figure 25 shows the activity of hLIV22 antibody on PC3 (DSMZ) prostate carcinoma model in

nude female mice. Dose days are indicated by triangles on the X-axis.

Figure 26 shows that activity of hLIV22 antibody on MCF7 (NCI) breast carcinoma tumors in nude mice.

Figure 27 compares the activity of hLIV22 and hLIV14 in the same model as **Figure 26**.

Figure 28 shows an analysis of LIV-1 protein expression by IHC on melanoma cancer patient samples.

DEFINITIONS

[0019] Monoclonal antibodies are typically provided in isolated form. This means that an antibody is typically at least 50% w/w pure of interfering proteins and other contaminants arising from its production or purification but does not exclude the possibility that the monoclonal antibody is combined with an excess of pharmaceutical acceptable carrier(s) or other vehicle intended to facilitate its use. Sometimes monoclonal antibodies are at least 60%, 70%, 80%, 90%, 95 or 99% w/w pure of interfering proteins and contaminants from production or purification.

[0020] Specific binding of a monoclonal antibody to its target antigen means an affinity of at least 10^6 , 10^7 , 10^8 , 10^9 , or 10^{10} M⁻¹. Specific binding is detectably higher in magnitude and distinguishable from non-specific binding occurring to at least one unrelated target. Specific binding can be the result of formation of bonds between particular functional groups or particular spatial fit (e.g., lock and key type) whereas nonspecific binding is usually the result of van der Waals forces. Specific binding does not however necessarily imply that a monoclonal antibody binds one and only one target.

[0021] The basic antibody structural unit is a tetramer of subunits. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. This variable region is initially expressed linked to a cleavable signal peptide. The variable region without the signal peptide is sometimes referred to as a mature variable region. Thus, for example, a light chain mature variable region, means a light chain variable region without the light chain signal peptide. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

[0022] Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a

"D" region of about 10 or more amino acids. (See *generally*, Fundamental Immunology (Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989, Ch. 7).

[0023] The mature variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD, 1987 and 1991), or Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987); Chothia et al., Nature 342:878-883 (1989). Kabat also provides a widely used numbering convention (Kabat numbering) in which corresponding residues between different heavy chains or between different light chains are assigned the same number.

[0024] The term "antibody" includes intact antibodies and binding fragments thereof. Typically, antibody fragments compete with the intact antibody from which they were derived for specific binding to the target including separate heavy chains, light chains Fab, Fab', F(ab')₂, F(ab)c, diabodies, Dabs, nanobodies, and Fv. Fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins. The term "antibody" also includes a diabody (homodimeric Fv fragment) or a minibody (V_L-V_H-C_H3), a bispecific antibody or the like. A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites (see, e.g., Songsivilai and Lachmann, Clin. Exp. Immunol., 79:315-321 (1990); Kostelny et al., J. Immunol., 148:1547-53 (1992)). The term "antibody" includes an antibody by itself (naked antibody) or an antibody conjugated to a cytotoxic or cytostatic drug.

[0025] The term "epitope" refers to a site on an antigen to which an antibody binds. An epitope can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of one or more proteins. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols, in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed. (1996).

[0026] Antibodies that recognize the same or overlapping epitopes can be identified in a simple immunoassay showing the ability of one antibody to compete with the binding of another antibody to a target antigen. The epitope of an antibody can also be defined by X-ray crystallography of the antibody bound to its antigen to identify contact residues. Alternatively, two antibodies have the same epitope if all amino acid mutations in the antigen that reduce or

eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0027] Competition between antibodies is determined by an assay in which an antibody under test inhibits specific binding of a reference antibody to a common antigen (see, e.g., Junghans et al., Cancer Res. 50:1495, 1990). A test antibody competes with a reference antibody if an excess of a test antibody (e.g., at least 2x, 5x, 10x, 20x or 100x) inhibits binding of the reference antibody by at least 50% but preferably 75%, 90% or 99% as measured in a competitive binding assay. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

[0028] The term "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[0029] For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

[0030] Percentage sequence identities are determined with antibody sequences maximally aligned by the Kabat numbering convention. After alignment, if a subject antibody region (e.g., the entire mature variable region of a heavy or light chain) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage.

[0031] Compositions or methods "comprising" one or more recited elements may include other elements not specifically recited. For example, a composition that comprises antibody may contain the antibody alone or in combination with other ingredients.

[0032] Designation of a range of values includes all integers within or defining the range.

[0033] An antibody effector function refers to a function contributed by an Fc domain(s) of an Ig. Such functions can be, for example, antibody-dependent cellular cytotoxicity, antibody-dependent cellular phagocytosis or complement-dependent cytotoxicity. Such function can be effected by, for example, binding of an Fc effector domain(s) to an Fc receptor on an immune

cell with phagocytic or lytic activity or by binding of an Fc effector domain(s) to components of the complement system. Typically, the effect(s) mediated by the Fc-binding cells or complement components result in inhibition and/or depletion of the LIV-1 targeted cell. Fc regions of antibodies can recruit Fc receptor (FcR)-expressing cells and juxtapose them with antibody-coated target cells. Cells expressing surface FcR for IgGs including FcγRIII (CD16), FcγRII (CD32) and FcγRI (CD64) can act as effector cells for the destruction of IgG-coated cells. Such effector cells include monocytes, macrophages, natural killer (NK) cells, neutrophils and eosinophils. Engagement of FcγR by IgG activates antibody-dependent cellular cytotoxicity (ADCC) or antibody-dependent cellular phagocytosis (ADCP). ADCC is mediated by CD16⁺ effector cells through the secretion of membrane pore-forming proteins and proteases, while phagocytosis is mediated by CD32⁺ and CD64⁺ effector cells (see *Fundamental Immunology*, 4th ed., Paul ed., Lippincott-Raven, N.Y., 1997, Chapters 3, 17 and 30; Uchida et al., 2004, *J. Exp. Med.* 199:1659-69; Akewanlop et al., 2001, *Cancer Res.* 61:4061-65; Watanabe et al., 1999, *Breast Cancer Res. Treat.* 53:199-207). In addition to ADCC and ADCP, Fc regions of cell-bound antibodies can also activate the complement classical pathway to elicit complement-dependent cytotoxicity (CDC). C1q of the complement system binds to the Fc regions of antibodies when they are complexed with antigens. Binding of C1q to cell-bound antibodies can initiate a cascade of events involving the proteolytic activation of C4 and C2 to generate the C3 convertase. Cleavage of C3 to C3b by C3 convertase enables the activation of terminal complement components including C5b, C6, C7, C8 and C9. Collectively, these proteins form membrane-attack complex pores on the antibody-coated cells. These pores disrupt the cell membrane integrity, killing the target cell (see *Immunobiology*, 6th ed., Janeway et al., Garland Science, N. Y., 2005, Chapter 2).

[0034] The term "antibody-dependent cellular cytotoxicity", or ADCC, is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells with immune cells possessing lytic activity (also referred to as effector cells). Such effector cells include natural killer cells, monocytes/macrophages and neutrophils. The effector cells attach to an Fc effector domain(s) of Ig bound to target cells via their antigen-combining sites. Death of the antibody-coated target cell occurs as a result of effector cell activity.

[0035] The term "antibody-dependent cellular phagocytosis", or ADCP, refers to the process by which antibody-coated cells are internalized, either in whole or in part, by phagocytic immune cells (e.g., macrophages, neutrophils and dendritic cells) that bind to an Fc effector domain(s) of Ig.

[0036] The term "complement-dependent cytotoxicity", or CDC, refers to a mechanism for inducing cell death in which an Fc effector domain(s) of a target-bound antibody activates a series of enzymatic reactions culminating in the formation of holes in the target cell membrane. Typically, antigen-antibody complexes such as those on antibody-coated target cells bind and activate complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement components on the target cell surface that facilitate ADCC by binding complement receptors (e.g., CR3) on leukocytes.

[0037] A "cytotoxic effect" refers to the depletion, elimination and/or the killing of a target cell. A "cytotoxic agent" refers to an agent that has a cytotoxic effect on a cell. Cytotoxic agents can be conjugated to an antibody or administered in combination with an antibody.

[0038] A "cytostatic effect" refers to the inhibition of cell proliferation. A "cytostatic agent" refers to an agent that has a cytostatic effect on a cell, thereby inhibiting the growth and/or expansion of a specific subset of cells. Cytostatic agents can be conjugated to an antibody or administered in combination with an antibody.

[0039] The term "pharmaceutically acceptable" means approved or approvable by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "pharmaceutically compatible ingredient" refers to a pharmaceutically acceptable diluent, adjuvant, excipient, or vehicle with which an anti-LIV-1 antibody.

[0040] The phrase "pharmaceutically acceptable salt," refers to pharmaceutically acceptable organic or inorganic salts of an anti-LIV-1 antibody or conjugate thereof or agent administered with an anti-LIV-1 antibody. Exemplary salts include sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p toluenesulfonate, and pamoate (i.e., 1,1' methylene bis -(2 hydroxy 3 naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counter ions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

[0041] Unless otherwise apparent from the context, the term "about" encompasses values within a standard deviation of a stated value.

DETAILED DESCRIPTION

I. General

[0042] The invention provides monoclonal antibodies that specifically bind to LIV-1. The antibodies are useful for treatment and diagnoses of various cancers as well as detecting LIV-1.

II. Target molecules

[0043] Unless otherwise indicated, LIV-1 means a human LIV-1. An exemplary human sequence is assigned Swiss Prot accession number Q13433. Q13433 is included herein as SEQ ID NO:83. Three variant isoforms and one polymorphism are known. A second version of the human LIV-1 protein, accession number AAA96258.2, is included herein as SEQ ID NO:84. Four extracellular domains are bounded by residues 29-325, 377-423, 679-686 and 746-755 of Q13433 respectively.

[0044] Unless otherwise apparent from the context reference LIV-1 means at least an extracellular domain of the protein and usually the complete protein other than a cleavable signal peptide (amino acids 1-28 of Q13433).

III. Antibodies

A. Binding specificity and functional properties

[0045] The disclosure provides humanized antibodies derived from two mouse antibodies, BR2-14a and BR2-22a. Unless specifically indicated otherwise, the present disclosures relate to both antibodies. The two mouse antibodies show 94% and 91% sequence identity to one another in the mature heavy and light chain variable regions. The two antibodies bind to the same or overlapping epitopes on human LIV-1. However, the BR2-22a antibody has about ten-fold higher affinity for human LIV-1 and about 3 -fold higher affinity for cynomolgus monkey LIV-1 than BR2-14a as shown in Fig. 22.

[0046] The affinity of humanized forms of the mouse BR2-14a antibody (i.e., K_a) is preferably within a factor of five or or a factor of two of that of the mouse antibody BR2-14a for human LIV-1. Humanized BR2-14a antibodies specifically bind to human LIV-1 in native form and/or recombinantly expressed from CHO cells as does the mouse antibody from which they were derived. Preferred humanized BR2-14a antibodies have an affinity the same as or greater than (i.e., greater than beyond margin of error in measurement) that of BR2-14a for human LIV-1 (e.g., 1.1-5 fold, 1.1 to 3 fold, 1.5 to 3-fold, 1.7 to 2.3-fold or 1.7-2.1-fold the affinity or about twice the affinity of BR2-14a). Preferred humanized BR2-14a antibodies bind to the same epitope and/or compete with BR2-14a for binding to human LIV-1. Preferred humanized BR2-14a antibodies also bind to the cyno-homolog of LIV-1 thus permitting preclinical testing in nonhuman primates.

[0047] The affinity of humanized forms of the mouse BR2-22a antibody (i.e., K_a) for human LIV-1, natively expressed or expressed from CHO cells, is preferably within a factor of five or a

factor of two of that of the mouse antibody BR2-22. Some humanized BR2-22a antibodies have an association constant that is essentially the same as that of BR2-22a (i.e., within experimental error). Some humanized BR2-22a antibodies have an association constant within a range of 0.5 to 1 or 0.5-1.5 that of the association constant of the BR2-22a antibody. Preferred humanized BR2-22a antibodies have an association constant greater than $5 \times 10^8 \text{ M}^{-1}$, or in a range of 0.5 to $2 \times 10^9 \text{ M}^{-1}$ or about $0.8 \times 10^9 \text{ M}^{-1}$ (+/- error in measurement) for human LIV-1 expressed from CHO cells. Here as elsewhere in this application, affinities can be measured in accordance with the methods of the Examples. Preferred humanized BR2-22a antibodies bind to the same epitope and/or compete with BR2-22a for binding to human LIV-1. Humanized BR2-22a antibodies bind to the cyno-homolog of LIV-1 as well as human LIV-1. Preferred humanized BR2-22a antibodies bind with essentially the same association constant to human and cynomolgus monkey LIV-1 both expressed from CHO cells (within experimental error) thus permitting and increasing the predictive accuracy of preclinical testing in nonhuman primates.

[0048] Preferred antibodies (both humanized BR2-14a and humanized BR2-22a) inhibit cancer (e.g., growth of cells, metastasis and/or lethality to the organisms) as shown on cancerous cells propagating in culture, in an animal model or clinical trial. Animal models can be formed by implanting LIV-1-expressing human tumor cell lines into appropriate immunodeficient rodent strains, e.g., athymic nude mice or SCID mice. These tumor cell lines can be established in immunodeficient rodent hosts either as solid tumor by subcutaneous injections or as disseminated tumors by intravenous injections. Once established within a host, these tumor models can be applied to evaluate the therapeutic efficacies of the anti-LIV-1 antibodies or conjugated forms thereof as described in the Examples.

B. Humanized Antibodies

[0049] A humanized antibody is a genetically engineered antibody in which the CDRs from a non-human "donor" antibody are grafted into human "acceptor" antibody sequences (see, e.g., Queen, US 5,530,101 and 5,585,089; Winter, US 5,225,539; Carter, US 6,407,213; Adair, US 5,859,205; and Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody sequence, a composite of such sequences, a consensus sequence of human antibody sequences, or a germline region sequence. A preferred acceptor sequence for the heavy chain is the germline V_H exon V_H1-2 (also referred to in the literature as HV1-2) (Shin et al., 1991, EMBO J. 10:3641-3645) and for the hinge region (J_H), exon J_H-6 (Mattila et al., 1995, Eur. J. Immunol. 25:2578-2582). For the light chain, a preferred acceptor sequence is exon V_K2-30 (also referred to in the literature as KV2-30) and for the hinge region exon J_K-4 (Hieter et al., 1982, J. Biol. Chem. 257:1516-1522). Thus, a humanized antibody is an antibody having some or all CDRs entirely or substantially from a donor antibody and variable region framework sequences and constant regions, if present, entirely or substantially from human antibody sequences. Similarly a humanized heavy chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody heavy chain, and a heavy chain

variable region framework sequence and heavy chain constant region, if present, substantially from human heavy chain variable region framework and constant region sequences. Similarly a humanized light chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody light chain, and a light chain variable region framework sequence and light chain constant region, if present, substantially from human light chain variable region framework and constant region sequences. Other than nanobodies and dAbs, a humanized antibody comprises a humanized heavy chain and a humanized light chain. A CDR in a humanized antibody is substantially from a corresponding CDR in a non-human antibody when at least 60%, 85%, 90%, 95% or 100% of corresponding residues (as defined by Kabat) are identical between the respective CDRs. The variable region framework sequences of an antibody chain or the constant region of an antibody chain are substantially from a human variable region framework sequence or human constant region respectively when at least 85%, 90%, 95% or 100% of corresponding residues defined by Kabat are identical.

[0050] Although humanized antibodies often incorporate all six CDRs (preferably as defined by Kabat) from a mouse antibody, they can also be made with less than all CDRs (e.g., at least 3, 4, or 5) CDRs from a mouse antibody (e.g., Pascalis et al., J. Immunol. 169:3076, 2002; Vajdos et al., Journal of Molecular Biology, 320: 415-428, 2002; Iwahashi et al., Mol. Immunol. 36:1079-1091, 1999; Tamura et al, Journal of Immunology, 164:1432-1441, 2000).

[0051] Certain amino acids from the human variable region framework residues can be selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is by modeling, examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of particular amino acids.

[0052] For example, when an amino acid differs between a murine variable region framework residue and a selected human variable region framework residue, the human framework amino acid can be substituted by the equivalent framework amino acid from the mouse antibody when it is reasonably expected that the amino acid:

1. (1) noncovalently binds antigen directly,
2. (2) is adjacent to a CDR region,
3. (3) otherwise interacts with a CDR region (e.g. is within about 6 Å of a CDR region); or
4. (4) mediates interaction between the heavy and light chains.

[0053] The disclosure provides humanized forms of the mouse BR2-14a antibody including five exemplified humanized heavy chain mature variable regions (HA-HE) and six exemplified humanized light chain mature variable regions (LA-LF). The permutations of these chains having the strongest binding (lowest EC₅₀) are HBLB, HBLF, HCLB, HCLF, HDLB, HDLF, HELE and HELF. Of these permutations, HBLB (also known as hLIV14) is preferred because it has the strongest binding, about 2 fold stronger than the mouse donor antibody, and has the

fewest back mutations (four).

[0054] The disclosure provides variants of the HBLB humanized antibody in which the humanized heavy chain mature variable region shows at least 90%, 95% or 99% identity to SEQ ID NO:10 and the humanized light chain mature variable region shows at least 90%, 95% or 99% sequence identity to SEQ ID NO:15. Preferably, in such antibodies some or all of the backmutations in HBLB are retained. In other words, at least 1, 2 or preferably all 3 of heavy chain positions H29, H30 and H76 are occupied by I and E and N, respectively. Likewise position L36 is preferably occupied by Y. The CDR regions of such humanized antibodies are preferably substantially identical to the CDR regions of HBLB, which are the same as those of the mouse donor antibody. The CDR regions can be defined by any conventional definition (e.g., Chothia) but are preferably as defined by Kabat. In one aspect, the humanized antibody comprises a heavy chain comprising the 3 CDRs of SEQ ID NO:10 and variable region frameworks with at least 95% identity to the variable region frameworks of SEQ ID NO: 10. In another aspect, the humanized antibody comprises a light chain comprising the 3 CDRs of SEQ ID NO:15 and variable region frameworks with at least 95% identity to variable region frameworks of SEQ ID NO:15. In a further aspect, the humanized antibody comprises a heavy chain comprising the 3 CDRs of SEQ ID NO: 10 and variable region frameworks with at least 95% identity to the variable region frameworks of SEQ ID NO: 10, and a light chain comprising the 3 CDRs of SEQ ID NO:15, and variable region frameworks with at least 95% identity to the variable region frameworks of SEQ ID NO:15.

[0055] Insofar as humanized antibodies show any variation from the exemplified HBLB humanized antibody, one possibility for such additional variation is additional backmutations in the variable region frameworks. Any or all of the positions backmutated in other exemplified humanized heavy or light chain mature variable regions can also be made (i.e., 1, 2, 3, 4, 5, 6, 7, 8 or all 9 of H27 occupied by F, H28 occupied by N, H48 occupied by I, H66 occupied by K, H67 occupied by A, H71 occupied by A, H76 occupied by N, H93 occupied by N and H94 occupied by V in the heavy chain and 1, 2, 3, 4 or all 5 of L37 occupied by L, L39 occupied by K, L45 occupied by K, and L46 occupied by L in the light chain. However, such additional backmutations are not preferred because they in general do not improve affinity and introducing more mouse residues may give increased risk of immunogenicity.

[0056] The disclosure provides humanized forms of the mouse BR2-22a antibody including three exemplified humanized heavy chain mature variable regions (HE, HF and HG) and two exemplified humanized light chain (LF and LG) which can be combined in different permutations with adequate binding (see Figure 21). Of these permutations, HGLG (also known as hLIV22) is preferred because it has the best combination of binding properties (essentially the same as the mouse BR2-22a antibody within experimental error) and fewest back mutations (seven).

[0057] The disclosure provides variants of the HGLG humanized antibody in which the humanized heavy chain mature variable region shows at least 90%, 95%, 98% or 99% identity to SEQ ID NO:53 and the humanized light chain mature variable region shows at least 90%,

95%, 98% or 99% sequence identity to SEQ ID NO:60. Preferably, in such antibodies some or all of the backmutations in HGLG are retained. In other words, at least 1, 2, 3, 4 or preferably all 5 of heavy chain positions H27, H29, H30, H76, and H94 are occupied by L, I, E, N and V (here, as elsewhere in this application Kabat numbering is used to describe positions in the mature variable heavy and light chain variable regions). Of these backmutations, H94 contributes the most to retention of binding affinity and H76 the least. Likewise positions L36 and L46 are preferably occupied by Y and P respectively. The CDR regions of such humanized antibodies are preferably substantially identical to the CDR regions of HGLG, which are the same as those of the mouse donor antibody. The CDR regions can be defined by any conventional definition (e.g., Chothia) but are preferably as defined by Kabat. In one embodiment, the humanized antibody comprises a heavy chain comprising the 3 CDRs of SEQ ID NO:53 and variable region frameworks with at least 95% identity to the variable region frameworks of SEQ ID NO:53, and a light chain comprising the 3 CDRs of SEQ ID NO:60, and variable region frameworks with at least 95% identity to the variable region frameworks of SEQ ID NO:60.

[0058] Insofar as humanized BR2-22a antibodies show any variation from the exemplified HGLG humanized antibody, one possibility for such additional variation is additional backmutations in the variable region frameworks. Any or all of the positions backmutated in other exemplified humanized heavy or light chain mature variable regions can also be made (i.e., 1, 2, 3, 4, 5, or all 6, of H28 occupied by N, H48 occupied by I, H66 occupied by K, H67 occupied by A, H71 occupied by A, H93 occupied by T in the heavy chain and 1 or, 2 of L37 occupied by L37 occupied by L,, and L45 occupied by K. However, such additional backmutations are not preferred because they in general do not improve affinity and introducing more mouse residues may give increased risk of immunogenicity.

[0059] Another possible variation is to substitute certain residues in the CDRs of the mouse antibody with corresponding residues from human CDRs sequences, typically from the CDRs of the human acceptor sequences used in designing the exemplified humanized antibodies. In some antibodies only part of the CDRs, namely the subset of CDR residues required for binding, termed the SDRs, are needed to retain binding in a humanized antibody. CDR residues not contacting antigen and not in the SDRs can be identified based on previous studies (for example residues H60-H65 in CDR H2 are often not required), from regions of Kabat CDRs lying outside Chothia hypervariable loops (Chothia, J. Mol. Biol. 196:901, 1987), by molecular modeling and/or empirically, or as described in Gonzales et al., Mol. Immunol. 41: 863 (2004). In such humanized antibodies at positions in which one or more donor CDR residues is absent or in which an entire donor CDR is omitted, the amino acid occupying the position can be an amino acid occupying the corresponding position (by Kabat numbering) in the acceptor antibody sequence. The number of such substitutions of acceptor for donor amino acids in the CDRs to include reflects a balance of competing considerations. Such substitutions are potentially advantageous in decreasing the number of mouse amino acids in a humanized antibody and consequently decreasing potential immunogenicity. However, substitutions can also cause changes of affinity, and significant reductions in affinity are preferably avoided. In a further variation, one or more residues in a CDR of a humanized BR2-

22a antibody (which would otherwise be the same as the CDR of the mouse BR2-22a antibody) can be replaced by corresponding residues from a CDR from the mouse BR2-14a antibody (or vice versa). Positions for substitution within CDRs and amino acids to substitute can also be selected empirically.

[0060] Although not preferred other amino acid substitutions can be made, for example, in framework residues not in contact with the CDRs, or even some potential CDR-contact residues amino acids within the CDRs. Often the replacements made in the variant humanized sequences are conservative with respect to the replaced HBLB amino acids (in the case of humanized BR2-14a) or HGLG amino acids (in the case of humanized BR2-22). Preferably, replacements relative to HBLB or HGLG (whether or not conservative) have no substantial effect on the binding affinity or potency of the humanized mAb, that is, its ability to bind human LIV-1 and inhibit growth of cancer cells.

[0061] Variants typically differ from the heavy and light chain mature variable region sequences of HBLB (hLIV14) or HGLG (hLIV22) by a small number (e.g., typically no more than 1, 2, 3, 5 or 10 in either the light chain or heavy chain mature variable region, or both) of replacements, deletions or insertions.

C. Selection of Constant Region

[0062] The heavy and light chain variable regions of humanized antibodies can be linked to at least a portion of a human constant region. The choice of constant region depends, in part, whether antibody-dependent cell-mediated cytotoxicity, antibody dependent cellular phagocytosis and/or complement dependent cytotoxicity are desired. For example, human isotopes IgG1 and IgG3 have strong complement-dependent cytotoxicity, human isotype IgG2 weak complement-dependent cytotoxicity and human IgG4 lacks complement-dependent cytotoxicity. Human IgG1 and IgG3 also induce stronger cell mediated effector functions than human IgG2 and IgG4. Light chain constant regions can be lambda or kappa. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab', F(ab')₂, and Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

[0063] Human constant regions show allotypic variation and isoallotypic variation between different individuals, that is, the constant regions can differ in different individuals at one or more polymorphic positions. Isoallotypes differ from allotypes in that sera recognizing an isoallotype binds to a non-polymorphic region of a one or more other isotypes.

[0064] One or several amino acids at the amino or carboxy terminus of the light and/or heavy chain, such as the C-terminal lysine of the heavy chain, may be missing or derivatized in a proportion or all of the molecules. Substitutions can be made in the constant regions to reduce or increase effector function such as complement-mediated cytotoxicity or ADCC (see, e.g., Winter et al., US Patent No. 5,624,821; Tso et al., US Patent No. 5,834,597; and Lazar et al.,

Proc. Natl. Acad. Sci. USA 103:4005, 2006), or to prolong half-life in humans (see, e.g., Hinton et al., J. Biol. Chem. 279:6213, 2004).

[0065] Exemplary substitution include the amino acid substitution of the native amino acid to a cysteine residue is introduced at amino acid position 234, 235, 237, 239, 267, 298, 299, 326, 330, or 332, preferably an S239C mutation in a human IgG1 isotype (US 20100158909). The presence of an additional cysteine residue allows interchain disulfide bond formation. Such interchain disulfide bond formation can cause steric hindrance, thereby reducing the affinity of the Fc region-FcγR binding interaction. The cysteine residue(s) introduced in or in proximity to the Fc region of an IgG constant region can also serve as sites for conjugation to therapeutic agents (i.e., coupling cytotoxic drugs using thiol specific reagents such as maleimide derivatives of drugs. The presence of a therapeutic agent causes steric hindrance, thereby further reducing the affinity of the Fc region-FcγR binding interaction. Other substitutions at any of positions 234, 235, 236 and/or 237 reduce affinity for Fcγ receptors, particularly FcγRI receptor (see, e.g., US 6,624,821, US 5,624,821.)

[0066] The in vivo half-life of an antibody can also impact on its effector functions. The half-life of an antibody can be increased or decreased to modify its therapeutic activities. FcRn is a receptor that is structurally similar to MHC Class I antigen that noncovalently associates with β2-microglobulin. FcRn regulates the catabolism of IgGs and their transcytosis across tissues (Ghetie and Ward, 2000, Annu. Rev. Immunol. 18:739-766; Ghetie and Ward, 2002, Immunol. Res. 25:97-113). The IgG-FcRn interaction takes place at pH 6.0 (pH of intracellular vesicles) but not at pH 7.4 (pH of blood); this interaction enables IgGs to be recycled back to the circulation (Ghetie and Ward, 2000, Ann. Rev. Immunol. 18:739-766; Ghetie and Ward, 2002, Immunol. Res. 25:97-113). The region on human IgG1 involved in FcRn binding has been mapped (Shields et al., 2001, J. Biol. Chem. 276:6591-604). Alanine substitutions at positions Pro238, Thr256, Thr307, Gln311, Asp312, Glu380, Glu382, or Asn434 of human IgG1 enhance FcRn binding (Shields et al., 2001, J. Biol. Chem. 276:6591-604). IgG1 molecules harboring these substitutions have longer serum half-lives. Consequently, these modified IgG1 molecules may be able to carry out their effector functions, and hence exert their therapeutic efficacies, over a longer period of time compared to unmodified IgG1. Other exemplary substitutions for increasing binding to FcRn include a Gln at position 250 and/or a Leu at position 428. EU numbering is used for all position in the constant region.

[0067] Oligosaccharides covalently attached to the conserved Asn297 are involved in the ability of the Fc region of an IgG to bind FcγR (Lund et al., 1996, J. Immunol. 157:4963-69; Wright and Morrison, 1997, Trends Biotechnol. 15:26-31). Engineering of this glycoform on IgG can significantly improve IgG-mediated ADCC. Addition of bisecting N-acetylglucosamine modifications (Umana et al., 1999, Nat. Biotechnol. 17:176-180; Davies et al., 2001, Biotech. Bioeng. 74:288-94) to this glycoform or removal of fucose (Shields et al., 2002, J. Biol. Chem. 277:26733-40; Shinkawa et al., 2003, J. Biol. Chem. 278:6591-604; Niwa et al., 2004, Cancer Res. 64:2127-33) from this glycoform are two examples of IgG Fc engineering that improves the binding between IgG Fc and FcγR, thereby enhancing Ig-mediated ADCC activity.

[0068] A systemic substitution of solvent-exposed amino acids of human IgG1 Fc region has generated IgG variants with altered FcγR binding affinities (Shields et al., 2001, J. Biol. Chem. 276:6591-604). When compared to parental IgG1, a subset of these variants involving substitutions at Thr256/Ser298, Ser298/Glu333, Ser298/Lys334, or Ser298/Glu333/Lys334 to Ala demonstrate increased in both binding affinity toward FcγR and ADCC activity (Shields et al., 2001, J. Biol. Chem. 276:6591-604; Okazaki et al., 2004, J. Mol. Biol. 336:1239-49).

[0069] Complement fixation activity of antibodies (both C1q binding and CDC activity) can be improved by substitutions at Lys326 and Glu333 (Idusogie et al., 2001, J. Immunol. 166:2571-2575). The same substitutions on a human IgG2 backbone can convert an antibody isotype that binds poorly to C1q and is severely deficient in complement activation activity to one that can both bind C1q and mediate CDC (Idusogie et al., 2001, J. Immunol. 166:2571-75). Several other methods have also been applied to improve complement fixation activity of antibodies. For example, the grafting of an 18-amino acid carboxyl-terminal tail piece of IgM to the carboxyl-termini of IgG greatly enhances their CDC activity. This is observed even with IgG4, which normally has no detectable CDC activity (Smith et al., 1995, J. Immunol. 154:2226-36). Also, substituting Ser444 located close to the carboxy-terminal of IgG1 heavy chain with Cys induced tail-to-tail dimerization of IgG1 with a 200-fold increase of CDC activity over monomeric IgG1 (Shopes et al., 1992, J. Immunol. 148:2918-22). In addition, a bispecific diabody construct with specificity for C1q also confers CDC activity (Kontermann et al., 1997, Nat. Biotech. 15:629-31).

[0070] Complement activity can be reduced by mutating at least one of the amino acid residues 318, 320, and 322 of the heavy chain to a residue having a different side chain, such as Ala. Other alkyl-substituted non-ionic residues, such as Gly, Ile, Leu, or Val, or such aromatic non-polar residues as Phe, Tyr, Trp and Pro in place of any one of the three residues also reduce or abolish C1q binding. Ser, Thr, Cys, and Met can be used at residues 320 and 322, but not 318, to reduce or abolish C1q binding activity. Replacement of the 318 (Glu) residue by a polar residue may modify but not abolish C1q binding activity. Replacing residue 297 (Asn) with Ala results in removal of lytic activity but only slightly reduces (about three fold weaker) affinity for C1q. This alteration destroys the glycosylation site and the presence of carbohydrate that is required for complement activation. Any other substitution at this site also destroys the glycosylation site. The following mutations and any combination thereof also reduce C1q binding: D270A, K322A, P329A, and P311S (see WO 06/036291).

[0071] Reference to a human constant region includes a constant region with any natural allotype or any permutation of residues occupying polymorphic positions in natural allotypes. Also, up to 1, 2, 5, or 10 mutations may be present relative to a natural human constant region, such as those indicated above to reduce Fcγ receptor binding or increase binding to FcRN.

D. Expression of Recombinant Antibodies

[0072] Humanized antibodies are typically produced by recombinant expression. Recombinant polynucleotide constructs typically include an expression control sequence operably linked to the coding sequences of antibody chains, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the crossreacting antibodies.

[0073] Mammalian cells are a preferred host for expressing nucleotide segments encoding immunoglobulins or fragments thereof. See Winnacker, *From Genes to Clones*, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include CHO cell lines (e.g., DG44), various COS cell lines, HeLa cells, HEK293 cells, L cells, and non-antibody-producing myelomas including Sp2/0 and NS0. Preferably, the cells are nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen et al., *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Preferred expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. See Co et al., *J. Immunol.* 148:1149 (1992).

[0074] Once expressed, antibodies can be purified according to standard procedures of the art, including HPLC purification, column chromatography, gel electrophoresis and the like (see generally, Scopes, *Protein Purification* (Springer-Verlag, NY, 1982)).

IV. Nucleic Acids

[0075] The invention further provides nucleic acids encoding any of the humanized heavy and light chains described above. Typically, the nucleic acids also encode a signal peptide fused to the mature heavy and light chains. Coding sequences on nucleic acids can be in operable linkage with regulatory sequences to ensure expression of the coding sequences, such as a promoter, enhancer, ribosome binding site, transcription termination signal and the like. The nucleic acids encoding heavy and light chains can occur in isolated form or can be cloned into one or more vectors. The nucleic acids can be synthesized by for example, solid state synthesis or PCR of overlapping oligonucleotides. Nucleic acids encoding heavy and light chains can be joined as one contiguous nucleic acid, e.g., within an expression vector, or can be separate, e.g., each cloned into its own expression vector.

V. Antibody Drug Conjugates

[0076] Anti-LIV-1 antibodies can be conjugated to cytotoxic or cytostatic moieties (including pharmaceutically compatible salts thereof) to form an antibody drug conjugate (ADC). Particularly suitable moieties for conjugation to antibodies are cytotoxic agents (e.g., chemotherapeutic agents), prodrug converting enzymes, radioactive isotopes or compounds, or toxins (these moieties being collectively referred to as a therapeutic agent). For example, an anti-LIV-1 antibody can be conjugated to a cytotoxic agent such as a chemotherapeutic agent, or a toxin (e.g., a cytostatic or cytotoxic agent such as, e.g., abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin).

[0077] An anti-LIV-1 antibody can be conjugated to a pro-drug converting enzyme. The pro-drug converting enzyme can be recombinantly fused to the antibody or chemically conjugated thereto using known methods. Exemplary pro-drug converting enzymes are carboxypeptidase G2, beta-glucuronidase, penicillin-V-amidase, penicillin-G-amidase, β -lactamase, β -glucosidase, nitroreductase and carboxypeptidase A.

[0078] Techniques for conjugating therapeutic agents to proteins, and in particular to antibodies, are well-known. (See, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in *Monoclonal Antibodies And Cancer Therapy* (Reisfeld et al. eds., Alan R. Liss, Inc., 1985); Hellstrom et al., "Antibodies For Drug Delivery," in *Controlled Drug Delivery* (Robinson et al. eds., Marcel Dekker, Inc., 2nd ed. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in *Monoclonal Antibodies '84: Biological And Clinical Applications* (Pinchera et al. eds., 1985); "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody In Cancer Therapy," in *Monoclonal Antibodies For Cancer Detection And Therapy* (Baldwin et al. eds., Academic Press, 1985); and Thorpe et al., 1982, *Immunol. Rev.* 62:119-58. See also, e.g., PCT publication WO 89/12624.)

[0079] The therapeutic agent can be conjugated in a manner that reduces its activity unless it is cleaved off the antibody (e.g., by hydrolysis, by antibody degradation or by a cleaving agent). Such therapeutic agent is attached to the antibody with a cleavable linker that is sensitive to cleavage in the intracellular environment of the LIV-1-expressing cancer cell but is not substantially sensitive to the extracellular environment, such that the conjugate is cleaved from the antibody when it is internalized by the LIV-1-expressing cancer cell (e.g., in the endosomal or, for example by virtue of pH sensitivity or protease sensitivity, in the lysosomal environment or in the caveolar environment).

[0080] Typically the ADC comprises a linker region between the therapeutic agent and the anti-LIV-1 antibody. As noted *supra*, typically, the linker is cleavable under intracellular conditions, such that cleavage of the linker releases the therapeutic agent from the antibody in the intracellular environment (e.g., within a lysosome or endosome or caveolea). The linker can be, e.g., a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including a lysosomal or endosomal protease. Typically, the peptidyl linker is at least two amino acids long or at least three amino acids long. Cleaving agents can include cathepsins B and D and plasmin (see, e.g., Dubowchik and Walker, 1999, *Pharm. Therapeutics* 83:67-123). Most

typical are peptidyl linkers that are cleavable by enzymes that are present in LIV-1-expressing cells. For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (e.g., a linker comprising a Phe-Leu or a Gly-Phe-Leu-Gly peptide). Other such linkers are described, e.g., in U.S. Patent No. 6,214,345. In specific embodiments, the peptidyl linker cleavable by an intracellular protease comprises a Val-Cit linker or a Phe-Lys dipeptide (see, e.g., U.S. patent 6,214,345, which describes the synthesis of doxorubicin with the Val-Cit linker). One advantage of using intracellular proteolytic release of the therapeutic agent is that the agent is typically attenuated when conjugated and the serum stabilities of the conjugates are typically high.

[0081] The cleavable linker can be pH-sensitive, *i.e.*, sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker is hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (e.g., a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) can be used. (See, e.g., U.S. Patent Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville et al., 1989, Biol. Chem. 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, e.g., a thioether attached to the therapeutic agent via an acylhydrazone bond (see, e.g., U.S. Patent No. 5,622,929)).

[0082] Other linkers are cleavable under reducing conditions (e.g., a disulfide linker). Disulfide linkers include those that can be formed using SATA (N-succinimidyl-S-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyldithio)toluene), SPDB and SMPT. (See, e.g., Thorpe et al., 1987, Cancer Res. 47:5924-5931; Wawrzynczak et al., In Immunoconjugates: Antibody Conjugates in Radioimaging and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987. See also U.S. Patent No. 4,880,935.)

[0083] The linker can also be a malonate linker (Johnson et al., 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1299-1304), or a 3'-N-amide analog (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1305-12).

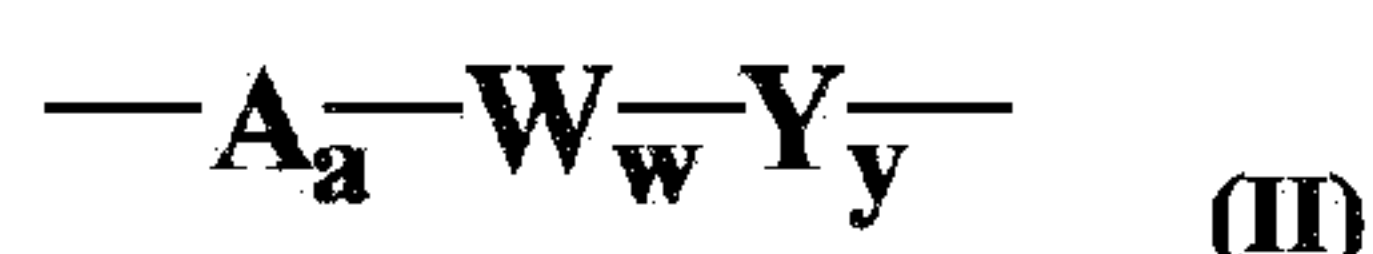
[0084] The linker also can be a non-cleavable linker, such as an maleimido-alkylene- or maleimide-aryl linker that is directly attached to the therapeutic agent (e.g., a drug). An active drug-linker is released by degradation of the antibody.

[0085] Typically, the linker is not substantially sensitive to the extracellular environment meaning that no more than about 20%, typically no more than about 15%, more typically no more than about 10%, and even more typically no more than about 5%, no more than about 3%, or no more than about 1% of the linkers in a sample of the ADC is cleaved when the ADC present in an extracellular environment (e.g., in plasma). Whether a linker is not substantially sensitive to the extracellular environment can be determined, for example, by incubating independently with plasma both (a) the ADC (the "ADC sample") and (b) an equal molar

amount of unconjugated antibody or therapeutic agent (the "control sample") for a predetermined time period (e.g., 2, 4, 8, 16, or 24 hours) and then comparing the amount of unconjugated antibody or therapeutic agent present in the ADC sample with that present in control sample, as measured, for example, by high performance liquid chromatography.

[0086] The linker can also promote cellular internalization. The linker can promote cellular internalization when conjugated to the therapeutic agent (*i.e.*, in the milieu of the linker-therapeutic agent moiety of the ADC or ADC derivative as described herein). Alternatively, the linker can promote cellular internalization when conjugated to both the therapeutic agent and the anti-LIV-1 antibody (*i.e.*, in the milieu of the ADC as described herein).

[0087] A variety of linkers that can be used with the present compositions are described in WO 2004-010957 and have the form



wherein:

-A- is a stretcher unit;

a is 0 or 1;

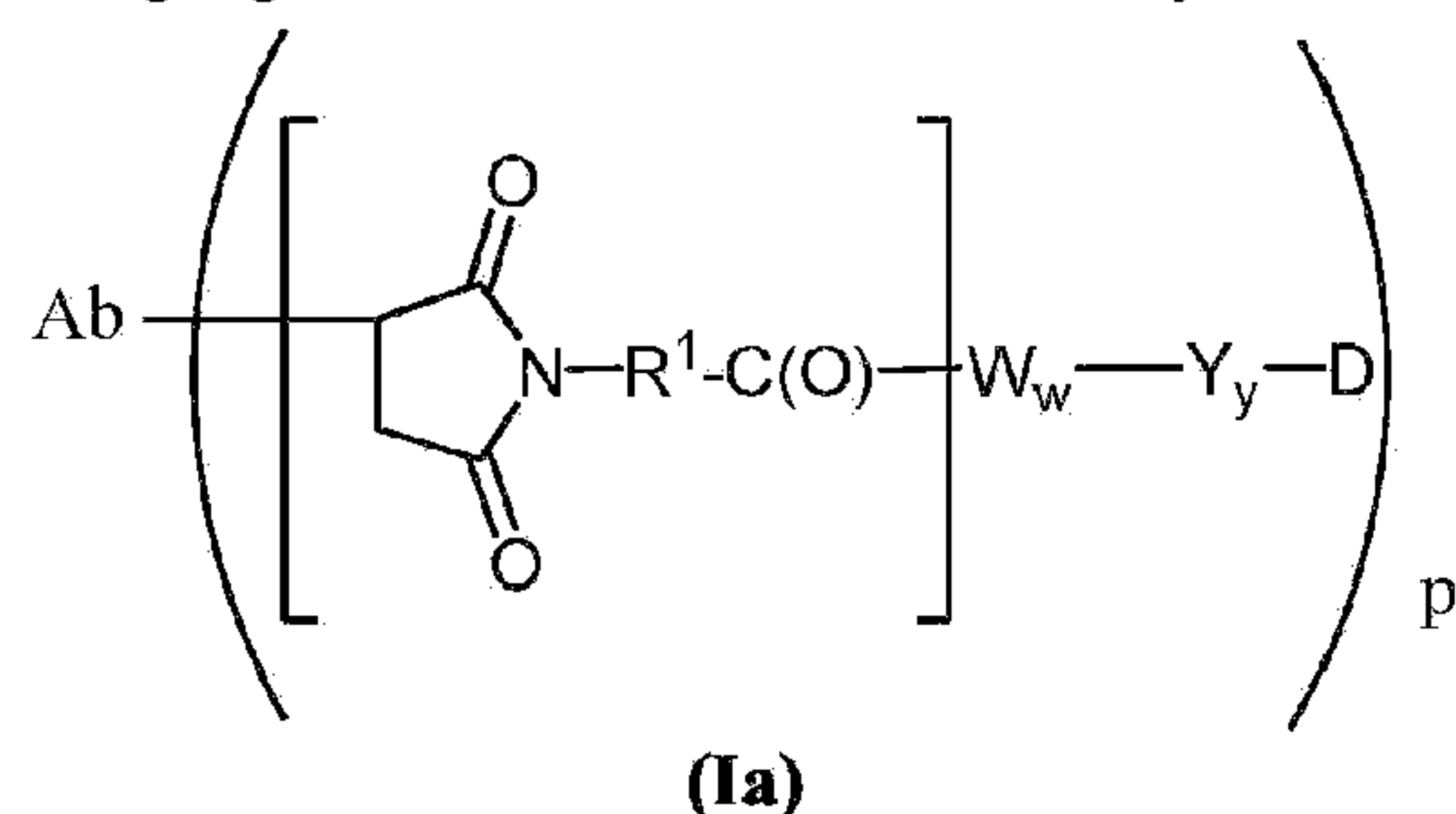
each -W- is independently an amino acid unit;

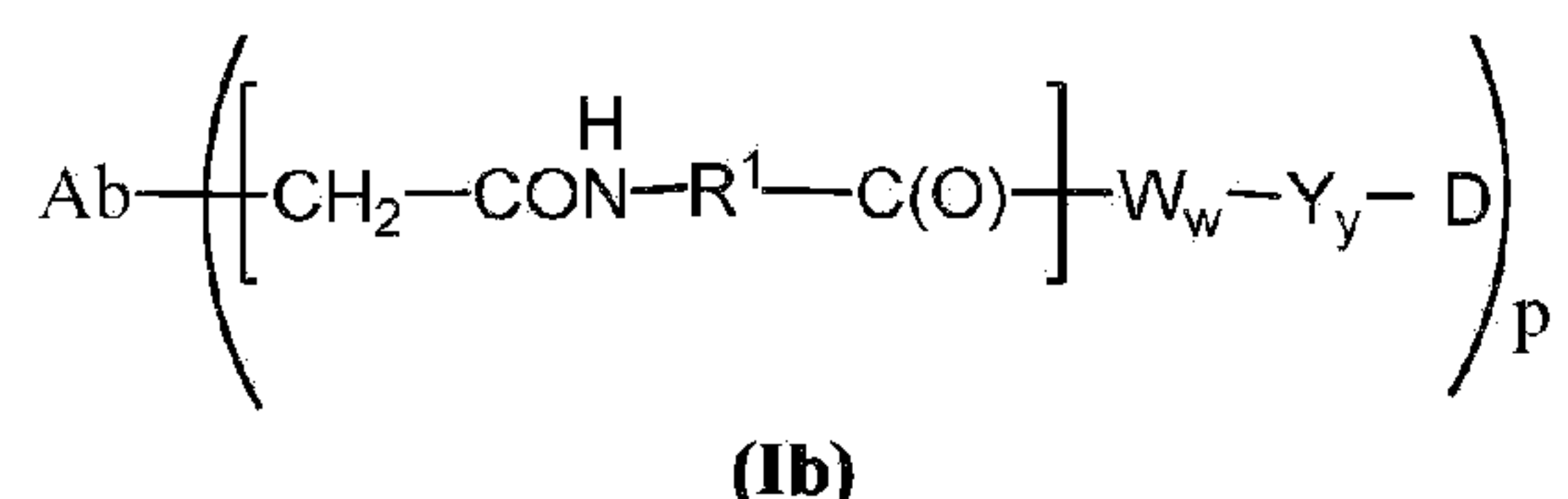
w is independently an integer ranging from 0 to 12;

-Y- is a spacer unit; and

y is 0, 1 or 2.

[0088] Representative stretcher units are depicted within the square brackets of Formulas (Ia) and (Ib; see *infra*), wherein A-, -W-, -Y-, -D, w and y are as defined above and R¹ is selected from -C₁-C₁₀ alkylene-, -C₃-C₈ carbocyclo-, -O-(C₁-C₈ alkyl)-, - arylene-, -C₁-C₁₀ alkylene-arylene-, -arylene-C₁-C₁₀ alkylene-, -C₁-C₁₀ alkylene-(C₃-C₈ carbocyclo)-, -(C₃-C₈ carbocyclo)-C₁-C₁₀ alkylene-, -C₃-C₈ heterocyclo-, -C₁-C₁₀ alkylene-(C₃-C₈ heterocyclo)-, -(C₃-C₈ heterocyclo)-C₁-C₁₀ alkylene-, -(CH₂CH₂O)_r-, and -(CH₂CH₂O)_r-CH₂-; and r is an integer ranging from 1-10. Ab is antibody.





[0089] The drug loading is represented by p , the number of drug-linker molecules per antibody. Depending on the context, p can represent the average number of drug-linker molecules per antibody, also referred to the average drug loading. P ranges from 1 to 20 and is preferably from 1 to 8. In some preferred embodiments, when p represents the average drug loading, p ranges from about 2 to about 5. In some embodiments, p is about 2, about 3, about 4, or about 5. The average number of drugs per antibody in a preparation may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC.

The Amino Acid unit ($-\text{W}-$), if present, links the Stretcher unit ($-\text{A}-$) to the Spacer unit ($-\text{Y}-$) if the Spacer unit is present, and links the Stretcher unit to the cytotoxic or cytostatic agent (Drug unit; D) if the spacer unit is absent.

[0090] If present, $-\text{W}_w-$ is preferably a dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit.

[0091] The Spacer unit ($-\text{Y}-$), when present, links an Amino Acid unit to the Drug unit. Spacer units are of two general types: self-immolative and non self-immolative. A non self-immolative spacer unit is one in which part or all of the Spacer unit remains bound to the Drug unit after enzymatic cleavage of an amino acid unit from the anti-LIV-1 antibody-linker-drug conjugate or the drug-linker compound. Examples of a non self-immolative Spacer unit include a (glycine-glycine) spacer unit and a glycine spacer unit. When an anti-LIV-1 antibody-linker-drug conjugate containing a glycine-glycine spacer unit or a glycine spacer unit undergoes enzymatic cleavage via a tumor-cell associated-protease, a cancer-cell-associated protease or a lymphocyte-associated protease, a glycine-glycine-drug moiety or a glycine-drug moiety is cleaved from $\text{Ab}-\text{A}_a-\text{W}_w-$. To liberate the drug, an independent hydrolysis reaction should take place within the target cell to cleave the glycine-drug unit bond.

[0092] Alternatively, an anti-LIV-1 antibody drug conjugate containing a self-immolative spacer unit can release the drug (D) without the need for a separate hydrolysis step. In some of these embodiments, $-\text{Y}-$ is a p -aminobenzyl alcohol (PAB) unit that is linked to $-\text{W}_w-$ via the nitrogen atom of the PAB group, and connected directly to $-\text{D}$ via a carbonate, carbamate or ether group. Other examples of self-immolative spacers include aromatic compounds that are electronically equivalent to the PAB group such as 2-aminoimidazol-5-methanol derivatives (see Hay et al., 1999, Bioorg. Med. Chem. Lett. 9:2237 for examples) and ortho or para-aminobenzylacetals. Spacers can be used that undergo facile cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al., 1995, Chemistry Biology 2:223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm et al., 1972, J. Amer. Chem. Soc. 94:5815) and 2-aminophenylpropionic acid

amides (Amsberry et al., 1990, J. Org. Chem. 55:5867). Elimination of amine-containing drugs that are substituted at the α -position of glycine (Kingsbury, et al., 1984, J. Med. Chem. 27:1447) are also examples of self-immolative spacer strategies that can be applied to the anti-LIV-1 antibody-linker-drug conjugates. Alternatively, the spacer unit is a branched bis(hydroxymethyl)styrene (BHMS) unit, which can be used to incorporate additional drugs.

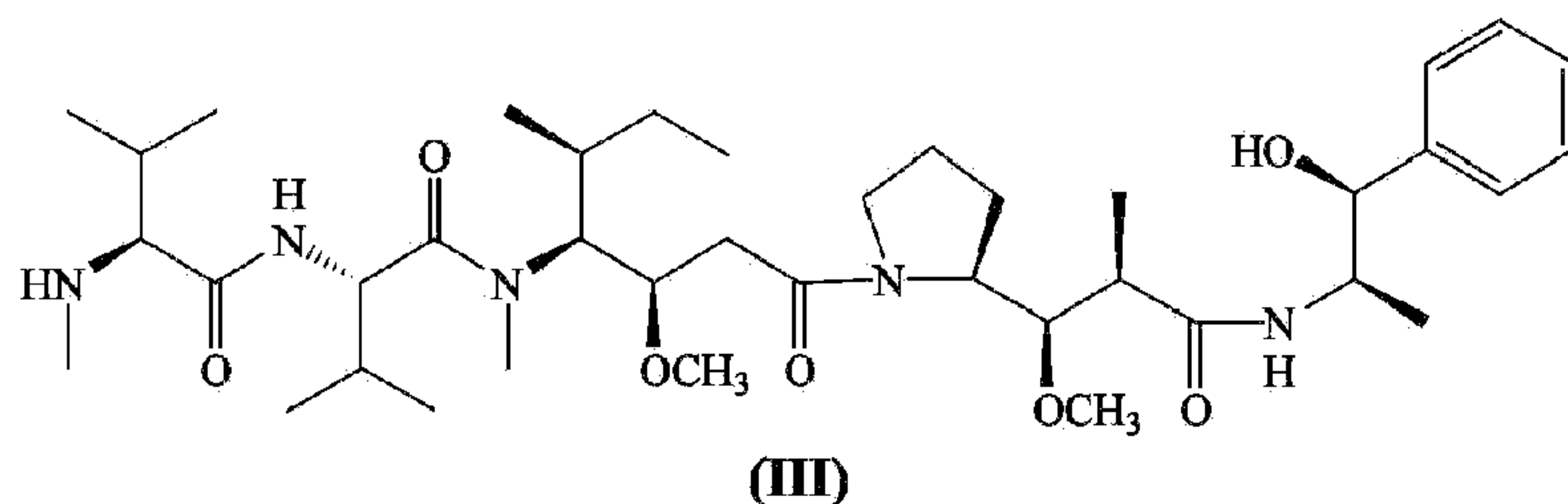
[0093] Useful classes of cytotoxic agents to conjugate to anti-LIV-1 antibodies include, for example, antitubulin agents, DNA minor groove binding agents, DNA replication inhibitors, chemotherapy sensitizers, or the like. Other exemplary classes of cytotoxic agents include anthracyclines, auristatins, camptothecins, duocarmycins, etoposides, maytansinoids and vinca alkaloids. Some exemplary cytotoxic agents include auristatins (e.g., auristatin E, AFP, MMAF, MMAE), DNA minor groove binders (e.g., enediynes and lexitropsins), duocarmycins, taxanes (e.g., paclitaxel and docetaxel), vinca alkaloids, doxorubicin, morpholino-doxorubicin, and cyanomorpholino-doxorubicin.

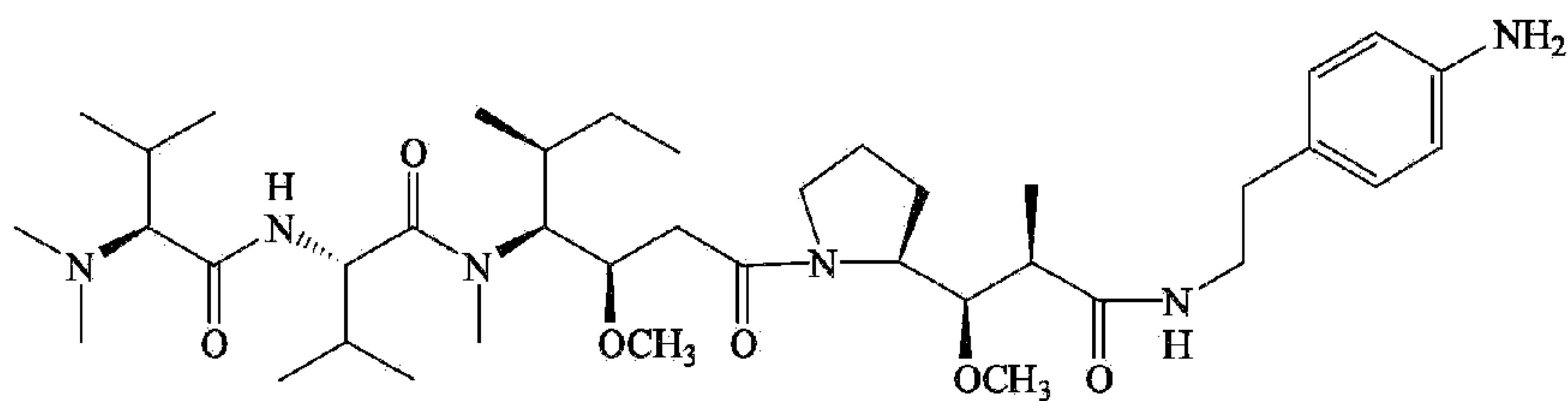
[0094] The cytotoxic agent can be a chemotherapeutic such as, for example, doxorubicin, paclitaxel, melphalan, vinca alkaloids, methotrexate, mitomycin C or etoposide. The agent can also be a CC-1065 analogue, calicheamicin, maytansine, an analog of dolastatin 10, rhizoxin, or palytoxin.

[0095] The cytotoxic agent can also be an auristatin. The auristatin can be an auristatin E derivative is, e.g., an ester formed between auristatin E and a keto acid. For example, auristatin E can be reacted with paraacetyl benzoic acid or benzoylvaleric acid to produce AEB and AEVB, respectively. Other typical auristatins include AFP, MMAF, and MMAE. The synthesis and structure of various auristatins are described in, for example, US 2005-0238649 and US2006-0074008.

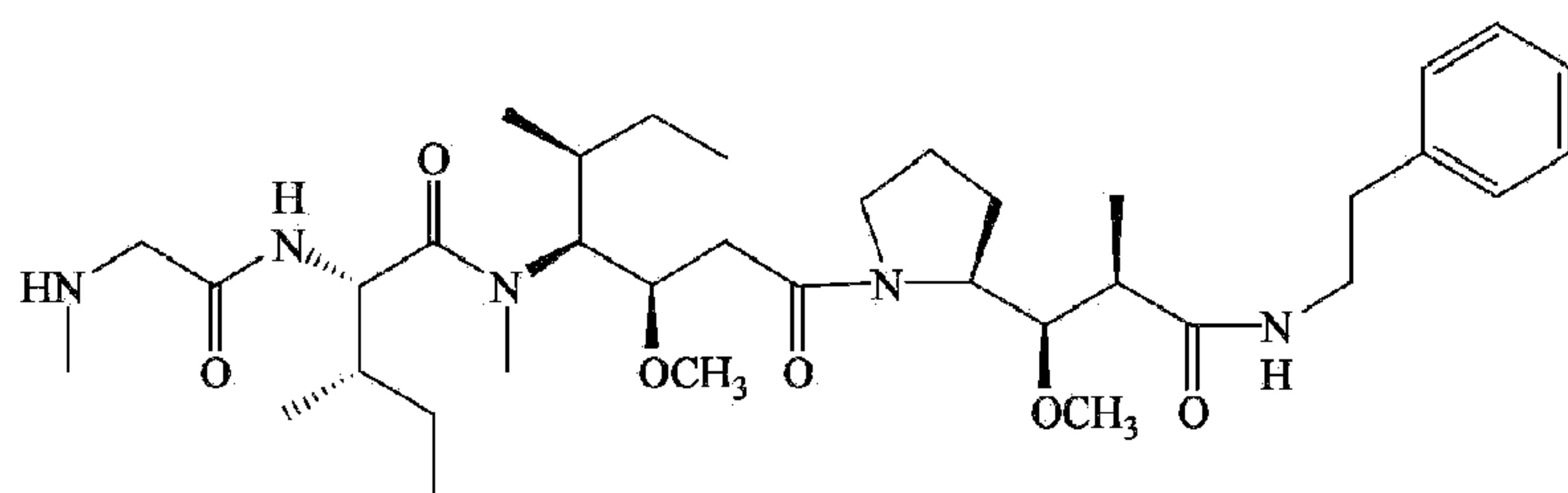
[0096] The cytotoxic agent can be a DNA minor groove binding agent. (See, e.g., U.S. Patent No. 6,130,237.) For example, the minor groove binding agent can be a CBI compound or an enediyne (e.g., calicheamicin).

[0097] The cytotoxic or cytostatic agent can be an anti-tubulin agent. Examples of anti-tubulin agents include taxanes (e.g., Taxol® (paclitaxel), Taxotere® (docetaxel)), T67 (Tularik), vinca alkyls (e.g., vincristine, vinblastine, vindesine, and vinorelbine), and auristatins (e.g., auristatin E, AFP, MMAF, MMAE, AEB, AEVB). (Exemplary auristatins are shown below in formulae III-XIII. Other suitable antitubulin agents include, for example, baccatin derivatives, taxane analogs (e.g., epothilone A and B), nocodazole, colchicine and colcimid, estramustine, cryptophysins, cemadotin, maytansinoids, combretastatins, discodermolide, and eleutherobin.

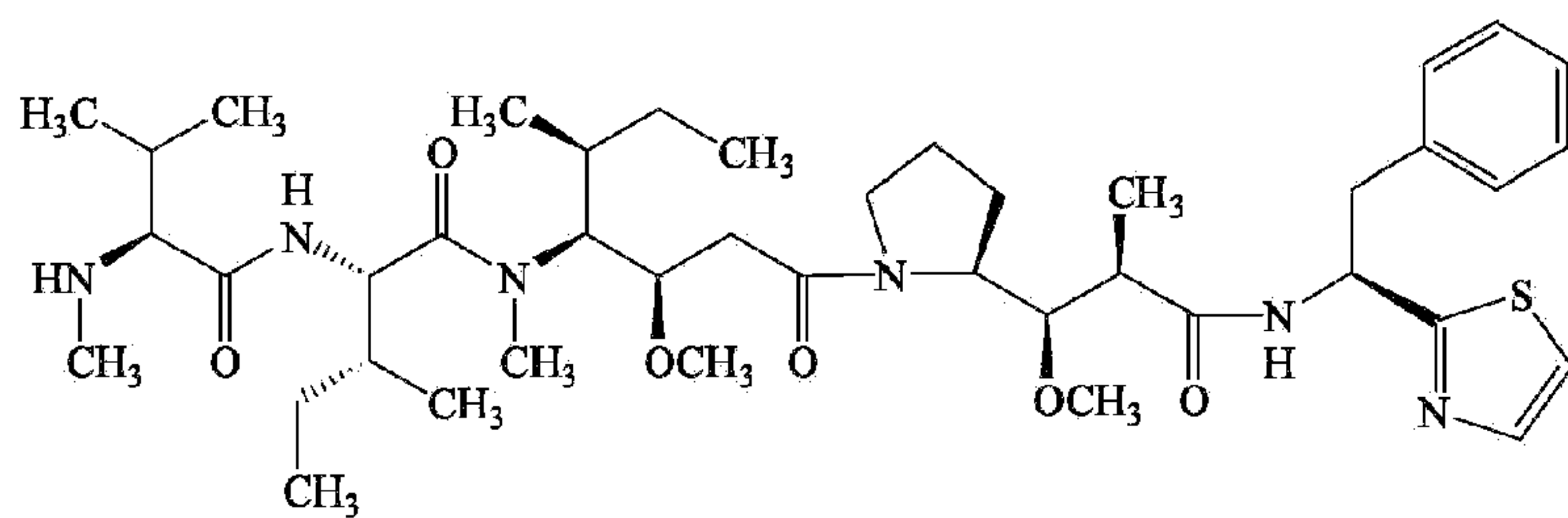




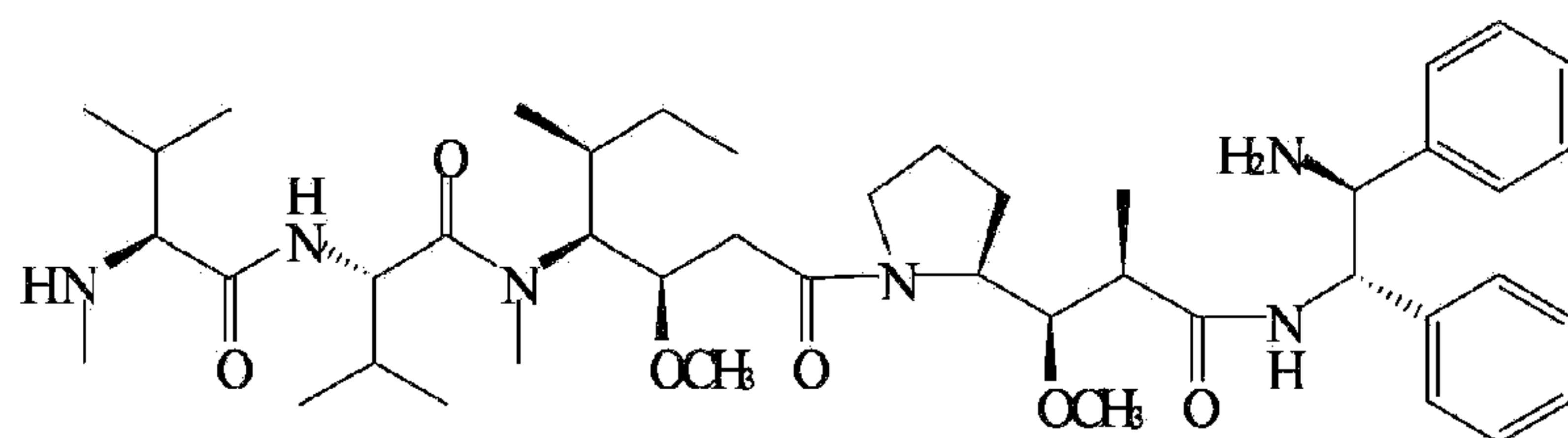
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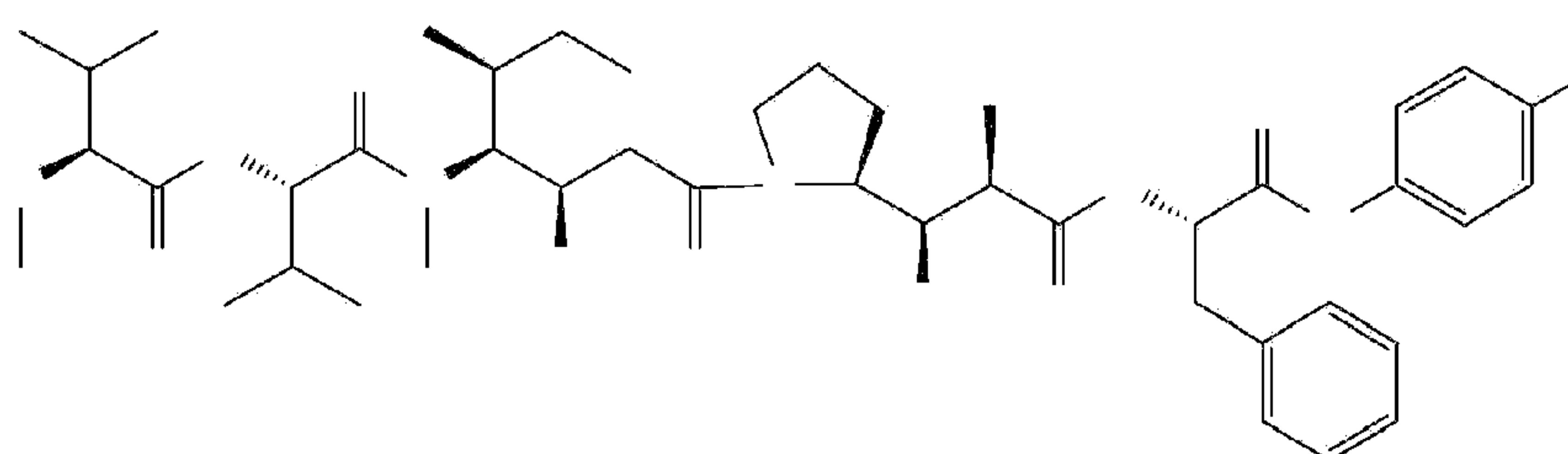
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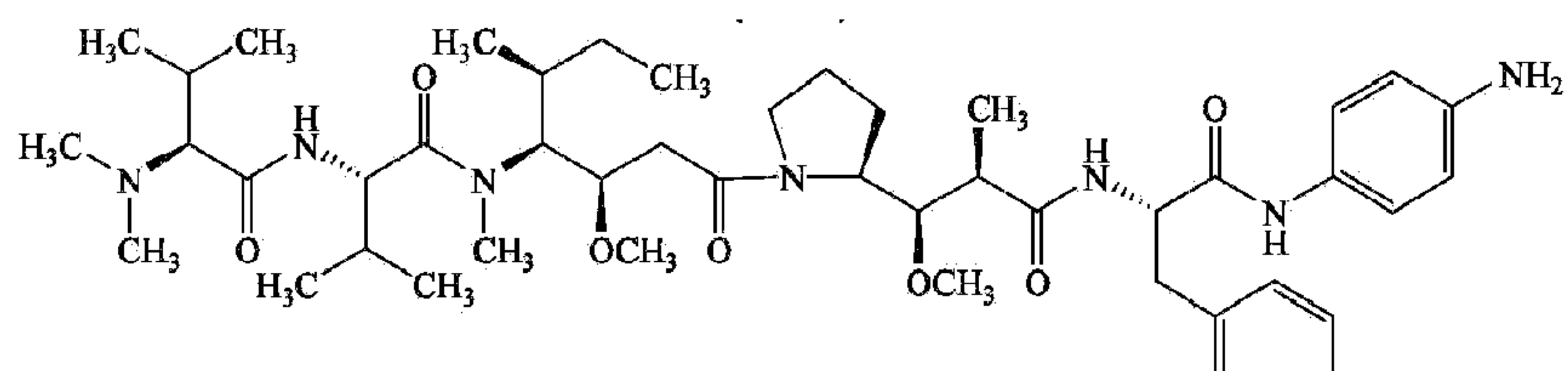
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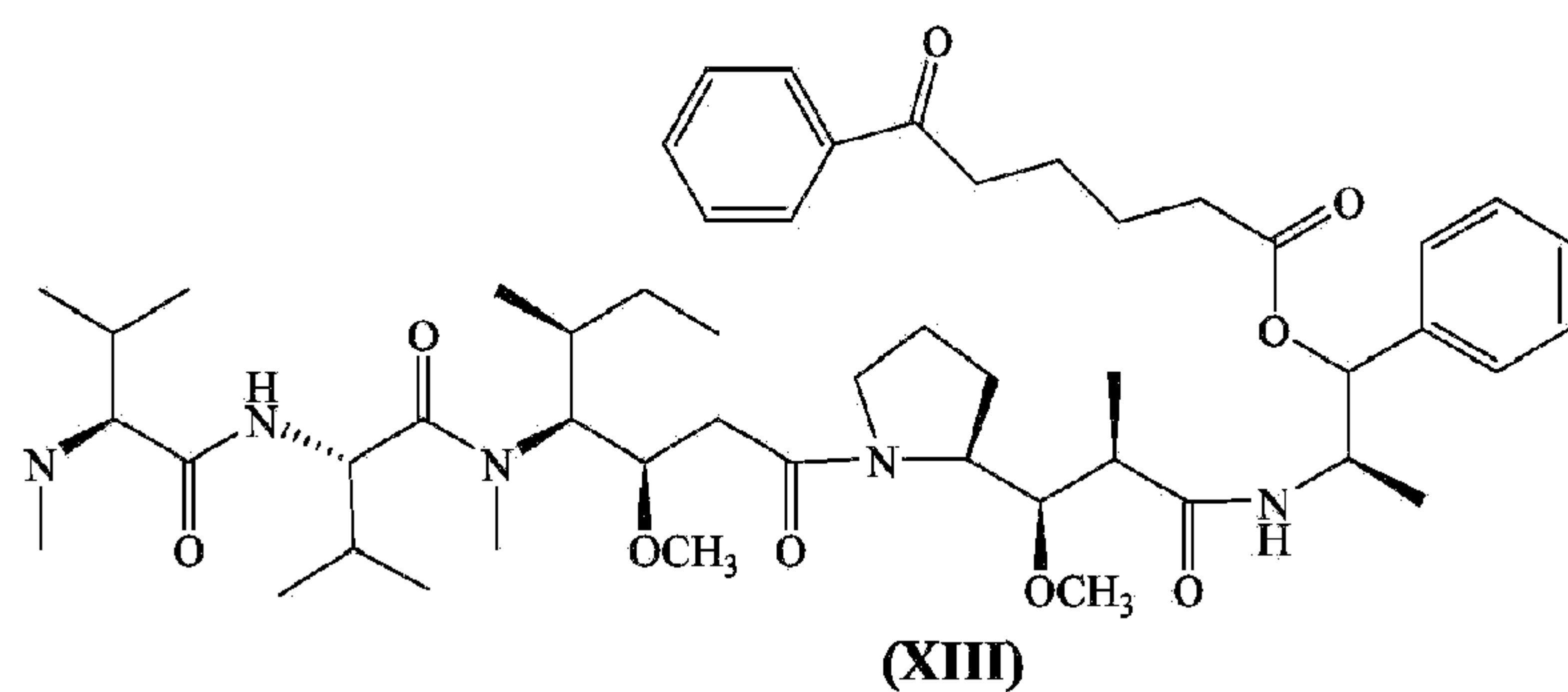
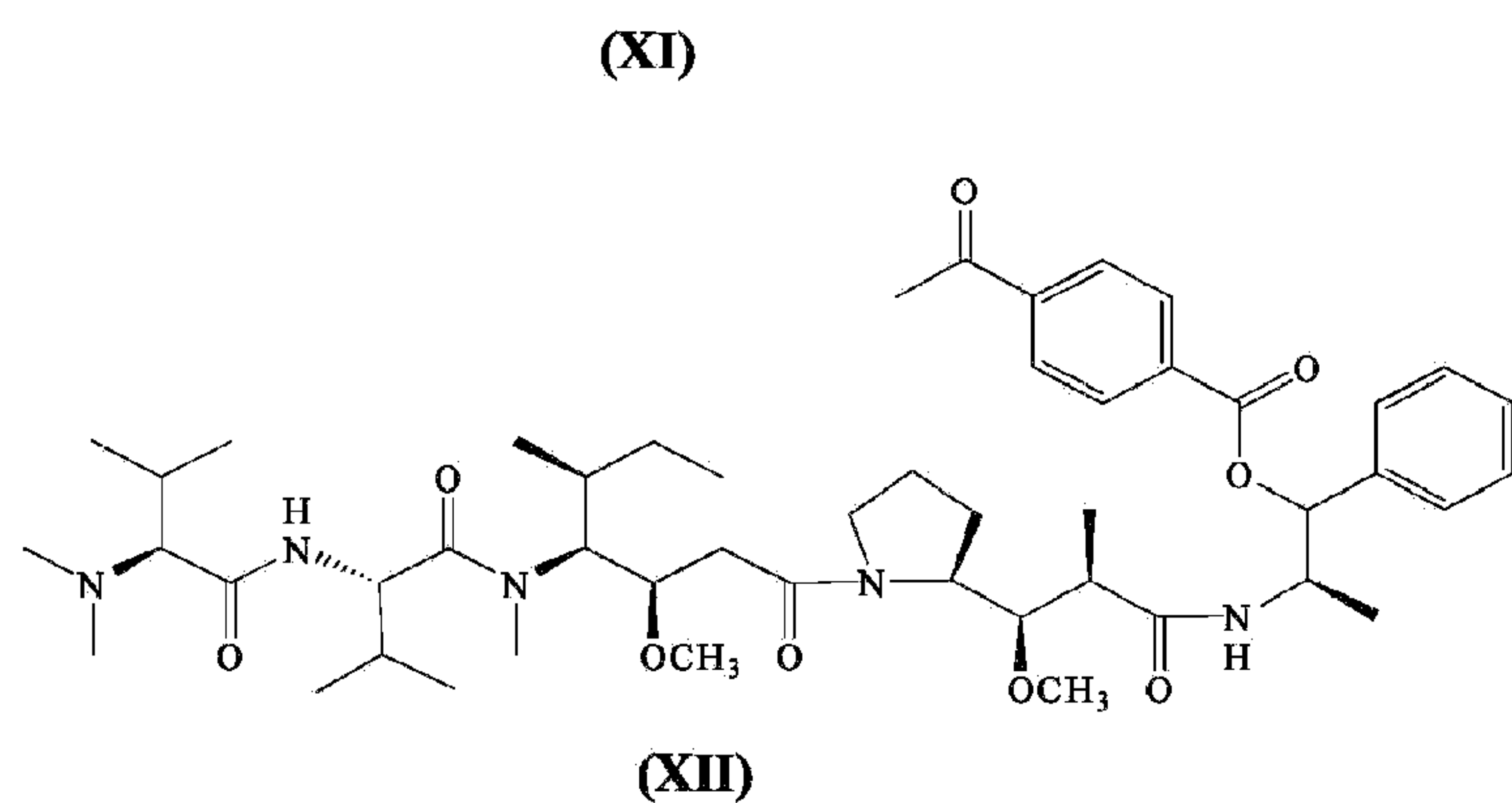
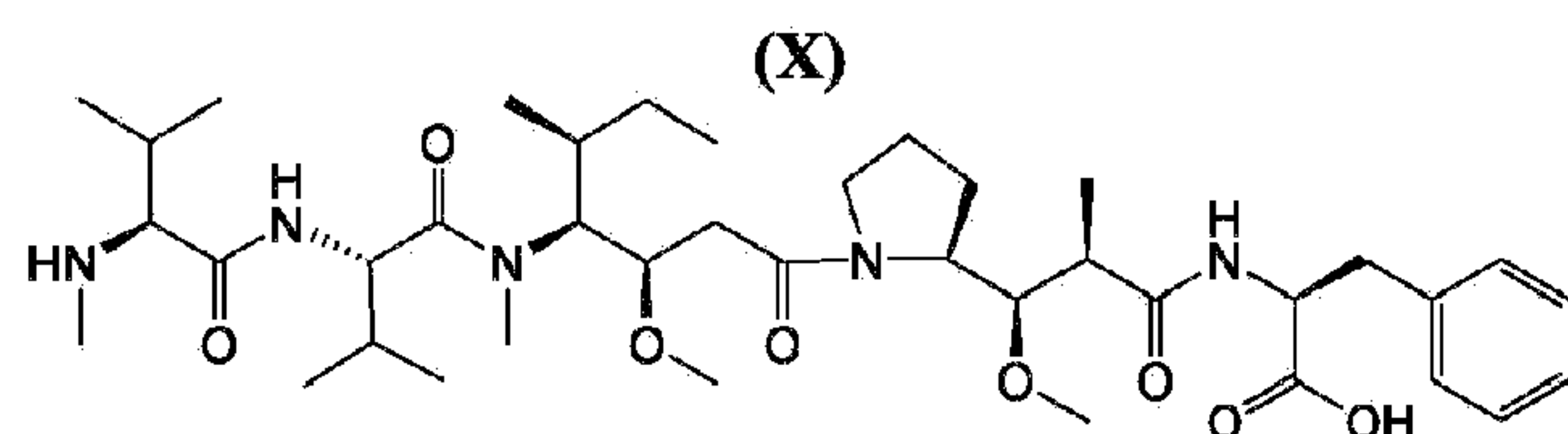
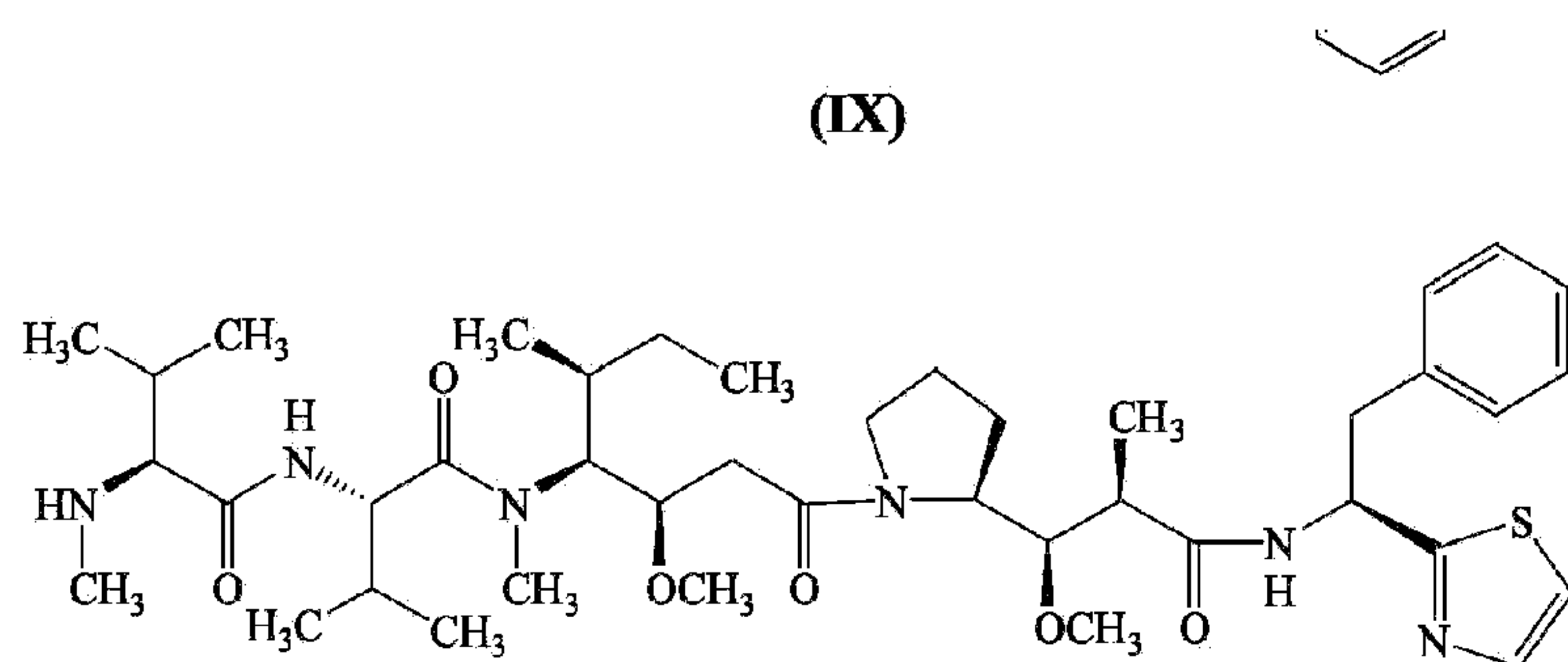


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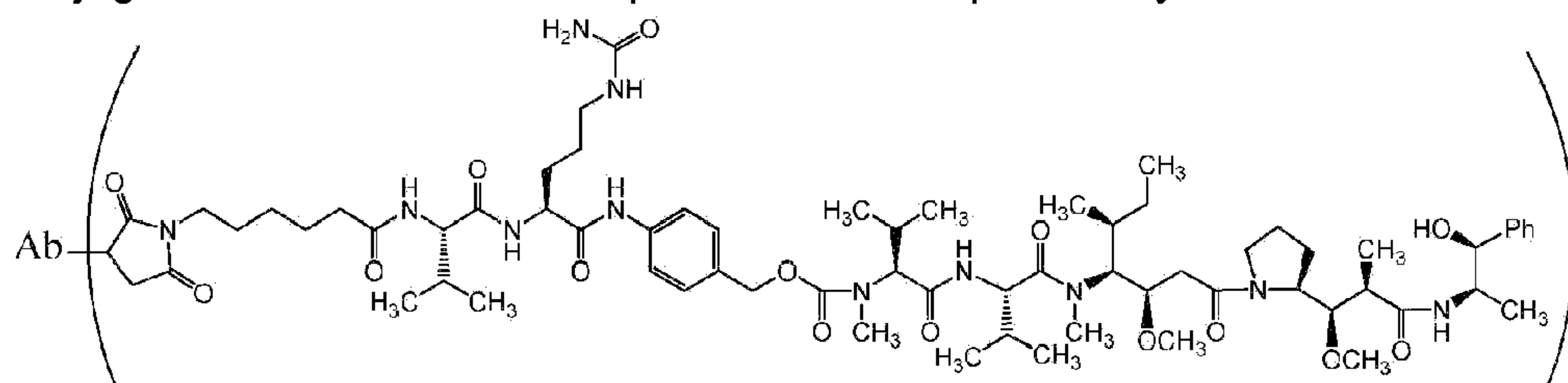
(VIII)





[0098] The cytotoxic agent can be a maytansinoid, another group of anti-tubulin agents. For example, the maytansinoid can be maytansine or a maytansine containing drug linker such as DM-1 or DM-4 (ImmunoGen, Inc.; see *a/so* Chari et al., 1992, Cancer Res. 52:127-131).

[0099] Exemplary antibody drug conjugates include vcMMAE and mcMMAF antibody drug conjugates as follows wherein p and Ab are as previously described herein:



CC(C)[C@@H](NC(=O)N[C@@H](C)[C@H](OC)C(=O)N1CC[C@H]1C(=O)O[C@H](C)[C@@H](C)C(=O)N[C@@H](C)[C@H](OC)C(=O)O[C@H](C)[C@@H](C)C(=O)NCCCCCNC2C(=O)CC(C2)C(=O)O)C(=O)N3C(=O)CC(C3)C(=O)O

[0102] Humanized, chimeric or veneered forms of non-human antibodies can be made. General methodology for producing humanized antibodies is described by Queen, US 5,530,101 and 5,585,089; Winter, US 5,225,539; Carter, US 6,407,213; Adair, US 5,859,205; and Foote, US 6,881,557). A chimeric antibody is an antibody in which the mature variable regions of light and heavy chains of a non-human antibody (e.g., a mouse) are combined with human light and heavy chain constant regions. Such antibodies substantially or entirely retain the binding specificity of the mouse antibody, and are about two-thirds human sequence. A veneered antibody is a type of humanized antibody that retains some and usually all of the CDRs and some of the non-human variable region framework residues of a non-human antibody but replaces other variable region framework residues that may contribute to B- or T-cell epitopes, for example exposed residues (Padlan, Mol. Immunol. 28:489, 1991) with residues from the corresponding positions of a human antibody sequence. The result is an

antibody in which the CDRs are entirely or substantially from a non-human antibody and the variable region frameworks of the non-human antibody are made more human-like by the substitutions.

[0103] Human antibodies against LIV-1 can be provided by a variety of techniques described below. Methods for producing human antibodies include the trioma method of Oestberg et al., Hybridoma 2:361-367 (1983); Oestberg, U.S. Patent No. 4,634,664; and Engleman et al., US Patent 4,634,666; use of transgenic mice including human immunoglobulin genes (see, e.g., Lonberg et al., WO93/12227 (1993); US 5,877,397, US 5,874,299, US 5,814,318, US 5,789,650, US 5,770,429, US 5,661,016, US 5,633,425, US 5,625,126, US 5,569,825, US 5,545,806, Nature 148, 1547-1553 (1994), Nature Biotechnology 14, 826 (1996), Kucherlapati, WO 91/10741 (1991) and phage display methods (see, e.g. Dower et al., WO 91/17271 and McCafferty et al., WO 92/01047, US 5,877,218, US 5,871,907, US 5,858,657, US 5,837,242, US 5,733,743 and US 5,565,332.

[0104] Any of the antibodies can be selected to have the same or overlapping epitope specificity as an exemplar antibody, such as the BR2-14a antibody, by a competitive binding assay or otherwise.

VII. Therapeutic Applications

[0105] The humanized antibodies of the invention, alone or as LIV-1 antibody drug conjugates thereof, can be used to treat cancer. Some such cancers show detectable levels of LIV-1 measured at either the protein (e.g., by immunoassay using one of the exemplified antibodies) or mRNA level. Some such cancers show elevated levels of LIV-1 relative to noncancerous tissue of the same type, preferably from the same patient. An exemplary level of LIV-1 on cancer cells amenable to treatment is 5000-150000 LIV-1 molecules per cell, although higher or lower levels can be treated. Optionally, a level of LIV-1 in a cancer is measured before performing treatment.

[0106] Examples of cancers associated with LIV-1 expression and amenable to treatment include breast cancer, prostate cancer, ovarian cancer, endometrial cancer, cervical, liver, gastric, kidney, and squamous cell carcinomas (e.g., bladder, head, neck and lung), skin cancers, e.g., melanoma, small lung cell carcinoma or lung carcinoid. The treatment can be applied to patients having primary or metastatic tumors of these kinds. The treatment can also be applied to patients who are refractory to conventional treatments (e.g., hormones, tamoxifen, herceptin), or who have relapsed following a response to such treatments. The methods can also be used on triple negative breast cancers. A triple negative breast cancer is a term of art for a cancer lacking detectable estrogen and progesterone receptors and lacking overexpression of HER2/neu when stained with an antibody to any of these receptors, such as described in the examples. Staining can be performed relative to an irrelevant control antibody and lack of expression shown from a background level of staining the same or similar to that of the control within experimental error. Likewise lack of overexpression is shown by staining at

the same or similar level within experimental error of noncancerous breast tissue, preferably obtained from the same patient. Alternatively or additionally, triple negative breast cancers are characterized by lack of responsiveness to hormones interacting with these receptors, aggressive behavior and a distinct pattern of metastasis. hLIV14 antibodies can be used to treat cancers that express LIV-1. hLIV22 antibodies can be used to treat cancers that express LIV-1. In one embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing breast cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing prostate cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing melanoma. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing ovarian cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing endometrial cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing cervical cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing liver cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing gastric cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing kidney cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing squamous cell carcinomas (e.g., bladder, head, neck and lung cancer). In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing breast cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing skin cancer. In another embodiment, an hLIV22 antibody is used to treat a subject with a LIV-1-expressing small lung cell carcinoma or lung carcinoid.

This application provides the first disclosure that LIV-1 protein is expressed on the surface of melanoma cells. Thus, antibodies that bind to LIV-1 can be used to treat patients that are afflicted with melanomas that express LIV-1. Such antibodies include antibodies disclosed herein, e.g., hLIV14 and hLIV22, but are not limited to the antibodies disclosed herein.

[0107] Humanized antibodies, alone or as conjugates thereof, are administered in an effective regime meaning a dosage, route of administration and frequency of administration that delays the onset, reduces the severity, inhibits further deterioration, and/or ameliorates at least one sign or symptom of cancer. If a patient is already suffering from cancer, the regime can be referred to as a therapeutically effective regime. If the patient is at elevated risk of the cancer relative to the general population but is not yet experiencing symptoms, the regime can be referred to as a prophylactically effective regime. In some instances, therapeutic or prophylactic efficacy can be observed in an individual patient relative to historical controls or past experience in the same patient. In other instances, therapeutic or prophylactic efficacy can be demonstrated in a preclinical or clinical trial in a population of treated patients relative to a control population of untreated patients.

[0108] Exemplary dosages for a monoclonal antibody are 0.1 mg/kg to 50 mg/kg of the patient's body weight, more typically 1 mg/kg to 30 mg/kg, 1 mg/kg to 20 mg/kg, 1 mg/kg to 15 mg/kg, 1 mg/kg to 12 mg/kg, or 1 mg/kg to 10 mg/kg, or 2 mg/kg to 30 mg/kg, 2 mg/kg to 20 mg/kg, 2 mg/kg to 15 mg/kg, 2 mg/kg to 12 mg/kg, or 2 mg/kg to 10 mg/kg, or 3 mg/kg to 30 mg/kg, 3 mg/kg to 20 mg/kg, 3 mg/kg to 15 mg/kg, 3 mg/kg to 12 mg/kg, or 3 mg/kg to 10

mg/kg . Exemplary dosages for a monoclonal antibody or antibody drug conjugates thereof are 1 mg/kg to 7.5 mg/kg, or 2 mg/kg to 7.5 mg/kg or 3 mg/kg to 7.5 mg/kg of the subject's body weight, or 0.1-20, or 0.5-5 mg/kg body weight (e.g., 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg) or 10-1500 or 200-1500 mg as a fixed dosage. In some methods, the patient is administered a dose of at least 1.5 mg/kg, at least 2 mg/kg or at least 3 mg/kg, administered once every three weeks or greater. The dosage depends on the frequency of administration, condition of the patient and response to prior treatment, if any, whether the treatment is prophylactic or therapeutic and whether the disorder is acute or chronic, among other factors.

[0109] Administration can be parenteral, intravenous, oral, subcutaneous, intra-arterial, intracranial, intrathecal, intraperitoneal, topical, intranasal or intramuscular. Administration can also be localized directly into a tumor. Administration into the systemic circulation by intravenous or subcutaneous administration is preferred. Intravenous administration can be, for example, by infusion over a period such as 30-90 min or by a single bolus injection.

[0110] The frequency of administration depends on the half-life of the antibody or conjugate in the circulation, the condition of the patient and the route of administration among other factors. The frequency can be daily, weekly, monthly, quarterly, or at irregular intervals in response to changes in the patient's condition or progression of the cancer being treated. An exemplary frequency for intravenous administration is between twice a week and quarterly over a continuous course of treatment, although more or less frequent dosing is also possible. Other exemplary frequencies for intravenous administration are between weekly or three out of every four weeks over a continuous course of treatment, although more or less frequent dosing is also possible. For subcutaneous administration, an exemplary dosing frequency is daily to monthly, although more or less frequent dosing is also possible.

[0111] The number of dosages administered depends on the nature of the cancer (e.g., whether presenting acute or chronic symptoms) and the response of the disorder to the treatment. For acute disorders or acute exacerbations of a chronic disorder between 1 and 10 doses are often sufficient. Sometimes a single bolus dose, optionally in divided form, is sufficient for an acute disorder or acute exacerbation of a chronic disorder. Treatment can be repeated for recurrence of an acute disorder or acute exacerbation. For chronic disorders, an antibody can be administered at regular intervals, e.g., weekly, fortnightly, monthly, quarterly, every six months for at least 1, 5 or 10 years, or the life of the patient.

[0112] Pharmaceutical compositions for parenteral administration are preferably sterile and substantially isotonic and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dosage form (i.e., the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries. The formulation depends on the route of administration chosen. For injection, antibodies can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline or acetate buffer (to reduce discomfort at the site of injection). The solution can contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Alternatively antibodies can be in lyophilized form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use. The concentration of antibody in a liquid formulation can be e.g., 1-100 mg/ml, such as 10 mg/ml.

[0113] Treatment with antibodies of the invention can be combined with chemotherapy, radiation, stem cell treatment, surgery other treatments effective against the disorder being treated. Useful classes of other agents that can be administered with humanized antibodies to LIV-1 include, for example, antibodies to other receptors expressed on cancerous cells, antitubulin agents (e.g., auristatins), DNA minor groove binders, DNA replication inhibitors, alkylating agents (e.g., platinum complexes such as *cis*-platin, mono(platinum), bis(platinum) and tri-nuclear platinum complexes and carboplatin), anthracyclines, antibiotics, antifolates, antimetabolites, chemotherapy sensitizers, duocarmycins, etoposides, fluorinated pyrimidines, ionophores, lexitropsins, nitrosoureas, platinols, pre-forming compounds, purine antimetabolites, puromycins, radiation sensitizers, steroids, taxanes, topoisomerase inhibitors, vinca alkaloids, and the like.

[0114] Treatment with the humanized anti-LIV-1 antibody, optionally in combination with any of the other agents or regimes described above alone or as an antibody drug conjugate, can increase the median progression-free survival or overall survival time of patients with tumors (e.g., breast, prostate, melanoma), especially when relapsed or refractory, by at least 30% or 40% but preferably 50%, 60% to 70% or even 100% or longer, compared to the same treatment (e.g., chemotherapy) but without an anti-LIV-1 antibody alone or as a conjugate. In addition or alternatively, treatment (e.g., standard chemotherapy) including the anti-LIV-1 antibody alone or as a conjugate can increase the complete response rate, partial response rate, or objective response rate (complete + partial) of patients with tumors by at least 30% or 40% but preferably 50%, 60% to 70% or even 100% compared to the same treatment (e.g., chemotherapy) but without the anti-LIV-1 antibody.

[0115] Typically, in a clinical trial (e.g., a phase II, phase II/III or phase III trial), the aforementioned increases in median progression-free survival and/or response rate of the patients treated with standard therapy plus the humanized anti-LIV-1 antibody, relative to the control group of patients receiving standard therapy alone (or plus placebo), are statistically significant, for example at the $p = 0.05$ or 0.01 or even 0.001 level. The complete and partial response rates are determined by objective criteria commonly used in clinical trials for cancer, e.g., as listed or accepted by the National Cancer Institute and/or Food and Drug Administration.

VIII. Other Applications

[0116] The anti-LIV-1 humanized antibodies can be used for detecting LIV-1 in the context of clinical diagnosis or treatment or in research. Expression of LIV-1 on a cancer provides an indication that the cancer is amenable to treatment with the antibodies of the present invention. The antibodies can also be sold as research reagents for laboratory research in detecting cells

bearing LIV-1 and their response to various stimuli. In such uses, monoclonal antibodies can be labeled with fluorescent molecules, spin-labeled molecules, enzymes or radioisotopes, and can be provided in the form of kit with all the necessary reagents to perform the assay for LIV-1. The antibodies described herein, BR2-14a, BR2-22a and humanized versions thereof, e.g., hLIV14 and hLIV22, can be used to detect LIV-1 protein expression and determine whether a cancer is amenable to treatment with LIV-1 ADCs. As an example, BR2-14a, BR2-22a and humanized versions thereof, e.g., hLIV14 and hLIV22 can be used to detect LIV-1 expression on breast cancer cells, melanoma cells, cervical cancer cells, or prostate cancer cells. The antibodies can also be used to purify LIV-1, e.g., by affinity chromatography.

IX. Cynomolgus monkey LIV-1

[0117] The disclosure further provides an amino acid sequence for LIV-1 (CY LIV-1) from cynomolgus monkeys at SEQ ID NO:85 with or without a signal peptide, which occupies approximately residues 1-28 of SEQ ID NO:85, as well as nucleic acids that encode that amino acid sequences. Variants differing by up to 1, 2, 3, 4, or 5 substitutions, deletions or insertions are also included provided CY variants do not include a natural human LIV-1 sequence. Analogous to human LIV-1, reference to CY-LIV-1 means at least an extracellular domain of the protein and usually the complete protein other than a cleavable signal peptide (amino acids 1-28). The disclosure further provides antibodies that specifically bind to SEQ ID NO:85 with or without specifically binding to human LIV-1 (i.e., binding to human LIV-1 at level of negative control irrelevant antibody). The disclosure further provides antibodies that preferentially bind CY-LIV-1 over human LIV-1 and vice versa. Preferential binding means an association higher beyond experimental error and preferably at least 2, 3 or 4 fold higher. The disclosure further provides antibodies that show the same binding profile to human and CY LIV-1 within experimental error as any of the exemplified antibodies described below. The disclosure further provides methods of analyzing binding of an antibody to CY LIV-1. Such methods involve contacting an antibody with CY LIV-1, determining whether the antibody specifically binds to CY LIV-1 and optionally determining a measure of binding strength, such as an association constant.

[0118] If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

EXAMPLES**I. Humanization of BR2-14a****Materials**

[0119] Cell lines described in the following examples were maintained in culture according to the conditions specified by the American Type Culture Collection (ATCC), the National Cancer Institute (NCI) or the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany (DMSZ). Cell culture reagents were obtained from Invitrogen Corp. (Carlsbad, CA.) or other suppliers.

Methodologies:**Saturation binding assays**

[0120] 1×10^5 antigen expressing cells (either MCF7 cells (ATCC) expressing human LIV-1, a transfected CHO cell line expressing human LIV-1 or a transfected CHO cell line expressing cyno LIV-1) were aliquoted per well of a 96-well v-bottom plates. AlexaFluor-647 labeled murine LIV-1 mAb, e.g., BR2-14a, was added in concentrations ranging from 0.66 pM to 690 nM and incubated on ice for 30 minutes. Cells were pelleted and washed 3X with PBS/BSA. The cells were then pelleted and resuspended in 125 μ L of PBS/BSA. Fluorescence was analyzed by flow cytometry, using percent of saturated fluorescent signal to determine percent bound and to subsequently calculate apparent K_d .

Competition binding assays

[0121] 1×10^5 CHO cells expressing recombinant human LIV-1 in PBS/BSA were aliquoted into each well of a 96-well v-bottom plates on ice. The cells were incubated for 1 hour with 5 nM AlexaFluor-647 (AF) labeled parental murine LIV-1 mAb and increasing concentrations (from 0.038 nM to 600 nM) of unlabeled humanized LIV-1 mAb, combinations of humanized light chains LA-LF and humanized heavy chains HA-HE. Cells were pelleted and washed 3 times with PBS/BSA. The cells were pelleted and resuspended in 125 μ L of PBS/BSA. Fluorescence was analyzed by flow cytometry, using percent of saturated fluorescent signal to determine percent labeled murine LIV-1 mAb bound and to subsequently extrapolate the EC_{50} by fitting

the data to a sigmoidal dose-response curve with variable slope.

[0122] 1×10^5 MCF7 cells expressing LIV-1 in PBS/BSA were aliquoted in each well of a 96-well v-bottom plates on ice. The cells were incubated for 1 hour with 5 nM AlexaFluor-647 labeled murine LIV-1 mAb and increasing concentrations (from 0.038 nM to 600 nM) of unlabeled humanized LIV-1 mAb, combinations of humanized light chains LA-LF and humanized heavy chains HA-HE. Cells were pelleted and washed 3 times with PBS. The cells were pelleted and resuspended in 125 μ L of PBS/BSA. Fluorescence was analyzed by flow cytometry, using percent of saturated fluorescent signal to determine percent labeled murine LIV-1 mAb bound and to subsequently extrapolate the EC50 by fitting the data to a sigmoidal dose-response curve with variable slope.

[0123] 1×10^5 CHO cells expressing recombinant cyno LIV-1 in PBS were aliquoted in each well of a 96-well v-bottom plates on ice. The cells were incubated for 1 hour with 5 nM AlexaFluor-647 labeled murine LIV-1 mAb and increasing concentrations (from 0.038 nM to 600 nM) of unlabeled humanized LIV-1 mAb, combinations of humanized light chains LA-LF and humanized heavy chains HA-HE. Cells were pelleted and washed 3 times with PBS. The cells were pelleted and resuspended in 125 μ L of PBS/BSA. Fluorescence was analyzed by flow cytometry, using percent of saturated fluorescent signal to determine percent labeled murine LIV-1 mAb bound and to subsequently extrapolate the EC50 by fitting the data to a sigmoidal dose-response curve with variable slope.

Quantitative Flow cytometric analysis

[0124] Quantification of LIV-1 copy number on the cell surfaces was determined using murine LIV-1 mAb as primary antibody and the DAKO QiFiKit flow cytometric indirect assay as described by the manufacturer (DAKO A/S, Glostrup, Denmark) and evaluated with a Becton Dickinson FACS[®]can flow cytometer.

Cytotoxicity assay

[0125] Tumor cells were incubated with LIV-1 antibody drug conjugates for 96-144 hours at 37°C. A non-binding (H00) ADC was used as a negative control. Cell viability was measured by resazurin (Sigma) at the final concentration of 50 μ M. Cells were incubated for four to six hours at 37°. Fluorescent signal was measured on a Fusion HT fluorescent plate reader (Perkin Elmer, Waltham, MA). Results are reported as IC₅₀, the concentration of compound needed to yield a 50% reduction in viability compared to vehicle-treated cells (control = 100%).

Production of antibody drug conjugates

[0126] Antibody drug conjugates of the LIV-1 antibodies were prepared as described in US20050238649. The drug linkers vcMMAE (also referred to as 1006) and mcMMAF (referred to as 1269) are both described in US20050238649. Preparation of cysteine mutants of IgG1 antibodies is generally described in US20100158919. US20050238649 and US20100158919 are herein incorporated by reference for all purposes.

Production of non-fucosylated anti-LIV-1 mAb

[0127] A CHO DG44 cell line producing the humanized IgG1 anti-LIV-1 monoclonal antibody, HBLB mAb (hLIV-14), was cultured at 3.0×10^5 cells per mL in 30 mL of CHO culture media at 37°, 5% CO₂ and shaking at 100 RPM in a 125 mL shake flask. Media was supplemented with insulin like growth factor (IGF), penicillin, streptomycin and 65 µM 2-fluorofucose peracetate (SGD-2084) (see US20090317869). Cultures were fed on day 3 with 2% volume of feed media. On day four, the culture was split 1:4 into fresh culture media. Cultures were fed with a 6% volume of production feed media on days 5, 7, 9 and 10. Conditioned media was collected on day 13 by passing the culture through a 0.2 µm filter. Antibody purification was performed by applying the conditioned media to a protein A column pre-equilibrated with IX phosphate buffered saline (PBS), pH 7.4.

[0128] After washing column with 20 column volumes of IX PBS, antibodies were eluted with 5 column volumes of Immunopure IgG elution buffer (Pierce Biotechnology, Rockford, IL). A 10% volume of 1M Tris pH 8.0 was added to eluted fraction. Sample was dialyzed overnight into 1x PBS.

Antibody-dependent Cellular Cytotoxicity (ADCC)

[0129] ADCC activity was measured using the standard ⁵¹Cr-release assay. Briefly, the MCF-7 target tumor cells were labeled with 100 µCi Na⁵¹CrO₄, washed, and preincubated with test antibodies prior to addition of effector (natural killer, NK) cells. NK (CD16⁺ CD56⁺) cells were prepared from non-adherent peripheral blood mononuclear cells (PBMCs) obtained from normal FcγRIIIA 158V/V donors (Lifeblood, Memphis, TN) with immunomagnetic beads (EasySep, StemCell Technologies, Vancouver, BC, Canada). Viable NK cells were added to target cells at an effector to target cell ratio of 10:1. A human IgG1κ (Ancell, Bayport, MN) was used as negative control in this assay. After 4 hours of incubation, supernatants were collected and dried overnight on Luma plates. Gamma radiation emitted from lysed MCF-7 cells was then detected using the TopCount Microplate Scintillation and Luminescence Counter (Perkin Elmer, Waltham, Massachusetts). ADCC activity is reported as % specific lysis.

***In Vivo* Activity Study**

[0130] Nude (*nu/nu*) mice (7-8 animals/group) were implanted with tumor cells grown in culture: MCF-7 from NCI (5×10^6 cells in 25% matrigel), PC3 from ATCC (2.5×10^6 cells in 25% matrigel), and PC3 from DSMZ (5×10^5 in 25% matrigel). For *in vivo* growth of MCF-7 cells, female mice also received estrogen supplementation by implanting a slow-release estrogen pellet (90 day release). Dosing with either chimeric or humanized LIV-1 ADC or nonbinding control ADC (3 mg/kg) started when tumors reached 100 mm³ (q4d x 4 intraperitoneal injections). Tumor volumes were monitored using calipers and animals were euthanized when tumor volume reached ~800 mm³. Median tumor volume plots were continued for each group until one or more animals were euthanized. All animal procedures were performed under a protocol approved by the Institutional Animal Care and Use Committee in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

LIV-1 immunohistochemical (IHC) staining

Method

[0131] Tumor microarrays (TMAs) and individual tumor samples were obtained from commercial sources. Tissue microarrays from normal or tumor formalin fixed and paraffin embedded (FFPE) tissues were purchased either from US Biomax Inc. or Cybrdi. A frozen array was purchased from BioChain. Single sections were purchased from NDRI, Asterand, Tissue Solution or CHTN. A set of 25 paraffin-embedded samples of metastatic hormone refractory prostate cancer (corresponding bone and soft tissue metastatic sites) was provided by Dr. R. Vessella, University of Washington, Genitourinary Cancer Department. All samples were processed on Bond-Max™ auto-stainer (Leica).

IHC staining of FFPE tissues:

[0132] FFPE slides or TMAs sectioned on glass slides were de-paraffinized using Bond™ Dewax solution (Leica, cat # AR9222) at 72°C and rehydrated. Antigen retrieval was performed using EDTA based Bond™ Epitope Retrieval Solution 2 (Leica, cat # AR9640) for 20 min at 95-100°C before incubation with the primary murine LIV-1 mAb (1-2 µg/ml for 30-45 minutes at 25°C). Isotype-matched murine IgG1 (Sigma; cat # M5284) was used as negative control for background staining. For automated IHC staining we used either a Refine DAB kit or an alkaline phosphatase based detection kit: Bond™ Polymer AP Red Detection kit (Leica, cat # DS9305). Slides were incubated with murine monoclonal primary antibodies against murine LIV-1 mAb for 45 min at 1 µg/ml with a preliminary 30 min protein block (DAKO cat #X0909). After chromogen development, sections were counterstained with hematoxylin and coverslipped. Slides were evaluated and scored by a pathologist and images were taken using

a Zeiss Axiovert 200M microscope (Carl Zeiss, Inc., Thornwood, NY).

IHC of frozen tissues:

[0133] 5 µm sections of frozen/OCT samples were acetone fixed for 10 min., air dried for 30 min, and pretreated 20 min with 1xMorphosave at room temperature. The slides were loaded into Bond-Max™ auto-stainer (Leica) and stained for 45 min with primary antibody with preliminary 30 min protein block (DAKO cat# X0909). Mouse IgG1 (BD Pharmingen cat #550878) was used as negative control. For detection we used DAB-based Bond Polymer Refine kit (Leica, cat # DS9800). After chromogen development, sections were counterstained with hematoxylin and coverslipped. Slides were evaluated and scored by pathologist.

Results

1. Binding of mouse antibody

[0134] The K_D for the murine LIV-1 monoclonal antibody BR2-14a antibody (US2004141983) was determined for human LIV-1 expressed as an endogenous protein in a human breast cancer cell line or as a recombinant protein in a CHO cell line. The K_D for the murine LIV-1 antibody BR2-14a was also determined for cyno LIV-1 expressed as a recombinant protein in a CHO cell line. MCF7 is a human breast cancer cell line. 293F is a human embryonic kidney cell line. Table 1 shows that the antibody had about 5-fold lower dissociation constant for non-recombinant LIV-1 expressed from a human cell line than recombinant LIV-1, whether human (hLIV-1) or from cynomolgus monkeys (cyLIV-1).

Table 1

Cell line	Antigen	Kd (nM)
MCF-7 (ATCC)	hLIV-1	2.4
293F (hLIV-1)	hLIV-1	2.7
CHO (hLIV-1)	hLIV-1	12.5
CHO (cyLIV-1) cLIV-1		14.0

2. Design and testing of humanized antibodies

[0135] The starting point or donor antibody for humanization in this Example is the mouse antibody BR2-14a produced by the hybridoma having ATCC Accession No. PTA-5705A and described in US2004141983. Suitable human acceptor sequences are genomic sequences

provided by VH1-02 and JH5 for the heavy chain and by VK2-30 and Jk4 for the light chain. The human acceptor sequences show 68 and 85 percentage identity to the donor sequences in the variable region frameworks. The light chain CDRs of the human acceptor sequences are of the same canonical type as the CDRs of the donor sequences. In contrast, the heavy chain CDRs of the human acceptor sequences differed in their canonical type (the germline was 1-3 versus 1-2 for the murine donor).

[0136] Alignment of the donor sequences identified eleven positions in the heavy chain (H27, H28, H29, H30, H48, H66, H67, H71, H76, H93 and H94) and five positions in the light chain (L36, L37, L45, L46 and L39) at which the human acceptor sequence differed from the donor sequence and that may affect antibody binding as a result of contacting antigen directly, affecting conformation of CDRs or affecting packing between heavy and light chains. Five humanized heavy chains and six humanized light chains were made incorporating back mutations at different permutations of these positions (Figure 1 (sequence alignment) and Table 2).

Table 2: Backmutations

V_H variant	V_H exon acceptor sequence	donor framework residues
hV _H A	VH1-02	none
hV _H B	VH1-02	H29, H30, H76
hV _H C	VH1-02	H66, H67, H71
hV _H D	VH1-02	H27, H93, H94
hV _H E	VH1-02	H27, H28, H29, H30, H48, H76, H66, H67, H71, H93, H94
V_L variant	V_L exon acceptor sequence	donor framework residues
hV _K A	VK2-30	none
hV _K B	VK2-30	L36
hV _K C	VK2-30	L37
hV _K D	VK2-30	L45
hV _K E	VK2-30	L46
hV _K F	VK2-30	L36, L37, L39, L45, L46

[0137] Humanized antibodies were then expressed representing every permutation of these chains (30 possibilities) of the humanized heavy and light chains. The binding curves for recombinant human LIV-1 expressed from CHO cells are shown in Figure 2. The EC₅₀'s are summarized in the Table 3 below.

Table 3: EC₅₀s for humanized LIV-1 mAb antibodies, derived from BR2-14a, on human LIV-1 expressed in CHO cells

Ab	EC50 (µg/mL)
HALA	DNB
HALB	37.8
HALC	25.5
HALD	4.9
HALE	DNB
HALF	8.8
HBLA	19.9
HBLB	0.3
HBLC	44.0
HBLD	17.4
HBLE	DNB
HBLF	0.7
HCLA	DNB
HCLB	1.8
HCLC	DNB
HCLD	66.6
HCLE	DNB
HCLF	1.3
HDLA	DNB
HDLB	2.3
HDLC	DNB
HDLD	67.9
HDLE	DNB
HDLF	1.4
HELA	12.5
HELB	173.3
HELC	DNB
HELD	24.2
HELE	0.3
HELF	1.5
DNB means "did not bind"	

[0138] These data indicate considerable variation of EC50 between the 30 humanized antibodies tested with HBLB and HELE showing at least two fold better binding than the next humanized antibody HBLF and larger margins over most of the humanized antibodies. The binding curves of Figure 2 show that both HBLB and HELE had stronger binding than the original mouse antibody.

[0139] The HBLB antibody was selected as the best of the humanized antibodies because it has (together with HELE) the strongest binding but has fewer backmutations versus HELE, there being four back mutations in HBLB and twelve in HELE.

[0140] The EC50s for the humanized LIV-1 mAb which bound human LIV-1 expressed on CHO cells were determined for human LIV-1 expressed as a native protein in an MCF7 cell line (Figure 3). Again, LIV-1 mAb HBLB and HELE were determined to be the tightest binders.

[0141] The Kd for HBLB to human LIV-1 on the MCF7 cell line was determined from the average of several saturation binding curves as 1.5 nM whereas that for the mouse antibody is 2.9 nM. In other words, the HBLB antibody has about twice the affinity for native human LIV-1 as the mouse antibody. The saturation binding curve shown in Figure 4 is a representative example.

[0142] Two forms of the HBLB were compared for binding to human LIV-1 recombinantly expressed from CHO cells. One form was expressed with wildtype human IgG1 and kappa constant regions. The other form was the same except for an S239C mutation (EU numbering) in the IgG1 heavy chain (referred to as LIV-14d or HBLB S239C), which reduces binding of the antibody to Fc gamma receptors. The binding curves and EC50's of these antibodies compared with the mouse donor antibody are shown in Figure 5. The EC50's of both forms of HBLB were similar to one another (within the error of the study), and both were stronger than the mouse antibody.

[0143] The EC50s for the humanized LIV-1 mAb HBLB and HBLB S239C were also determined for cyno LIV-1 expressed as a recombinant protein in a CHO cell line. Both antibodies bound with equal affinity (better than murine LIV-1 mAb).

Expression Data for LIV-1

[0144] Murine LIV-1 mAbs (at least 2 for concordance) were used for immunohistochemical analysis of various tumor types using formalin-fixed paraffin embedded tissues.

Table 4: Summary of the expression data for LIV-1 in tumor samples

Origin	Type	LIV-1+	# cases	%
Breast	Primary & metastatic (TMA)			28-46

Origin	Type	LIV-1+	# cases	%
	Primary tumors	12	12	100
	Metastatic tumors	17	19	89
	Post-hormone treatment	19	22	86
	Triple Negative	13	20	65
Prostate	Metastatic hormone refractory: bone mets	15	25	60
	soft tissue mets	21	25	84
Ovarian	Primary (TMA)	9	72	13
	Metastatic (TMA)	4	11	36
	Post-chemo treated	5	17	29
Endometrial		7	56	12
Squamous cell carcinoma (uterine and multiple organs)	Primary tumors	8	114	7
Pancreatic	Primary tumors	9	95	9
Lung	Primary tumors (TMA)	3	192	2

[0145] We observed lower LIV-1 IHC positivity in studies done using tissue microarrays compared to large tissue sections. The difference in expression is highly significant suggesting analysis of LIV-1 expression in larger tissue sections is preferred. There was good concordance of expression using at least 2 different anti-LIV-1 mAbs. Figures 6 and 7 show a high level of LIV-1 expression in post-hormone (tamoxifen or aromatase inhibitors) treated breast and prostate tumors providing a strong rationale to target these tumors using a LIV-1 ADC. Figure 8 shows detectable LIV-1 expression in triple negative (ER-,PgR-, Her2-) breast cancer tissues. The LIV-1 level of expression in triple negative breast cancer by immunohistochemistry staining was comparable to the level in the PC3 animal model, where we demonstrated anti-tumor activity of LIV-1 ADC. Triple negative breast cancers are therefore a potential target population, particularly triple negative breast cancers which have been found to express LIV-1.

***In Vitro* Anti-tumor Activity of hLIV-14 mAb as ADC and Effector Function Enhanced mAb (SEA)**

[0146] Anti-tumor activity of LIV-1 ADCs in vitro was measured using both cytotoxicity assays (Figure 9) and antibody dependent cell cytotoxicity (ADCC) (Figures 10 and 11). First, we performed a survey of LIV-1 expression in various cell lines by quantitative FACS analysis. The breast cancer cell line MCF-7 from ATCC had the highest level of LIV-1 binding sites/cell, as compared to the MCF-7 cell line from other sources (data not shown). We used this cell line for

both assays *in vitro*. Referring to Figure 9, various hLIV-14 ADCs (the HBLB antibody conjugated with vcMMAE (referred to as 1006) or mcMMAF (referred to as 1269) (both small molecules and/or linkers described in US20050238649)) were highly effective in killing MCF-7 cells, as compared with the nonbinding and murine control conjugates (mIgG-1006, mIgG-1269, hIgG-1006 and hIgG-1269). In addition, cysteine mutant LIV-14d ADCs, having an average of two drug linkers per antibody were also highly effective in killing MCF-7 cells as measured by the cytotoxic assay. Referring to Figures 10 and 11, in ADCC assays the activity of the fucosylated/wild-type (WT) mAb and ADCs were compared with the effector-function enhanced versions (non-fucosylated mAbs and ADCs, referred to as SEA). The results demonstrated that effector function enhanced LIV-1 mAbs and ADCs have good ADCC activity against MCF-7 cells, as compared to non-effector function enhanced mAbs or ADCs (compare, for example, Figure 10 hLIV-1 SEA vcMMAE with hLIV-1 vcMMAE). Referring again to Figure 9, an effector function enhanced LIV-1 ADC (indicated as SEA) also had a similar level of cytotoxic activity as wildtype (non-fucosylated) ADCs (compare hLIV-1 SEA 1006 (vcMMAE) with hLIV-1 1006 (vcMMAE)). Thus cytotoxicity can be affected by both effector function and conjugate action.

***In Vivo* Anti-tumor Activity of hLIV-14 ADC**

[0147] Using breast cancer (MCF-7) and prostate cancer (PC-3) models, we determined the anti-tumor activity of LIV-1 ADCs (chimeric and humanized (HBLB) mAbs with an average of 4 drugs per antibody) *in vivo* (Figs. 12-15). LIV-1 ADCs conjugated to vcMMAE showed significant tumor delay compared to untreated and control ADCs. At least one complete regression (CR) was observed in all the studies using LIV-1-vcMMAE at 3 mg/kg with a number of animals having tumors that were static or grew slowly compared to controls. Referring to Figure 12, a chimeric form of the parental murine antibody conjugated to vcMMAE resulted in complete regressions in 3 out of 7 mice. Referring to Figure 13, the same chimeric ADC produced a complete regression in 1 out of 8 mice. Referring to Figure 14, a humanized ADC (HBLB) conjugated to vcMMAE (hLIV-14-vcMMAE(4)) produced a complete regression in 1 out of 8 mice. In addition, a cysteine mutant form of the HBLB antibody, a vcMMAE drug linker conjugated to each heavy chain at position 239, producing a conjugate with an average drug load of 2 drug linkers per antibody; designated hLIV-14d-vcMMAE(2)) exhibited similar activity as the 4-loaded form. Referring to Figure 15, the humanized ADC (HBLB) conjugated to vcMMAE (hLIV-14-vcMMAE(4)) produced a complete regression in 1 out of 8 mice in a prostate carcinoma model. In contrast, the activity of the two loaded cysteine mutants was not as pronounced in this model (compare hLIV-14-vcMMAE(4) with hLIV-14d-vcMMAE(2), and hLIV-14-mcMMAF(4) with hLIV-14d-mcMMAF(2)). In summary, these studies demonstrate that LIV-1 ADC can stop or delay growth of LIV-1 expressing cancers including breast and prostate.

II. Humanization of BR2-22a

[0148] BR2-22a, sometimes also referred to as mAb2, is a mouse monoclonal antibody of isotype IgG1 Kappa.

Methodologies:

[0149] Unless otherwise stated below, methods described for humanization and testing of BR2-14a are also applicable to BR2-22.

Saturation binding assays

[0150] 1×10^5 antigen expressing cells (either MCF7 cells expressing human LIV-1, 293F cells, a transfected CHO cell line expressing human LIV-1 or a transfected CHO cell line expressing cyno LIV-1) were aliquoted per well of a 96-well v-bottom plates. AlexaFluor-647 labeled murine BR2-22a was added in concentrations ranging from 0.66 pM to 690 nM and incubated on ice for 30 minutes. Cells were pelleted and washed 3X with PBS/BSA. The cells were then pelleted and resuspended in 125 μ L of PBS/BSA. Fluorescence was analyzed by flow cytometry, using percent of saturated fluorescent signal to determine percent bound and to subsequently calculate apparent Kd.

Competition binding assays

[0151] 1×10^5 CHO cells expressing recombinant LIV-1 in PBS were aliquoted into each well of a 96-well v-bottom plates on ice. The cells were incubated for 1 hour with 5 nM AlexaFluor-647 (AF) labeled parental BR2-22a and increasing concentrations (from 0.038 nM to 600 nM) of unlabeled humanized BR2-22a antibody in all combinations of humanized light Chains LA-LG and humanized heavy chains HA-HG. Cells were pelleted and washed 3 times with PBS. The cells were then pelleted and resuspended in 125 μ L of PBS/BSA. Fluorescence was analyzed by flow cytometry, using percent of saturated fluorescent signal to determine percent labeled humanized BR2-22a antibody bound and to subsequently extrapolate the EC50 by fitting the data to a sigmoidal dose-response curve with variable slope.

In Vivo Activity Study

[0152] Nude (*nulnu*) mice (7-8 animals/group) were implanted with tumor cells grown in culture: MCF-7 (NCI) at 5×10^6 in 25% matrigel, PC3 from ATCC (2.5×10^6 cells in 25% matrigel), and PC3 from DSMZ (5×10^5 in 25% matrigel). For *in vivo* growth of MCF-7 cells, female mice also received estrogen supplementation by implanting a slow-release estrogen pellet (90 day release). Dosing with either chimeric or humanized LIV-1 ADC or nonbinding

control ADC (3 mg/kg) started when tumors reached 100 mm³ (q4d x 4 intraperitoneal injections). Tumor volumes were monitored using calipers and animals were euthanized when tumor volume reached ~800 mm³. Median tumor volume plots were continued for each group until one or more animals were euthanized. All animal procedures were performed under a protocol approved by the Institutional Animal Care and Use Committee in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Summary of Results and Discussion

Saturation binding

[0153] BR2-22a shows 94% identity to BR2-14a in the mature heavy chain variable region and 91% identity in the mature light chain variable region. The KD for the murine Liv1 of BR2-22a (Table 5) was determined for human LIV-1 expressed as an endogenous protein in a human breast cancer cell line, in 293F cells or as a recombinant protein in a CHO cell line. The KD for BR2-22a was also determined for cyno LIV-1 expressed as a recombinant protein in a CHO cell line.

Table 5: Affinity measurements of BR2-22a for human (hLIV-1) and cyno LIV-1 (cyLIV-1).

Cell line	Antigen	Kd (nM)
MCF7 (ATCC)	hLIV-1	1.1
293F (hLIV-1)	hLIV-1	0.5
Cho hLIV-1	hLIV-1	1.5
Cho cyLIV-1	cLIV-1	4.2

Humanization strategy

[0154] The BR2-22a antibody was humanized using a VH1-02 JH5 germline acceptor sequence for the heavy chain and a VK2-30 JK4acceptor sequence for the light chain. These acceptor sequences were chosen based on their having the highest sequence identity to the mature variable region frameworks of BR2-22A heavy and light chains. Initially five variant heavy chains were constructed. Each included the three Kabat CDRs from the heavy chain of BR2-22a, the chains differing in having from zero (VA) to 11 (VE) backmutations. Initially six variant light chains were constructed. Each included the three Kabat CDRs from the light chain of BR2-22a and from zero (LA) to four backmutations (LF). These backmutations were chosen as a result of modeling the BR2-22A antibody to identify positions with potential to interact with antigen directly, affect CDR conformation or affect the interface between heavy and light chains and based on prior experience in humanizing BR2-14a because of the high sequence identity

between BR2-14a and BR2-22a. In fact, the same eleven positions in the heavy chain and same four positions in the light chain were considered for backmutation in both BR2-14a and BR2-22a (L39 was not considered in BR2-22a because the mouse residue is the same as the human residue). The back mutations present in each variant of humanized BR2-22a are shown in Tables 6 and 7 below.

Table 6

V_H variant	VH exon acceptor sequence	donor framework residues
hV _H A	VH1-02	None
hV _H B	VH1-02	H29, H30, H76
hV _H C	VH1-02	H66, H67, H71
hV _H D	VH1-02	H27, H93, H94
hV _H E	VH1-02	H27, H28, H29, H30, H48, H66, H67, H71, H76, H93, H94
hV _H F	VH1-02	H27, H29, H30, H94
hV _H G	VH1-02	H27, H29, H30, H76, H94

Table 7

V_L variant	VL exon acceptor sequence	donor framework residues
hV _K A	VK2-30	None
hV _K B	VK2-30	L36
hV _K C	VK2-30	L37
hV _K D	VK2-30	L45
hV _K E	VK2-30	L46
hV _K F	VK2-30	L36, L37, L45, L46
hV _K G	VK2-30	L36, L46

[0155] The full sequence of the mature variable region of each variant is shown in Figures 16A and 16B.

[0156] All permutations of these five heavy chains and six light chains were then tested in a competition assay compared with BR2-22a (see Figure 17). Surprisingly, in view of the experience with the BR2-14a antibody in which improved binding relative to the mouse antibody was obtained with only four backmutations and further backmutations did not necessarily improve binding affinity, the only combination of humanized chains that showed binding affinity approximating that of BR2-22a was HELF with 15 backmutations. The other permutations showed poor or no significant binding to LIV-1. The EC₅₀s of the different permutations are shown in Table 8 below.

Table 8: EC50s for humanized BR2-22a antibodies

Ab	EC50 (µg/mL)
HALA	DNB
HALB	DNB
HALC	DNB
HALD	DNB
HALE	DNB
HALF	33.2
HBLA	DNB
HBLB	4.9
HBLC	DNB
HBLD	DNB
HBLE	DNB
HBLF	6.5
HCLA	DNB
HCLB	>100
HCLC	DNB
HCLD	DNB
HCLE	DNB
HCLF	>100
HDLA	DNB
HDLB	DNB
HDLC	DNB
HDLD	DNB
HDLE	DNB
HDLF	14.4
HELA	68.2
HELB	>100
HELC	65.7
HELD	>100
HELE	25.1
HELF	0.3
HELG	0.2
HFLF	0.8
HFLG	0.8
HGLF	0.4

Ab	EC50 (µg/mL)
HGLG	0.5
DNB means did not bind	

[0157] Although HELF shows satisfactory binding, the antibody contains a total of 15 backmutations, a number larger than ideal with respect to potential immunogenicity. Therefore, the HE and LF chains were systematically varied to test the effect of removing individual backmutations. Figure 18 shows the variants tested. LF-1 through LF-4 differ from LF in each lacking a different backmutation present in LF. Similarly, HE-1 through HE-11 lack one of the backmutations present in HE. Figure 19 compares LF-1 through LF-4 (each paired with HE). Figure 19 shows that LF-2 and LF-3 lose substantial binding affinity relative to LF (indicated as HELF historic control in the graph), whereas LF-1 and LF-4 do not. It is concluded that backmutations L36 and L46 contribute substantially to retention of binding affinity, while backmutations at positions L37 and L45 can be dispensed without significant effect on binding. Figure 20 shows similar binding curves for the HE variants. Figure 20 shows that HE-11 lost most of its binding indicating that backmutation at position H94 has the greatest effect on binding affinity of the backmutations tested. Loss of backmutations at positions H27, H29 and H30 also caused significant loss of affinity. The role of H30 can be rationalized by the mouse residue being the result of somatic mutation. Loss of a back mutation at position H76 caused some loss of affinity. The other back mutations at positions H28, H48, H66, H67, H71 and H93 could be dispensed with little or no effect on binding affinity.

[0158] In light of these experiments, heavy chains HF and HG were constructed as was light chain LG. HF included backmutations at H27, H29, H30 and H94 and HG included these mutations and a backmutation at H76. LG contains backmutations at L36 and L46. Several permutations of HF, HG, LE and LF were tested for competition binding as shown in Figure 21 and all showed binding within a factor of three of that of mouse BR2-22a.

[0159] In light of this experiment, HGLG was selected for further experiments as representing the best combination of binding affinity and fewest backmutations. This antibody is hereafter referred to as hLIV22. The saturation binding affinity of hLIV22 for human and cyno LIV-1 expressed from CHO cells is shown in Figure 22 compared with that of hLIV14. Figure 22 shows that hLIV22 has about four fold higher affinity (inverse of dissociation constant) for human LIV-1 than does hLIV14. Furthermore, the affinity of hLIV22 for human LIV-1 is the same within experimental error as its affinity for cynomolgus LIV-1, whereas hLIV14 shows twice the affinity for human LIV-1 as for cynomolgus LIV-1. The affinity of hLIV22 for human LIV-1 is the same within experimental error as that of the parent mouse antibody, BR2-22a.

***In Vitro* Anti-tumor Activity of hLIV22 ADCs**

[0160] Anti-tumor activity of hLIV22 ADC in vitro was measured using cytotoxicity assays. First,

we performed a survey of LIV-1 expression in various cell lines by quantitative FACS analysis. The breast cancer cell line MCF-7 from ATCC had the highest level of LIV-1 binding sites/cell, as compared to the MCF-7 cell line from other sources (data not shown). We used this cell line for in vitro assays. We observed that various hLIV22 ADCs (conjugated with vcMMAE (referred to as 1006) or mcMMAF (referred to as 1269) (both small molecules described in US 2005-0238649)) were highly effective in killing MCF-7 cells as measured by the *in vitro* cytotoxic assay. Figures 23 and 24 compare hLIV22-conjugated to 1006 or 1269 with a nonbinding control antibody conjugated to 1006 or 1269.

***In Vivo* Anti-tumor Activity of LIV-1 ADC**

[0161] Using prostate cancer (PC-3) and breast cancer (MCF-7) models as shown in Figures 25 and 26, we determined the anti-tumor activity of hLIV22 ADCs (with an average of 4 drugs per antibody) *in vivo*. hLIV22 ADCs conjugated to vcMMAE showed significant tumor delay compared to untreated and control ADCs. There were multiple complete regressions was observed in the MCF-7 study using hLIV22-vcMMAE at 3 mg/kg. Additionally, in all studies there were a number of animals that had tumors that were static or grew slowly compared to controls. These studies demonstrate that hLIV22 ADC can stop or delay growth of LIV-1 expressing cancers, including breast and prostate. Figure 27 compares the activity of hLIV22 and hLIV14 ADCs in the MCF-7 model. Although both antibodies were effective, hLIV22 was slightly more effective. hLIV22 ADCs were also tested in a model of cervical cancer. A HeLA cell xenograft model was used for the assay. After tumors grew to an appropriate size, hLIV22 conjugated to vcMMAE was administered to animals at 3 mg/kg and 1 mg/kg. A control antibody conjugate was administered at 3 mg/kg. Complete and partial regression were observed in animals that received 3 mg/kg hLIV22 vc MMAE conjugate. (Data not shown.) Thus, LIV-1 antibodies and antibody drug conjugates can be used to treat LIV-1 expressing cervical cancers.

III. Treatment of skin cancer using anti-LIV-1 antibodies

Expression of LIV-1 Protein on Melanoma Tumor Samples

[0162] Melanoma samples from patients were assessed for LIV-1 expression, using IHC staining. FFPE slides were de-paraffinized using Bond™ Dewax solution (Leica, cat # AR9222) at 72°C. Antigen retrieval was performed using EDTA based Bond™ Epitope Retrieval Solution 2 (Leica, cat # AR9640) for 20 min at 100°C. For IHC staining we used alkaline phosphatase based detection kit: Bond™ Polymer Refine Red Detection kit (Leica, cat # DS9390). Slides were incubated with murine monoclonal primary antibodies against LIV-1 (BR2-14a) for 45 min at 1 µg/ml with preliminary 30 min protein block (DAKO cat #X0909). Mouse IgG (Sigma, cat # M5284) was used as negative control. After chromogen development, sections were

counterstained with hematoxylin and coverslipped. Slides were evaluated and scored by pathologist.

[0163] Results are shown in Figure 28. Seventy-two percent of the tested melanoma patient samples (21/29) were positive for LIV-1 expression. This indicates that LIV-1 inhibitors, e.g., anti-LIV-1 antibodies, can be used to treat melanoma cancers.

***In vivo* anti-melanoma activity of LIV-1 ADC**

[0164] Nude (nu/nu) mice (7-8 animals/group) are implanted with 10×10^6 SK-MEL-5 cells (a melanoma tumor-derived cell line) grown in culture. Tumors are allowed to grow *in vivo* until they are 100 mm^3 , as measured using a caliper. Humanized LIV-1 ADCs, e.g., hLIV14 or hLIV22, are administered at 3 mg/kg. Drug conjugates are, e.g., vcMMAE or mcMMAF. Control ADC's are also administered to control animals at 3 mg/kg. ADC's are given as q4d x 4 intraperitoneal injections. Tumor volumes are monitored using calipers and animals are euthanized when tumor volume reaches $\sim 800 \text{ mm}^3$. Administration of hLIV14 ADC or hLIV22 ADC greatly reduces tumor growth in animals as compared to those animals that received control ADC's.

Sequence listing

[0165]

SEQ ID NO:1 <LIV-1 mAb light chain leader;PRT/1;mus musculus>

MKLPVRLLVLMFWIPVSTS

SEQ ID NO:2<LIV-1 mAb heavy chain leader;PRT/1;mus musculus>

MKCSWWIFFLMAVVLGINS

SEQ ID NO:3<replacement heavy chain leader sequence;PRT/1;mus musculus>

MAWWWTLLFLMAAAQSAQA

SEQ ID NO:4<Light chain constant region;PRT/1;homo sapiens>

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC

SEQ ID NO:5<CH1-CH3;PRT/1;homo sapiens>

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CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGOPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWOOGNVF

SCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:6<heavy chain CH1 - CH3 (no c-term
K);PRT/1;homo sapiens>

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CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF
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SEQ ID NO:7<S239C heavy chain CH1 - CH3;PRT/1;homo
sapiens>

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KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF
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SEQ ID NO:8<S239C heavy chain CH1 - CH3 (no c-term
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SEQ ID NO:9<hLIV-1 mAb HA;PRT/1;artificial>

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YGTWFAYWGQGTTLVTVSS

SEQ ID NO:10<hLIV-1 mAb HB;PRT/1;artificial>

QVQLVQSGAEVKKPGASVKVSCKASGYTIEDYYMHWVRQAPGQGLEWMGWI
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SEQ ID NO:11<hLIV-1 mAb HC;PRT/1;artificial>

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YGTWFAYWGQGTTLVTVSS

SEQ ID NO:12<hLIV-1 mAb HD;PRT/1;artificial>

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SEQ ID NO:13<hLIV-1 mAb HE;PRT/1;artificial>
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 DPENGDTEYAPTFQGKATMTADTSINTAYMELSRRLRSDDTAVYYCNVHDAH
 YGTWFAYWGQGTLLVTVSS

SEQ ID NO:14<hLIV-1 mAb LA;PRT/1;artificial>
 DVVMTQSPLSLPVTLGQPASISCRSSQSIIRNDGNTYLEWFQQRPGQSPRR
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 FGGGTKVEIKR

SEQ ID NO:15<hLIV-1 mAb LB;PRT/1;artificial>
 DVVMTQSPLSLPVTLGQPASISCRSSQSIIRNDGNTYLEWYQQRPGQSPRR
 LIYRVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHPYPT
 FGGGTKVEIKR

SEQ ID NO:16<hLIV-1 mAb LC;PRT/1;artificial>
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 LIYRVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHPYPT
 FGGGTKVEIKR

SEQ ID NO:17<hLIV-1 mAb LD;PRT/1;artificial>
 DVVMTQSPLSLPVTLGQPASISCRSSQSIIRNDGNTYLEWFQQRPGQSPKR
 LIYRVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHPYPT
 FGGGTKVEIKR

SEQ ID NO:18<hLIV-1 mAb LE;PRT/1;artificial>
 DVVMTQSPLSLPVTLGQPASISCRSSQSIIRNDGNTYLEWFQQRPGQSPRL
 LIYRVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHPYPT
 FGGGTKVEIKR

SEQ ID NO:19<hLIV-1 mAb LF;PRT/1;artificial>
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 LIYRVSNRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHPYPT
 FGGGTKVEIKR

DNA sequences:

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 aattca

SEQ ID NO:21<LIV-1 mAb light chain leader;DNA;mus
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SEQ ID NO:22<replacement heavy chain leader
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 caagca

SEQ ID NO:23<light chain constant region;DNA;mus musculus>

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SEQ ID NO:27<S239C CH1-CH3 (w/o c-term
K);DNA;artificial>

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SEQ ID NO:28<hLIV-1 mAb HA;DNA;artificial>

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SEQ ID NO:29<hLIV-1 mAb HB;DNA;artificial>

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SEQ ID NO:30<hLIV-1 mAb HC;DNA;artificial>

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SEQ ID NO:31<hLIV-1 mAb HD;DNA;artificial>

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SEQ ID NO:32<hLIV-1 mAb HE;DNA;artificial>

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SEQ ID NO:33<hLIV-1 mAb LA;DNA;artificial>

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SEQ ID NO:34<hLIV-1 mAb LB;DNA;artificial>

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SEQ ID NO:35<hLIV-1 mAb LC;DNA;artificial>

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SEQ ID NO:36<hLIV-1 mAb LD;DNA;artificial>

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SEQ ID NO:37<hLIV-1 mAb LE;DNA;artificial>

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SEQ ID NO:38<hLIV-1 mAb LF;DNA;artificial>

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SEQ ID NO:39<Liv1 mAb2 light chain leader;PRT/1;mus musculus>

MKLPVRLLVLMFWIPVATSS

SEQ ID NO:40<Liv1 mAb2 heavy chain leader;PRT/1;mus musculus>

MKCSWWIFFLMAVVIGINS

SEQ ID NO:41<replacement heavy chain leader sequence;PRT/1;mus musculus>

MAWWWTLFLMAAAQSAQA

SEQ ID NO:42<Light chain constantregion;PRT/1;homo sapiens>

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS
QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN
RGEC

SEQ ID NO:43<CH1-CH3;PRT/1;homo sapiens>

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK*

SEQ ID NO:44<heavy chain CH1 - CH3 (no c-term K);PRT/1;homo sapiens>

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:45<S239C heavy chain CH1 - CH3;PRT/1;homo sapiens>

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS
CDKTHTCPPCPAPELLGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
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KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:46<S239C heavy chain CH1 - CH3 (no c-term K);PRT/1;homo sapiens>

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS

CDKTHTCPPCPAPELLGGPCVFLFPPKPKDTLMISRTPEVTCVVVDVSHED
PEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
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SCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO:47<hLiv1 mAb2 HA;PRT/1;artificial>

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYMHWVRQAPGQGLEWMGWI
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YGTWFAYWGQGTLLVTVSS

SEQ ID NO:48<hLiv1 mAb2 HB;PRT/1;artificial>

QVQLVQSGAEVKKPGASVKVSCKASGYTIEDYMHWVRQAPGQGLEWMGWI
DPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRRLRSDDTAVYYCARHNAH
YGTWFAYWGQGTLLVTVSS

SEQ ID NO:49<hLiv1 mAb2 HC;PRT/1;artificial>

QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYMHWVRQAPGQGLEWMGWI
DPENGDTEYGPKFQGKATMTADTSISTAYMELSRRLRSDDTAVYYCARHNAH
YGTWFAYWGQGTLLVTVSS

SEQ ID NO:50<hLiv1 mAb2 HD;PRT/1;artificial>

QVQLVQSGAEVKKPGASVKVSCKASGLTFTDYMHWVRQAPGQGLEWMGWI
DPENGDTEYGPKFQGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCTVHNAH
YGTWFAYWGQGTLLVTVSS

SEQ ID NO:51<hLiv1 mAb2 HE;PRT/1;artificial>

QVQLVQSGAEVKKPGASVKVSCKASGLNIEDYMHWVRQAPGQGLEWIGWI
DPENGDTEYGPKFQGKATMTADTSINTAYMELSRRLRSDDTAVYYCTVHNAH
YGTWFAYWGQGTLLVTVSS

SEQ ID NO:52<hLiv1 mAb2 HF;PRT/1;artificial>

QVQLVQSGAEVKKPGASVKVSCKASGLTIEDYMHWVRQAPGQGLEWMGWI
DPENGDTEYGPKFQGRVTMTRDTSISTAYMELSRRLRSDDTAVYYCAVHNAH
YGTWFAYWGQGTLLVTVSS

SEQ ID NO:53<hLiv1 mAb2 HG;PRT/1;artificial>

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DPENGDTEYGPKFQGRVTMTRDTSINTAYMELSRRLRSDDTAVYYCAVHNAH
YGTWFAYWGQGTLLVTVSS

SEQ ID NO:54<hLiv1 mAb2 LA;PRT/1;artificial>

DVVMTQSPLSLPVTLGQPASISCRSSQSLHSSGNTYLEWFQQRPGQSPRR
LIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYT
FGGGTKVEIKR

SEQ ID NO:55<hLiv1 mAb2 LB;PRT/1;artificial>

DVVMTQSPLSLPVTLGQPASISCRSSQSLHSSGNTYLEWYQQRPGQSPRR
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FGGGTKVEIKR

SEQ ID NO:56<hLiv1 mAb2 LC;PRT/1;artificial>

DVVMTQSPLSLPVTLGQPASISCRSSQSLHSSGNTYLEWFLQRPQSPRR
LIYKISTRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYT
FGGGTKVETKR

SEQ ID NO:57<hLiv1 mAb2 LD;PRT/1;artificial>
 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWFQQRPGQSPKR
 LIYKISTRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYT
 FGGGTKVEIKR

SEQ ID NO:58<hLiv1 mAb2 LE;PRT/1;artificial>
 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWFQQRPGQSPRP
 LIYKISTRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYT
 FGGGTKVEIKR

SEQ ID NO:59<hLiv1 mAb2 LF;PRT/1;artificial>
 DVVMTQSPLSLPVTLGQPASISCRSSQSLLHSSGNTYLEWYLQRPQGSPKP
 LIYKISTRFSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCFQGSHVPYT
 FGGGTKVEIKR

SEQ ID NO:60<hLiv1 mAb2 LG;PRT/1;artificial>
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DNA sequences:

SEQ ID NO:61<Liv1 mAb2 heavy chain leader;DNA;mus
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SEQ ID NO:62<Liv1 mAb2 light chain leader;DNA;mus
 musculus>
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SEQ ID NO:63<replacement heavy chain leader
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SEQ ID NO:64<light chain constant region;DNA;homo
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SEQ ID NO:65<CH1-CH3;DNA;homo sapiens>
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SEQ ID NO:66<CH1-CH3 (w/o c-term K);DNA;homo
sapiens>

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SEQ ID NO:67<S239C CH1-CH3;DNA;artificial>

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SEQ ID NO:68<S239C CH1-CH3 (w/o c-term
K);DNA;artificial>

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SEQ ID NO:69<hLiv1 mAb2 HA;DNA;artificial>

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SEQ ID NO:70<hLiv1 mAb2 HB;DNA;artificial>

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FLETIETPRP GKLFPKDVSS STPPSVTSKS RVSRLAGRKT
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LVALAVGTLS GDAFLHLLPH SHASHHSHS HEEPAMEMKR
GPLFSHLSSQ NIEESAYFDS TWKGLTALGG LYFMFLVEHV
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YHHILHHHHH QNHHPHSHSQ RYSREELKDA GVATLAWMVI
MGDGLHNFSD GLAIGAAFTE GLSSGLSTSV AVFCHELPHE
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EHHS DHNHAA SGKNKRKALC PDHSDSSGK DPRNSQKGGA
HRPEHASGRR NVKDSVSASE VTSTVYNTVS EGTHFLETIE
TPRPGKLF PK DVSSSTPPSV TSKSRVSRLA GRKTNESVSE
PRKGFMYSRN TNENPQECFN ASKLLTSHGM GIQVPLNATE
FNYLCPAIIN QIDARSCLIH TSEKKAEIPP KTYSLQIAWV
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GTLSGDAFLH LLPHSHASHH HSHSHEEPAM EMKRGPLFSH
LSSQNIEESA YFDSTWKGLT ALGGLYFMFL VEHVLTLIKQ
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<151> 2010-12-06

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35 40 45

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
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Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
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Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
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Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
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Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
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Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
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Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
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Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
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Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
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Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
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Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
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Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
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Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
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Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
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<213> homo sapiens

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Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser

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Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
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Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
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Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
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Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130              135              140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145              150              155              160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165              170              175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180              185              190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195              200              205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
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Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
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Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275              280              285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
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Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
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Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser
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Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser
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Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr
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Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys
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Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys
			100					105					110		

Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Cys	Val	Phe	Leu	Phe	Pro	Pro
		115					120					125			

Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys
	130					135					140				

Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp
145					150					155					160

Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu
			165						170					175	

Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu
			180					185					190		

His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn
		195					200					205			

Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly
	210					215					220				

Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu
225					230					235					240

Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr
			245						250					255	

Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn
			260					265					270		

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
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Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
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Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
 100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Cys Val Phe Leu Phe Pro Pro
 115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
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Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
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Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
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His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
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Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
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Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 305 310 315 320

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

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Thr 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
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Ala Arg His Asp Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
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Gln Phe Lys Val Phe Val Ser Ser

Gly Thr Leu Val Thr Val Ser Ser
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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 Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Thr Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Asn 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 His Asp Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 11

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> humanized heavy chain

<400> 11

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 Asp Tyr
20 25 30

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

Glv Trp Ile Asp Pro Glu Asn Glv Asp Thr Glu Tyr Ala Pro Thr Phe

50 55 60
 Gln Gly Lys Ala Thr Met Thr Ala 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 His Asp Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 12

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> humanized heavy chain

<400> 12

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 Phe Thr Phe Thr Asp 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 Asp Pro Glu Asn Gly Asp Thr Glu Tyr Ala Pro Thr 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
 Asn Val His Asp Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 13

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> humanized heavy chain

<400> 13

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	Phe	Asn	Ile	Glu	Asp	Tyr	20	25	30	
Tyr	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	35	40	45	
Gly	Trp	Ile	Asp	Pro	Glu	Asn	Gly	Asp	Thr	Glu	Tyr	Ala	Pro	Thr	Phe	50	55	60	
Gln	Gly	Lys	Ala	Thr	Met	Thr	Ala	Asp	Thr	Ser	Ile	Asn	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	
Asn	Val	His	Asp	Ala	His	Tyr	Gly	Thr	Trp	Phe	Ala	Tyr	Trp	Gly	Gln	100	105	110	
Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	115	120										

<210> 14

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> humanized light chain

<400> 14

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

100 - - 105 - - 110 -

Arg

<210> 15

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> humanized light chain

<400> 15

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Ile Arg Asn
20 25 30

Asp Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Arg Pro Gly Gln Ser
35 40 45

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

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 16

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> humanized light chain

<400> 16

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Ile Arg Asn
20 25 30

Asp Gly Asn Thr Tyr Leu Glu Trp Phe Leu Gln Arg Pro Gly Gln Ser
35 40 45

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

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
 85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

Arg

<210> 17

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> humanized light chain

<400> 17

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Ile Arg Asn
 20 25 30

Asp Gly Asn Thr Tyr Leu Glu Trp Phe Gln Gln Arg Pro Gly Gln Ser
 35 40 45

Pro Lys Arg Leu Ile Tyr Arg Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
 85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
 100 105 110

Arg

<210> 18

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> humanized light chain

<400> 18

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Leu	Gly
1				5					10					15	

Gln	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Ile	Ile	Arg	Asn
			20					25					30		

Asp	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Phe	Gln	Gln	Arg	Pro	Gly	Gln	Ser
		35					40					45			

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

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

Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Phe	Gln	Gly
				85					90					95	

Ser	His	Val	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
			100					105					110		

Arg

<210> 19

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> humanized light chain

<400> 19

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Leu	Gly
1				5					10					15	

Gln	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Ile	Ile	Arg	Asn
			20					25					30		

Asp	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser
		35					40					45			

Pro	Lys	Leu	Leu	Ile	Tyr	Arg	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				

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

Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Phe	Gln	Gly
				85					90					95	

Ser	His	Val	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
			...					...					...		

100	105	110
Arg		
<210> 20		
<211> 57		
<212> DNA		
<213> mus musculus		
<400> 20		
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<210> 21		
<211> 57		
<212> DNA		
<213> mus musculus		
<400> 21		
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<212> DNA		
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<211> 318		
<212> DNA		
<213> mus musculus		
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aaggtggata acgccctcca atcgggtaac tcccaggaga gtgtcacaga gcaggacagc		180
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cacaaagtct acgcctgcga agtcacccat cagggcctga gctcgcccgt cacaaagagc		300
ttcaacaggg gagagtgt		318
<210> 24		
<211> 990		
<212> DNA		
<213> homo sapiens		
<400> 24		
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ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg caccagacc 240
tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc 300
aaatcttgtg acaaaactca cacatgcccc ccgtgccag cacctgaact cctgggggga 360
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ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 780
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ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa gagcaggtgg 900
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacaca 960

cagaagagcc tctccctgtc tccgggtaaa 990

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<210> 25

<211> 987

<212> DNA

<213> homo sapiens

<400> 25

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gctagcacca agggcccatc tgtcttcccc ctggcacctt cctccaagag cacctctggg 60
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tggaaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180
ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg caccagacc 240
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tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 540
agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 600
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aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggatgag 720
ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 780
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 840
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa gagcaggtgg 900

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cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacaca 960
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<210> 26

<211> 990

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 26

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 tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180
 ggactctact ccctcagcag cgtgggtgacc gtgccctcca gcagcttggg caccagacc 240
 tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc 300
 aaatcttgtg acaaaactca cacatgcccc cctgcccag cacctgaact cctgggggga 360
 ccgtgtgtct tctcttccc cccaaaaccc aaggacacc tcctgatctc ccggaccct 420
 gaggtcacat gcgtgggtgt ggacgtgagc cagcaagacc ctgaggtcaa gttcaactgg 480
 tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac 540
 agcacgtacc gtgtgggtcag cgtcctcacc gtctcgcacc aggactggct gaatggcaag 600
 gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 660
 aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggatgag 720
 ctgaccaaga accaggtcag cctgacctgc ctgggtcaaag gcttctatcc cagcgacatc 780
 gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 840
 ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa gagcaggtgg 900
 cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacaca 960
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<210> 27

<211> 987

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 27

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 tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180
 ggactctact ccctcagcag cgtgggtgacc gtgccctcca gcagcttggg caccagacc 240

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ccgtgtgtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 420
gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 480
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ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 780
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cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacaca 960
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<210> 28

<211> 360

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 28

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cctggacaag ggcttgagtg gatgggatgg attgatcctg agaatgggtga tactgaatat 180
gccccacct tccagggcag ggtcaccatg accagggaca cctccatcag cacagcctac 240
atggagctga gcaggctgag atctgatgac acagctgtgt attactgtgc cagacatgat 300
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<210> 29

<211> 360

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 29

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tcctgcaagg cttctggata caccattgaa gactactata tgcactgggt gaggcaggcc 120
cctggacaag ggcttgagtg gatgggatgg attgatcctg agaatgggtga tactgaatat 180

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gccccacacct tccagggcag ggtcaccatg accagggaca cctccatcaa cacagcctac 240
atggagctga gcaggctgag atctgatgac acagctgtgt attactgtgc cagacatgat 300
gctcactatg ggacctggtt tgcttactgg ggccaaggaa ccctgggtcac agtctcctca 360

<210> 30
<211> 360
<212> DNA
<213> Artificial Sequence

<220>
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<400> 30
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cctggacaag ggcttgagtg gatgggatgg attgatcctg agaatgggtga tactgaatat 180
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<210> 31
<211> 360
<212> DNA
<213> Artificial Sequence

<220>
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<400> 31
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<210> 32
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<400> 32
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cctggacaag ggcttgagtg gattggatgg attgatcctg agaatgggtga tactgaatat	180
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atggagctga gcaggctgag atctgatgac acagctgtgt attactgtaa tgtccatgat	300
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<210> 33

<211> 339

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 33

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tttcagcaga ggccaggcca atctccaagg aggctaattt atagagtttc caacaggttt	180
tctggggctcc cagacagatt ctctggcagt gggtcaggca ctgatttcac actgaaaatc	240
agcaggggtgg aggctgagga tgttgggggtt tattactgct ttcaagggtc acatgttccc	300
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<210> 34

<211> 339

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 34

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taccagcaga ggccaggcca atctccaagg aggctaattt atagagtttc caacaggttt	180
tctggggctcc cagacagatt ctctggcagt gggtcaggca ctgatttcac actgaaaatc	240
agcaggggtgg aggctgagga tgttgggggtt tattactgct ttcaagggtc acatgttccc	300
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<210> 35

<211> 339

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 35

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tttctgcaga ggccaggcca atctccaagg aggctaattt atagagtttc caacaggttt	180
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agcaggggtgg aggctgagga tgttgggggtt tattactgct ttcaagggtc acatgttccc	300
tacacctttg gaggagggac caaggtggag atcaaacgt	339

<210> 36

<211> 339

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 36

gatgttgtga tgactcagtc tccactctcc ctgcctgtca cccttggaca gcctgcctcc	60
atctcctgca gatctagtca gagcattata aggaatgatg gaaacaccta tttggaatgg	120
tttcagcaga ggccaggcca atctccaaag aggctaattt atagagtttc caacaggttt	180
tctgggggtcc cagacagatt ctctggcagt gggtcaggca ctgatttcac actgaaaatc	240
agcaggggtgg aggctgagga tgttgggggtt tattactgct ttcaagggtc acatgttccc	300
tacacctttg gaggagggac caaggtggag atcaaacgt	339

<210> 37

<211> 339

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 37

gatgttgtga tgactcagtc tccactctcc ctgcctgtca cccttggaca gcctgcctcc	60
atctcctgca gatctagtca gagcattata aggaatgatg gaaacaccta tttggaatgg	120
tttcagcaga ggccaggcca atctccaagg ctccctaattt atagagtttc caacaggttt	180
tctgggggtcc cagacagatt ctctggcagt gggtcaggca ctgatttcac actgaaaatc	240
agcaggggtgg aggctgagga tgttgggggtt tattactgct ttcaagggtc acatgttccc	300
tacacctttg gaggagggac caaggtggag atcaaacgt	339

<210> 38

<211> 339

<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 38
gatgttgtga tgactcagtc tccactctcc ctgcctgtca cccttggaca gcctgcctcc 60
atctcctgca gatctagtca gagcattata aggaatgatg gaaacaccta tttggaatgg 120
tacctgcaga aaccaggcca atctccaaag ctcttaattt atagagtttc caacaggttt 180
tctgggggtcc cagacagatt ctctggcagt gggtcaggca ctgatttcac actgaaaatc 240
agcaggggtgg aggctgagga tgttgggggtt tattactgct ttcaaggttc acatgttccc 300
tacacctttg gaggagggac caaggtggag atcaaacgt 339

<210> 39
<211> 20
<212> PRT
<213> mus musculus

<400> 39
Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Val
1 5 10 15

Ala Thr Ser Ser
20

<210> 40
<211> 19
<212> PRT
<213> mus musculus

<400> 40
Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Ile Gly
1 5 10 15

Ile Asn Ser

<210> 41
<211> 19
<212> PRT
<213> mus musculus

<400> 41
Met Ala Trp Val Trp Thr Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ala Gln Ala

<210> 42
<211> 106

<212> PRT

<213> homo sapiens

<400> 42

Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln
1				5					10					15	

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

Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser
		35					40					45			

Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr
	50					55					60				

Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys
65					70					75					80

His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro
				85					90					95	

Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys
			100					105	

<210> 43

<211> 330

<212> PRT

<213> homo sapiens

<400> 43

Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys
1				5					10					15	

Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr
			20					25					30		

Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser
		35					40					45			

Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser
	50					55					60				

Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr
65					70				75						80

Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys
				85					90					95	

Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys
			100					105					110		

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

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 130 135 140
 Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
 145 150 155 160
 Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 165 170 175
 Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
 180 185 190
 His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 195 200 205
 Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
 210 215 220
 Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
 225 230 235 240
 Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 245 250 255
 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 260 265 270
 Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 275 280 285
 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 290 295 300
 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 305 310 315 320
 Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 325 330

<210> 44

<211> 329

<212> PRT

<213> homo sapiens

<400> 44

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

50		55		60													
Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr		
65					70					75					80		
Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys		
			85						90					95			
Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys		
			100					105					110				
Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro		
		115					120					125					
Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys		
	130					135					140						
Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp		
145					150					155					160		
Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu		
			165						170					175			
Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu		
			180					185					190				
His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn		
		195					200					205					
Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly		
	210					215					220						
Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu		
225					230					235					240		
Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr		
			245						250					255			
Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn		
		260						265					270				
Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe		
		275					280					285					
Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn		
	290					295					300						
Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr		
305					310					315					320		
Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly									
			325														

<210> 45

<211> 330

<212> PRT

<213> homo sapiens

<400> 45

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Cys Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225 230 235 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
325 330

<210> 46

<211> 329

<212> PRT

<213> homo sapiens

<400> 46

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
35 40 45

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
85 90 95

Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Cys Val Phe Leu Phe Pro Pro
115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
195 200 205

Lvs Ala Leu Pro Ala Pro Ile Glu Lvs Thr Ile Ser Lvs Ala Lvs Glv

```

      210              215              220
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
225              230              235              240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245              250              255

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260              265              270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
275              280              285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290              295              300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305              310              315              320

Gln Lys Ser Leu Ser Leu Ser Pro Gly
325

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<210> 47

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 47

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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 Asp 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 Asp Pro Glu Asn Gly Asp Thr Glu Tyr Gly Pro 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 His Asn Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
100             105             110

Gly Thr Leu Val Thr Val Ser Ser
115              120

```

115 120

<210> 48
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic sequence

<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 Ile Glu Asp 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 Asp Pro Glu Asn Gly Asp Thr Glu Tyr Gly Pro Lys Phe
 50 55 60
 Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Asn 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 His Asn Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
 100 105 110
 Gly Thr Leu Val Thr Val Ser Ser
 115 120

<210> 49
 <211> 120
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> synthetic sequence

<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 Thr Asp 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 Asp Pro Glu Asn Gly Asp Thr Glu Tyr Gly Pro Lys Phe
 50 55 60

Gln Gly Lys Ala Thr Met Thr Ala 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 His Asn Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 50

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 50

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 Leu Thr Phe Thr Asp 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 Asp Pro Glu Asn Gly Asp Thr Glu Tyr Gly Pro 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

Thr Val His Asn Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 51

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 51

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 Leu Asn Ile Glu Asp Tyr
20 25 30

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

Gly Trp Ile Asp Pro Glu Asn Gly Asp Thr Glu Tyr Gly Pro Lys Phe
50 55 60

Gln Gly Lys Ala Thr Met Thr Ala Asp Thr Ser Ile Asn 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

Thr Val His Asn Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 52

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 52

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 Leu Thr Ile Glu Asp 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 Asp Pro Glu Asn Gly Asp Thr Glu Tyr Gly Pro 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 Val His Asn Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 53

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 53

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 Leu Thr Ile Glu Asp 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 Asp Pro Glu Asn Gly Asp Thr Glu Tyr Gly Pro Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Asn 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 Val His Asn Ala His Tyr Gly Thr Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 54

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 54

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Ser Gly Asn Thr Tyr Leu Glu Trp Phe Gln Gln Arg Pro Gly Gln Ser
35 40 45

Pro Arg Arg Leu Ile Tyr Lys Ile Ser Thr Arg Phe Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 55

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 55

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Arg Pro Gly Gln Ser
35 40 45

Pro Arg Arg Leu Ile Tyr Lys Ile Ser Thr Arg Phe Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 56

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 56

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Leu	Gly
1				5				10						15	

Gln	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser
			20					25					30		

Ser	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Phe	Leu	Gln	Arg	Pro	Gly	Gln	Ser
		35					40					45			

Pro	Arg	Arg	Leu	Ile	Tyr	Lys	Ile	Ser	Thr	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				

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

Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Phe	Gln	Gly
				85					90					95	

Ser	His	Val	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
			100					105					110		

Arg

<210> 57

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 57

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Leu	Gly
1				5				10						15	

Gln	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser
			20					25					30		

Ser	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Phe	Gln	Gln	Arg	Pro	Gly	Gln	Ser
		35					40					45			

Pro	Lys	Arg	Leu	Ile	Tyr	Lys	Ile	Ser	Thr	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				

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

Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Phe	Gln	Gly
				85					90					95	

Ser	His	Val	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
			100					105					110		

Arg

<210> 58

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 58

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Leu	Gly
1				5					10					15	

Gln	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser
			20					25					30		

Ser	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Phe	Gln	Gln	Arg	Pro	Gly	Gln	Ser
		35					40					45			

Pro	Arg	Pro	Leu	Ile	Tyr	Lys	Ile	Ser	Thr	Arg	Phe	Ser	Gly	Val	Pro
	50					55					60				

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

Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Phe	Gln	Gly
				85					90					95	

Ser	His	Val	Pro	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys
			100					105					110		

Arg

<210> 59

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 59

Asp	Val	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Leu	Pro	Val	Thr	Leu	Gly
1				5					10					15	

Gln	Pro	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Leu	His	Ser
			20					25					30		

Ser	Gly	Asn	Thr	Tyr	Leu	Glu	Trp	Tyr	Leu	Gln	Arg	Pro	Gly	Gln	Ser
		35					40					45			

Pro Lys Pro Leu Ile Tyr Lys Ile Ser Thr Arg Phe Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 60

<211> 113

<212> PRT

<213> Artificial Sequence

<220>

<223> snythetic sequence

<400> 60

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Leu Gly
1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Ser Gly Asn Thr Tyr Leu Glu Trp Tyr Gln Gln Arg Pro Gly Gln Ser
35 40 45

Pro Arg Pro Leu Ile Tyr Lys Ile Ser Thr Arg Phe Ser Gly Val Pro
50 55 60

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

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Phe Gln Gly
85 90 95

Ser His Val Pro Tyr Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
100 105 110

Arg

<210> 61

<211> 57

<212> DNA

<213> mus musculus

<400> 61

atgaaatgca gctgggtcat cttcttcctg atggcagtgg ttataggaat caattca 57

<210> 62
 <211> 57
 <212> DNA
 <213> mus musculus

<400> 62
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<210> 63
 <211> 57
 <212> DNA
 <213> mus musculus

<400> 63
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<210> 64
 <211> 324
 <212> DNA
 <213> homo sapiens

<400> 64
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 ggaactgcct ctgttgtgtg cctgctgaat aacttctatc ccagagaggc caaagtacag 120
 tggaagggtgg ataacgccct ccaatcgggt aactcccagg agagtgtcac agagcaggac 180
 agcaaggaca gcacctacag cctcagcagc accctgacgc tgagcaaagc agactacgag 240
 aaacacaaag tctacgcctg cgaagtcacc catcagggcc tgagctcgcc cgtcacaaag 300
 agcttcaaca ggggagagtg ttag 324

<210> 65
 <211> 990
 <212> DNA
 <213> homo sapiens

<400> 65
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 ggcacagctg ccctgggctg cctggtcaag gactacttcc ctgaacctgt gacagtgtcc 120
 tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca 180
 ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg caccagacc 240
 tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc 300
 aaatcttgtg acaaaactca cacatgcccc ccgtgcccag cacctgaact cctgggggga 360
 ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 420
 gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 480

tacgtggacg gcggtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac	540
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag	600
gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc	660
aaagccaaag ggcagccccc agaaccacag gtgtacaccc tgcccccatc ccgggatgag	720
ctgaccaaga accaggtcag cctgacctgc ctgggtcaaag gcttctatcc cagcgacatc	780
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg	840
ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg	900
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacaca	960
cagaagagcc tctccctgtc tccgggtaaa	990

<210> 66

<211> 987

<212> DNA

<213> homo sapiens

<400> 66

gctagcacca agggcccatc tgtcttcccc ctggcaccct cctccaagag cacctctggg	60
ggcacagctg ccctgggctg cctgggtcaag gactacttcc ctgaacctgt gacagtgtcc	120
tggaaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca	180
ggactctact ccctcagcag cgtgggtgacc gtgccctcca gcagcttggg caccagacc	240
tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc	300
aaatcttgtg acaaaactca cacatgccc aacgtgcccag cacctgaact cctgggggga	360
ccgtcagttc tctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccct	420
gaggtcacat gcggtgggtg ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg	480
tacgtggacg gcggtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac	540
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag	600
gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc	660
aaagccaaag ggcagccccc agaaccacag gtgtacaccc tgcccccatc ccgggatgag	720
ctgaccaaga accaggtcag cctgacctgc ctgggtcaaag gcttctatcc cagcgacatc	780
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg	840
ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg	900
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacaca	960
cagaagagcc tctccctgtc tccgggt	987

<210> 67

<211> 990

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 67

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ggcacagctg ccctgggctg cctgggtcaag gactacttcc ctgaacctgt gacagtgtcc	120
tggaaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca	180
ggactctact ccctcagcag cgtgggtgacc gtgccctcca gcagcttggg caccagacc	240
tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc	300
aaatcttgtg acaaaactca cacatgccc cctgcccag cacctgaact cctgggggga	360
ccgtgtgtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccct	420
gaggtcacat gcgtgggtgg ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg	480
tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac	540
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag	600
gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc	660
aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgccccatc ccgggatgag	720
ctgaccaaga accaggtcag cctgacctgc ctgggtcaaag gcttctatcc cagcgacatc	780
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg	840
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa gagcaggtgg	900
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacaca	960
cagaagagcc tctccctgtc tccgggtaaa	990

<210> 68

<211> 987

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 68

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ggcacagctg ccctgggctg cctgggtcaag gactacttcc ctgaacctgt gacagtgtcc	120
tggaaactcag gcgccctgac cagcggcgtg cacaccttcc cggctgtcct acagtcctca	180
ggactctact ccctcagcag cgtgggtgacc gtgccctcca gcagcttggg caccagacc	240
tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagaa agttgagccc	300
aaatcttgtg acaaaactca cacatgccc cctgcccag cacctgaact cctgggggga	360
ccgtgtgtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccct	420
gaggtcacat gcgtgggtgg ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg	480
tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacaac	540
agcacgtacc gtgtgggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag	600
gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc	660

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aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggatgag      720
ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc      780
gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg      840
ctggactccg acggctcctt ctctctctac agcaagctca ccgtggacaa gagcaggtgg      900
cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacaca      960
cagaagagcc tctccctgtc tccgggt                                     987

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<210> 69

<211> 360

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 69

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caggtgcagc tgggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg cttctggata caccctcaca gactactata tgcactgggt gaggcaggcc      120
cctggacaag ggcttgagtg gatgggatgg attgatcctg aaaatgggtga tactgaatat      180
ggcccgaagt tccagggcag ggtcaccatg accagggaca cctccatcag cacagcctac      240
atggagctga gcaggctgag atctgatgac acagctgtgt attactgtgc cagacataat      300
gctcactacg ggacctgggt tgcttactgg ggccaaggaa ccctggtcac agtctcctca      360

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<210> 70

<211> 360

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 70

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caggtgcagc tgggtgcagtc tggggctgag gtgaagaagc ctggggcctc agtgaaggtc      60
tcctgcaagg cttctggata caccattgaa gactactata tgcactgggt gaggcaggcc      120
cctggacaag ggcttgagtg gatgggatgg attgatcctg aaaatgggtga tactgaatat      180
ggcccgaagt tccagggcag ggtcaccatg accagggaca cctccatcaa cacagcctac      240
atggagctga gcaggctgag atctgatgac acagctgtgt attactgtgc cagacataat      300
gctcactacg ggacctgggt tgcttactgg ggccaaggaa ccctggtcac agtctcctca      360

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<210> 71

<211> 360

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 71

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tcctgcaagg	cttctggata	caccttcaca	gactactata	tgcactgggt	gaggcaggcc	120
cctggacaag	ggcttgagtg	gatgggatgg	attgacccctg	aaaatgggtga	tactgaatat	180
ggcccgaagt	tccagggcaa	ggccaccatg	accgcagaca	cctccatcag	cacagcctac	240
atggagctga	gcaggctgag	atctgatgac	acagctgtgt	attactgtgc	cagacataat	300
gctcactacg	ggacctgggt	tgcttactgg	ggccaaggaa	ccctgggtcac	agtctcctca	360

<210> 72

<211> 360

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 72

caggtgcagc	tgggtgcagtc	tggggctgag	gtgaagaagc	ctggggcctc	agtgaaggtc	60
tcctgcaagg	cttctggact	caccttcaca	gactactata	tgcactgggt	gaggcaggcc	120
cctggacaag	ggcttgagtg	gatgggatgg	attgacccctg	aaaatgggtga	tactgaatat	180
ggcccgaagt	tccagggcag	ggtcaccatg	accagggaca	cctccatcag	cacagcctac	240
atggagctga	gcaggctgag	atctgatgac	acagctgtgt	attactgtac	tgtccataat	300
gctcactacg	ggacctgggt	tgcttactgg	ggccaaggaa	ccctgggtcac	agtctcctca	360

<210> 73

<211> 360

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 73

caggtgcagc	tgggtgcagtc	tggggctgag	gtgaagaagc	ctggggcctc	agtgaaggtc	60
tcctgcaagg	cttctggact	caacattgaa	gactactata	tgcactgggt	gaggcaggcc	120
cctggacaag	ggcttgagtg	gattggatgg	attgacccctg	aaaatgggtga	tactgaatat	180
ggcccgaagt	tccagggcaa	ggccaccatg	accgcagaca	cctccatcaa	cacagcctac	240
atggagctga	gcaggctgag	atctgatgac	acagctgtgt	attactgtac	tgtccataat	300
gctcactacg	ggacctgggt	tgcttactgg	ggccaaggaa	ccctgggtcac	agtctcctca	360

<210> 74

<211> 360

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 74

caggtgcagc	tgggtgcagtc	tggggctgag	gtgaagaagc	ctggggcctc	agtgaaggtc	60
tcctgcaagg	cttctggact	caccattgaa	gactactata	tgcactgggt	gaggcaggcc	120
cctggacaag	ggcttgagtg	gatgggatgg	attgatcctg	aaaatgggtga	tactgaatat	180
ggcccgaagt	tccagggcag	ggtcaccatg	accagggaca	cctccatcag	cacagcctac	240
atggagctga	gcaggctgag	atctgatgac	acagctgtgt	attactgtgc	cgtccataat	300
gctcactacg	ggacctgggt	tgcttactgg	ggccaaggaa	ccctggtcac	agtctcctca	360

<210> 75

<211> 360

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 75

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tcctgcaagg	cttctggact	caccattgaa	gactactata	tgcactgggt	gaggcaggcc	120
cctggacaag	ggcttgagtg	gatgggatgg	attgatcctg	aaaatgggtga	tactgaatat	180
ggcccgaagt	tccagggcag	ggtcaccatg	accagggaca	cctccatcaa	cacagcctac	240
atggagctga	gcaggctgag	atctgatgac	acagctgtgt	attactgtgc	cgtccataat	300
gctcactacg	ggacctgggt	tgcttactgg	ggccaaggaa	ccctggtcac	agtctcctca	360

<210> 76

<211> 370

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 76

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gcctgtcacc	cttggacagc	ctgcctccat	ctcctgcaga	tctagtcaga	gccttttaca	120
cagtagtgga	aacacctatt	tagaatgggt	tcagcagagg	ccaggccaat	ctccaaggag	180
gctaatttat	aaaatttcca	cccgattttc	tgggggccca	gacagattct	ctggcagtgg	240
atcaaacact	aatttcacac	taaaaatcaa	caaaatgaaa	actaaagata	ttaaaattta	300

ttactgcttt caaggttcac atgttcccta cacctttgga ggagggacca aggtggagat 360
caaacgtacg 370

<210> 77
<211> 370
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 77
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gcctgtcacc cttggacagc ctgcctccat ctctgcaga tctagtcaga gccttttaca 120
cagtagtgga aacacctatt tagaatggta ccagcagagg ccaggccaat ctccaaggag 180
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gtcaggcact gatttcacac tgaaaatcag cagggtggag gctgaggatg ttggggttta 300
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caaacgtacg 370

<210> 78
<211> 370
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 78
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gcctgtcacc cttggacagc ctgcctccat ctctgcaga tctagtcaga gccttttaca 120
cagtagtgga aacacctatt tagaatggtt tctgcagagg ccaggccaat ctccaaggag 180
gctaatttat aaaatttcca cccgattttc tgggggtcca gacagattct ctggcagtgg 240
gtcaggcact gatttcacac tgaaaatcag cagggtggag gctgaggatg ttggggttta 300
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caaacgtacg 370

<210> 79
<211> 370
<212> DNA
<213> Artificial Sequence

<220>
<223> synthetic sequence

<400> 79

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gcctgtcacc cttggacagc ctgcctccat ctctgcaga tctagtcaga gccttttaca	120
cagtagtgga aacacctatt tagaatgggt tcagcagagg ccaggccaat ctccaaagag	180
gctaatttat aaaatttcca cccgattttc tgggggtcca gacagattct ctggcagtgg	240
gtcaggcact gatttcacac tgaaaatcag caggggtggag gctgaggatg ttgggggttta	300
ttactgcttt caagggtcac atgttcccta cacctttgga ggagggacca aggtggagat	360
caaacgtacg	370

<210> 80

<211> 370

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 80

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cagtagtgga aacacctatt tagaatgggt tcagcagagg ccaggccaat ctccaaggcc	180
cctaatttat aaaatttcca cccgattttc tgggggtcca gacagattct ctggcagtgg	240
gtcaggcact gatttcacac tgaaaatcag caggggtggag gctgaggatg ttgggggttta	300
ttactgcttt caagggtcac atgttcccta cacctttgga ggagggacca aggtggagat	360
caaacgtacg	370

<210> 81

<211> 339

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 81

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tacctgcaga ggccaggcca atctccaaag cccctaattt ataaaatttc cacccgattt	180
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agcaggggtgg aggctgagga tgttgggggt tattactgct ttcaagggtc acatgttccc	300
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<210> 82

<211> 339

<212> DNA

<213> Artificial Sequence

<220>

<223> synthetic sequence

<400> 82

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taccagcaga ggccaggcca atctccaagg cccctaattt ataaaatttc cacccgattt      180
tctgggggtcc cagacagatt ctctggcagt gggtcaggca ctgatttcac actgaaaatc      240
agcaggggtgg aggctgagga tgttgggggtt tattactgct ttcaaggttc acatgttccc      300
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<210> 83

<211> 755

<212> PRT

<213> Homo sapiens

<400> 83

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Met Ala Arg Lys Leu Ser Val Ile Leu Ile Leu Thr Phe Ala Leu Ser
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Val Thr Asn Pro Leu His Glu Leu Lys Ala Ala Ala Phe Pro Gln Thr
          20          25          30

Thr Glu Lys Ile Ser Pro Asn Trp Glu Ser Gly Ile Asn Val Asp Leu
          35          40          45

Ala Ile Ser Thr Arg Gln Tyr His Leu Gln Gln Leu Phe Tyr Arg Tyr
          50          55          60

Gly Glu Asn Asn Ser Leu Ser Val Glu Gly Phe Arg Lys Leu Leu Gln
65          70          75          80

Asn Ile Gly Ile Asp Lys Ile Lys Arg Ile His Ile His His Asp His
          85          90          95

Asp His His Ser Asp His Glu His His Ser Asp His Glu Arg His Ser
          100          105          110

Asp His Glu His His Ser Glu His Glu His His Ser Asp His Asp His
          115          120          125

His Ser His His Asn His Ala Ala Ser Gly Lys Asn Lys Arg Lys Ala
          130          135          140

Leu Cys Pro Asp His Asp Ser Asp Ser Ser Gly Lys Asp Pro Arg Asn
145          150          155          160

Ser Gln Gly Lys Gly Ala His Arg Pro Glu His Ala Ser Gly Arg Arg

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				165						170						175
Asn	Val	Lys	Asp	Ser	Val	Ser	Ala	Ser	Glu	Val	Thr	Ser	Thr	Val	Tyr	
			180					185					190			
Asn	Thr	Val	Ser	Glu	Gly	Thr	His	Phe	Leu	Glu	Thr	Ile	Glu	Thr	Pro	
		195					200					205				
Arg	Pro	Gly	Lys	Leu	Phe	Pro	Lys	Asp	Val	Ser	Ser	Ser	Thr	Pro	Pro	
	210					215					220					
Ser	Val	Thr	Ser	Lys	Ser	Arg	Val	Ser	Arg	Leu	Ala	Gly	Arg	Lys	Thr	
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Asn	Glu	Ser	Val	Ser	Glu	Pro	Arg	Lys	Gly	Phe	Met	Tyr	Ser	Arg	Asn	
				245					250					255		
Thr	Asn	Glu	Asn	Pro	Gln	Glu	Cys	Phe	Asn	Ala	Ser	Lys	Leu	Leu	Thr	
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Ser	His	Gly	Met	Gly	Ile	Gln	Val	Pro	Leu	Asn	Ala	Thr	Glu	Phe	Asn	
		275						280					285			
Tyr	Leu	Cys	Pro	Ala	Ile	Ile	Asn	Gln	Ile	Asp	Ala	Arg	Ser	Cys	Leu	
	290					295					300					
Ile	His	Thr	Ser	Glu	Lys	Lys	Ala	Glu	Ile	Pro	Pro	Lys	Thr	Tyr	Ser	
305					310					315					320	
Leu	Gln	Ile	Ala	Trp	Val	Gly	Gly	Phe	Ile	Ala	Ile	Ser	Ile	Ile	Ser	
				325					330					335		
Phe	Leu	Ser	Leu	Leu	Gly	Val	Ile	Leu	Val	Pro	Leu	Met	Asn	Arg	Val	
			340					345					350			
Phe	Phe	Lys	Phe	Leu	Leu	Ser	Phe	Leu	Val	Ala	Leu	Ala	Val	Gly	Thr	
		355					360					365				
Leu	Ser	Gly	Asp	Ala	Phe	Leu	His	Leu	Leu	Pro	His	Ser	His	Ala	Ser	
	370					375					380					
His	His	His	Ser	His	Ser	His	Glu	Glu	Pro	Ala	Met	Glu	Met	Lys	Arg	
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Gly	Pro	Leu	Phe	Ser	His	Leu	Ser	Ser	Gln	Asn	Ile	Glu	Glu	Ser	Ala	
				405					410					415		
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			420				425						430			
Phe	Met	Phe	Leu	Val	Glu	His	Val	Leu	Thr	Leu	Ile	Lys	Gln	Phe	Lys	
		435					440					445				
Asp	Lys	Lys	Lys	Lys	Asn	Gln	Lys	Lys	Pro	Glu	Asn	Asp	Asp	Asp	Val	
	450					455					460					

Glu Ile Lys Lys Gln Leu Ser Lys Tyr Glu Ser Gln Leu Ser Thr Asn
465 470 475 480

Glu Glu Lys Val Asp Thr Asp Asp Arg Thr Glu Gly Tyr Leu Arg Ala
485 490 495

Asp Ser Gln Glu Pro Ser His Phe Asp Ser Gln Gln Pro Ala Val Leu
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Glu Glu Glu Glu Val Met Ile Ala His Ala His Pro Gln Glu Val Tyr
515 520 525

Asn Glu Tyr Val Pro Arg Gly Cys Lys Asn Lys Cys His Ser His Phe
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His Asp Thr Leu Gly Gln Ser Asp Asp Leu Ile His His His His Asp
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Tyr His His Ile Leu His His His His His Gln Asn His His Pro His
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Ser His Ser Gln Arg Tyr Ser Arg Glu Glu Leu Lys Asp Ala Gly Val
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Ala Thr Leu Ala Trp Met Val Ile Met Gly Asp Gly Leu His Asn Phe
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Ser Asp Gly Leu Ala Ile Gly Ala Ala Phe Thr Glu Gly Leu Ser Ser
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Gly Leu Ser Thr Ser Val Ala Val Phe Cys His Glu Leu Pro His Glu
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Leu Gly Asp Phe Ala Val Leu Leu Lys Ala Gly Met Thr Val Lys Gln
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Ala Val Leu Tyr Asn Ala Leu Ser Ala Met Leu Ala Tyr Leu Gly Met
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Ala Thr Gly Ile Phe Ile Gly His Tyr Ala Glu Asn Val Ser Met Trp
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Ile Phe Ala Leu Thr Ala Gly Leu Phe Met Tyr Val Ala Leu Val Asp
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Met Val Pro Glu Met Leu His Asn Asp Ala Ser Asp His Gly Cys Ser
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Arg Trp Gly Tyr Phe Phe Leu Gln Asn Ala Gly Met Leu Leu Gly Phe
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Ile Asn Phe
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Gly Glu Asn Asn Ser Leu Ser Val Glu Gly Phe Arg Lys Leu Leu Gln
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Thr His Phe Leu Glu Thr Ile Glu Thr Pro Arg Pro Gly Lys Leu Phe
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Pro Lys Asp Val Ser Ser Ser Thr Pro Pro Ser Val Thr Ser Lys Ser
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Arg Val Ser Arg Leu Ala Gly Arg Lys Thr Asn Glu Ser Val Ser Glu
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Glu Cys Phe Asn Ala Ser Lys Leu Leu Thr Ser His Gly Met Gly Ile
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 Ile Asn Gln Ile Asp Ala Arg Ser Cys Leu Ile His Thr Ser Glu Lys
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 Lys Ala Glu Ile Pro Pro Lys Thr Tyr Ser Leu Gln Ile Ala Trp Val
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 Gly Gly Phe Ile Ala Ile Ser Ile Ile Ser Phe Leu Ser Leu Leu Gly
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 Ser Phe Leu Val Ala Leu Ala Val Gly Thr Leu Ser Gly Asp Ala Phe
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 Lys Gly Leu Thr Ala Leu Gly Gly Leu Tyr Phe Met Phe Leu Val Glu
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Ala Val Phe Cys His Glu Leu Pro His Glu Leu Gly Asp Phe Ala Val
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Leu Ser Ala Met Leu Ala Tyr Leu Gly Met Ala Thr Gly Ile Phe Ile
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Gly His Tyr Ala Glu Asn Val Ser Met Trp Ile Phe Ala Leu Thr Ala
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Gly Leu Phe Met Tyr Val Ala Leu Val Asp Met Val Pro Glu Met Leu
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His Asn Asp Ala Ser Asp His Gly Cys Ser Arg Trp Gly Tyr Phe Phe
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Leu Ala Ile Thr Thr Arg Gln Tyr His Leu Gln Gln Leu Phe Tyr Arg
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Tyr Gly Glu Asn Asn Ser Leu Ser Val Glu Gly Phe Arg Lys Leu Leu
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Gln Asn Ile Gly Ile Asp Lys Ile Lys Arg Ile His Ile His His Asp
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His Asp His His Ser Asp His Glu His His Ser Asp His Glu His His
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Ser Asp His Glu His His Ser His Arg Asn His Ala Ala Ser Gly Lys
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130 135 140

Lys Asp Pro Arg Asn Ser Gln Gly Lys Gly Ala His Arg Pro Glu His
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Pro Pro Ser Val Thr Glu Lys Ser Leu Val Ser Arg Leu Ala Gly Arg

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Cys Leu Ile His Thr Ser Glu Lys Lys Ala Glu Ile Pro Pro Lys Thr
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 Cys Ser Arg Trp Gly Tyr Phe Phe Leu Gln Asn Ala Gly Met Leu Leu
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 Gly Phe Gly Ile Met Leu Leu Ile Ser Ile Phe Glu His Lys Ile Val
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 Phe Arg Ile Asn Phe
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PATENTKRAV

1. Humaniseret antistof mod LIV-1 omfattende et modent variabelt område af tungkæden, der omfatter de tre Kabat CDR'er ifølge SEQ ID NO: 53 og har en
5 aminosyresekvens, der er mindst 95 % identisk med SEQ ID NO: 53, forudsat positionen H27 er besat af L, position H29 er besat af I, position H30 er besat af E og position H94 er besat af V, og et modent variabelt område af letkæden, der omfatter de tre Kabat CDR'er ifølge SEQ ID NO: 60 og har en aminosyresekvens, der er mindst 95 % identisk med SEQ ID NO: 60, forudsat position L36 er besat
10 af Y og position L46 er besat af P.

2. Humaniseret antistof ifølge krav 1, der omfatter et modent variabelt område af tungkæden med en aminosyresekvens, der er mindst 98 % identisk med SEQ ID NO: 53, og et modent variabelt område af letkæden, der er mindst 98 % identisk med SEQ ID NO: 60; eller
15 der omfatter et modent variabelt område af tungkæden med en aminosyresekvens, der er mindst 99 % identisk med SEQ ID NO: 53, og et modent variabelt område af letkæden, der er mindst 99 % identisk med SEQ ID NO: 60.

3. Humaniseret antistof ifølge krav 1 eller krav 2, hvor det modne variable område af tungkæden er kondenseret til et konstant område af tungkæden og det modne variable område af letkæden er kondenseret til et konstant område af letkæden, eventuelt hvor det konstante område af tungkæden er en mutant form af et naturligt humant konstant område, der har reduceret binding til en Fc-gamma-receptor i forhold til det naturlige humane konstante område.
20

4. Humaniseret antistof ifølge krav 3, hvor det konstante område af tungkæden er af IgG1-isotype.
25

5. Humaniseret antistof ifølge et hvilket som helst af kravene 1-4, hvor antistoffet er konjugeret til et cytotoxisk eller cytostatisk middel.

6. Humaniseret antistof ifølge krav 5, hvor det humaniserede antistof er konjugeret til det cytotoxiske eller cytostatiske middel via en linker, eventuelt hvor linkeren er en fraspaltelig peptid-linker, hvor linkeren er fraspaltelig under intracellulære forhold.

5 **7.** Humaniseret antistof ifølge krav 6, hvor linkeren er en fraspaltelig peptid- linker med formelen: $-A_a-W_w-Y_y-$,

hvor:

-A- er en forlængelsesenhed;

a er 0 eller 1;

10 hver -W- uafhængigt er en aminosyreenhed;

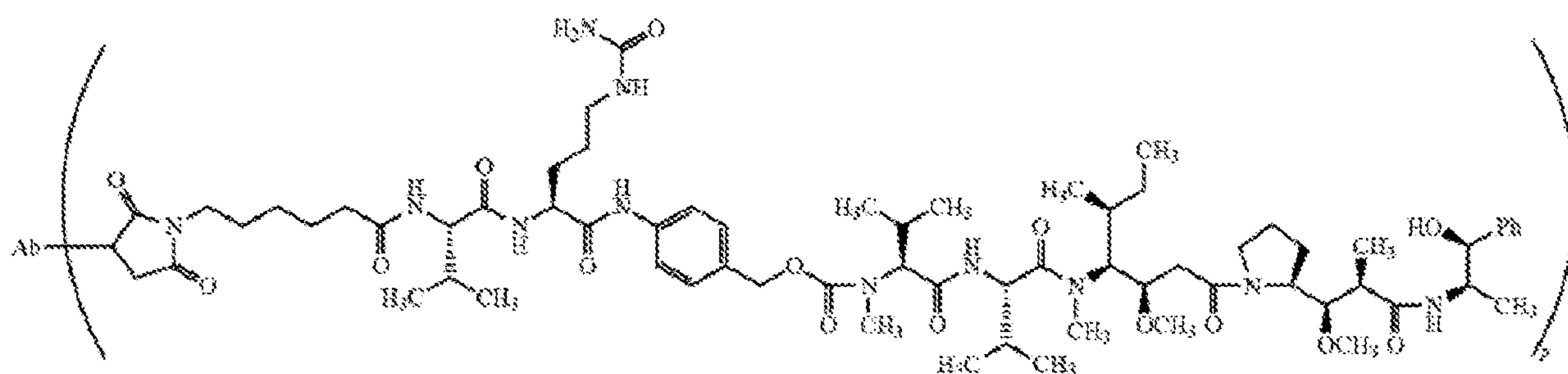
w uafhængigt er et heltal i intervallet fra 0 til 12;

-Y- er en afstandsenhed; og

y er 0, 1 eller 2.

15 **8.** Humaniseret antistof ifølge et hvilket som helst af kravene 5-7, hvor det cytotoxiske eller cytostatiske middel er et antitubulinmiddel, eller hvor det cytotoxiske eller cytostatiske middel er et auristatin, eventuelt hvor auristatinet er monomethyl-auristatin F eller monomethyl-auristatin E.

20 **9.** Humaniseret antistof ifølge krav 8, hvor linkeren er bundet til monomethyl-auristatin E, hvorved der dannes et antistoflægemiddelkonjugat med strukturen:



hvor p angiver det gennemsnitlige antal lægemiddel-linker-molekyler pr. antistof i en population af antistoflægemiddelkonjugatet, hvor p ligger i intervallet fra 1 til 8 og Ab betegner det humaniserede antistof, hvor p eventuelt er 4.

10. Nukleinsyre eller nukleinsyrer, der koder for et modent variabelt område af tungkæden og et modent variabelt område af letkæden som defineret i et hvilket som helst af kravene 1-4.

11. Vektor, der omfatter nukleinsyren eller nukleinsyrerne ifølge krav 10, eventuelt hvor vektoren er en ekspressionsvektor.

12. Eukaryotisk værtscelle, der omfatter nukleinsyren ifølge krav 10 eller vektoren ifølge krav 11, eventuelt hvor værtscellen er en kinesisk hamsterovarie-(CHO) celle.

13. Fremgangsmåde til fremstilling af et anti-LIV-1-antistof, der omfatter dyrkning af værtscellen ifølge krav 12 under forhold, der er egnet til produktion af anti-LIV-1-antistoffet, eventuelt hvor fremgangsmåden endvidere omfatter isolering af anti-LIV-1-antistoffet produceret af værtscellen.

14. Fremgangsmåde til fremstilling af et anti-LIV-1-antistof-lægemiddelkonjugat omfattende dyrkning af værtscellen ifølge krav 12 under forhold, der er egnet til produktion af et anti-LIV-1-antistof; isolering af anti-LIV-1-antistoffet produceret fra værtscellen og konjugering af anti-LIV-1-antistoffet til et cytotoxisk eller cytostatisk middel.

15. Humaniseret antistof ifølge et hvilket som helst af kravene 1-9 til anvendelse i behandling af en patient, der har eller har risiko for at få cancer, hvor anvendelsen omfatter administration af det humaniserede antistof til patienten, eventuelt hvor canceren er en brystcancer, en prostatacancer, en cervixcancer eller et melanom, eventuelt hvor brystcanceren er en triple negativ brystcancer.

16. Farmaceutisk sammensætning, der omfatter det humaniserede antistof ifølge et hvilket som helst af kravene 1-9.

DRAWINGS

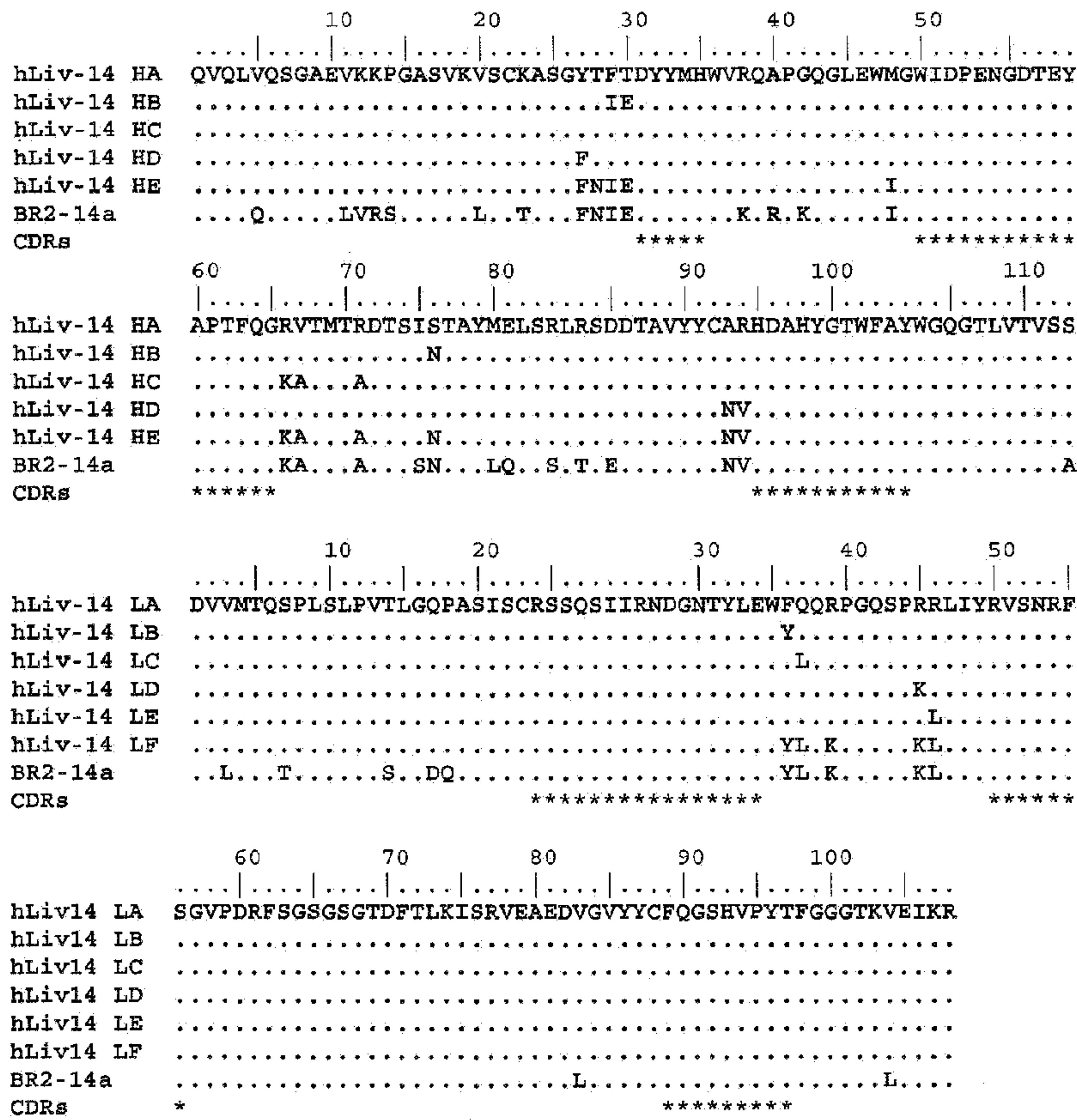
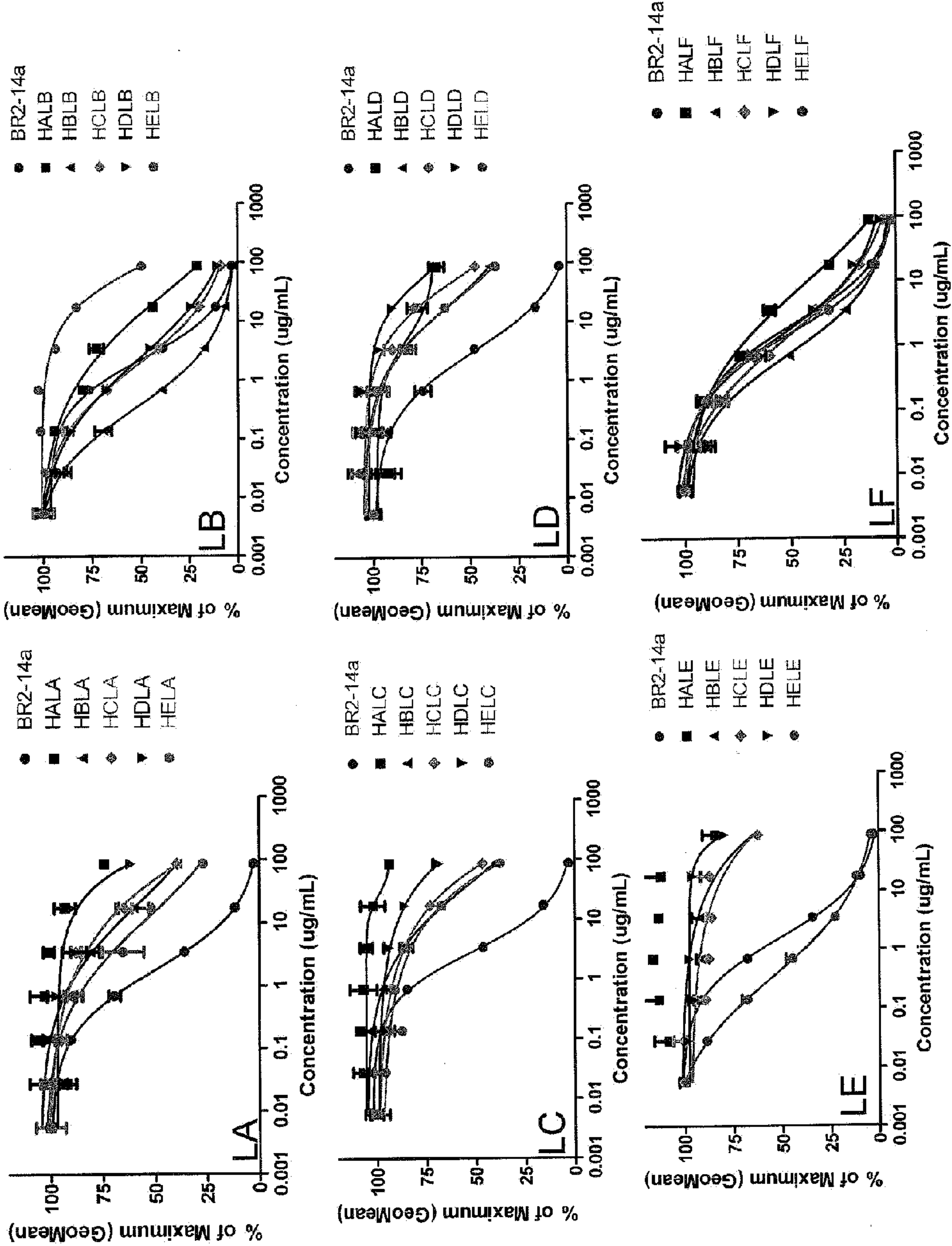


FIGURE 1

Antigen binding by humanized
BR2-14a antibodies

FIGURE 2



Competition binding on MCF-7 ATCC cells

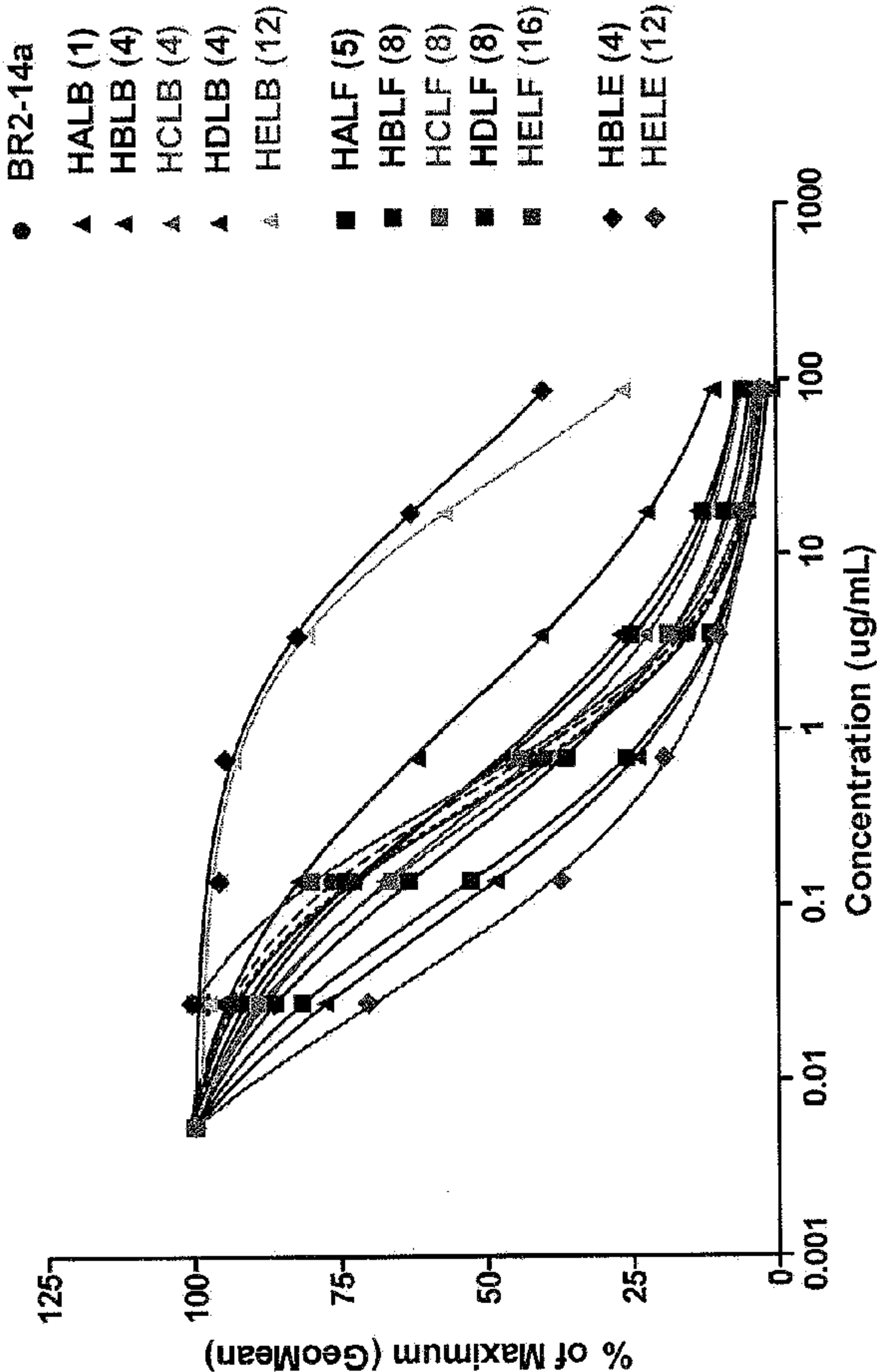


FIGURE 3

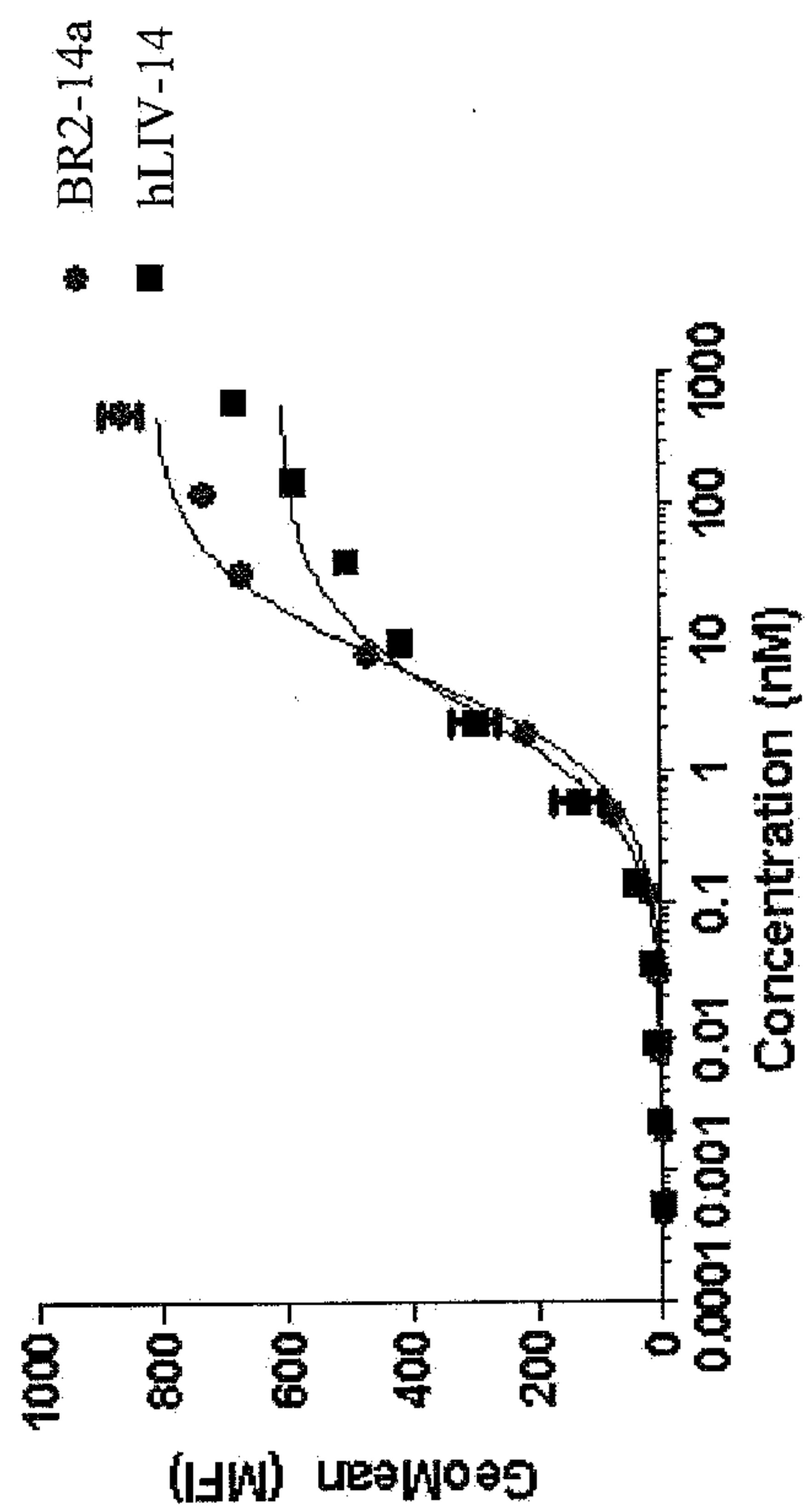
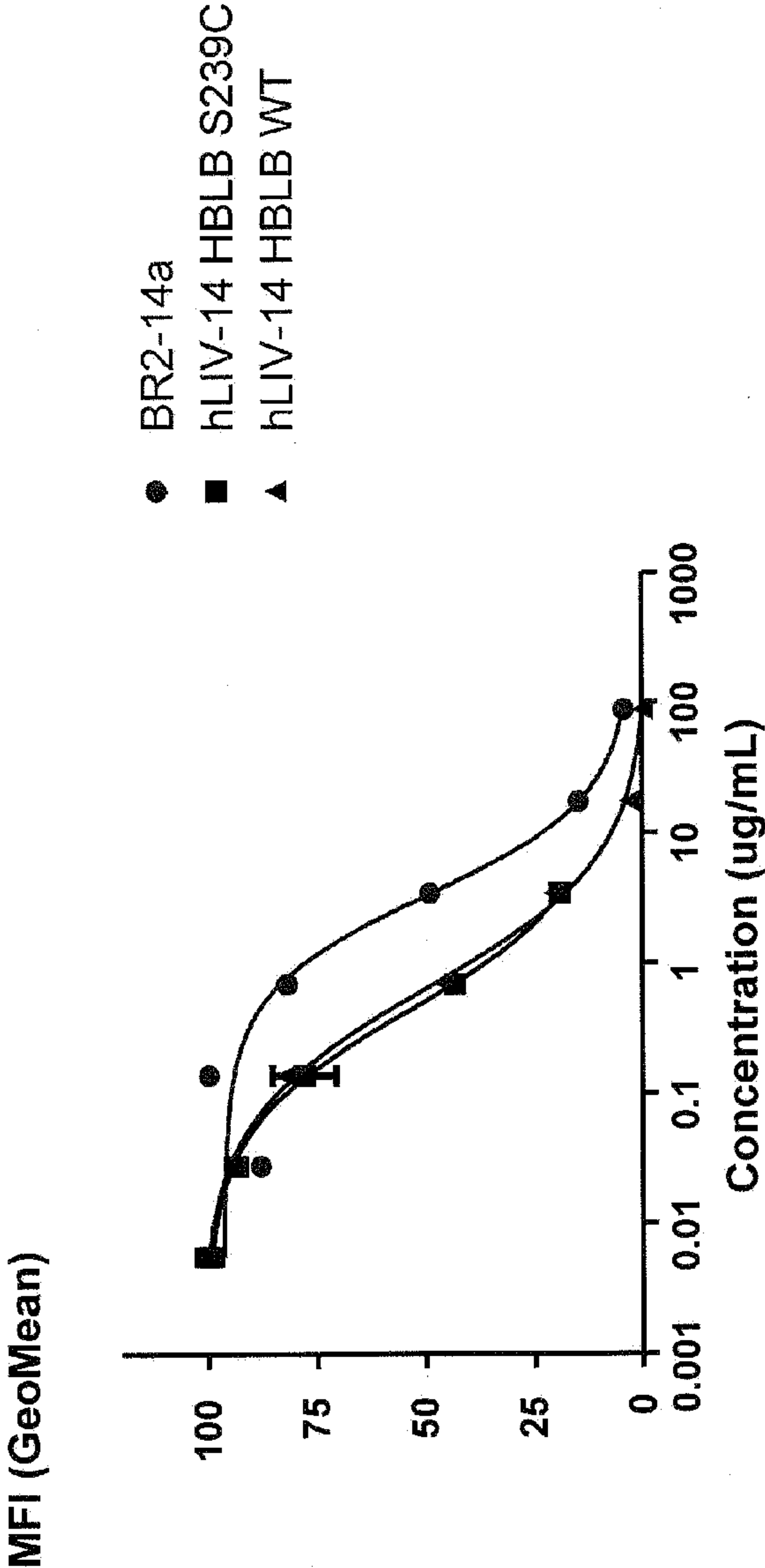


FIGURE 4

	BR2-14a	hLIV-14
Kd (nM)	5.8	2.9
Kd (ug/mL)	0.8	0.4

Competition Binding on CHO hLIV1 pool



	BR2-14a	hLIV-14 HBLB S239C	hLIV-14 HBLB WT
EC50	3.421	0.4195	0.6416

FIGURE 5

High LIV-1 Expression in Post-Hormone Treated Breast Cancers

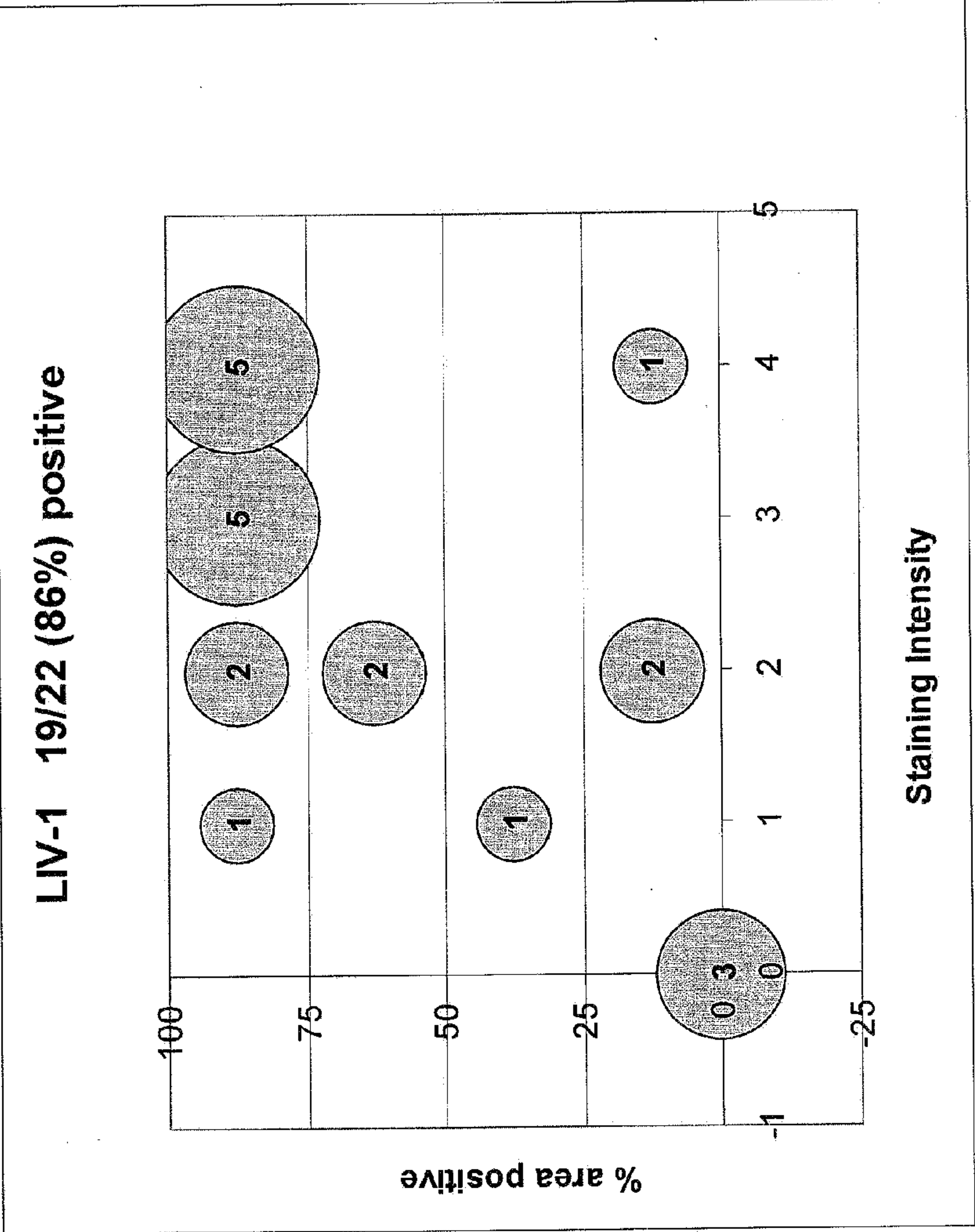


FIGURE 6

- Samples of primary and metastatic lesions from patients previously treated with hormonal therapy, including tamoxifen and aromatase inhibitors
- Tissue samples from 5 commercial sources

LIV-1 Expression in Hormone Refractory Metastatic Prostate Cancer
(Sorted By Site)

	LIV-1	
	#	%
Total Number of Samples+ (intensity 1-4+)	36/50	72
Bone Mets	15/25	60
Soft Tissue Mets	21/25	82

FIGURE 7

LIV-1 Expression In Triple Negative Breast Cancer Cases

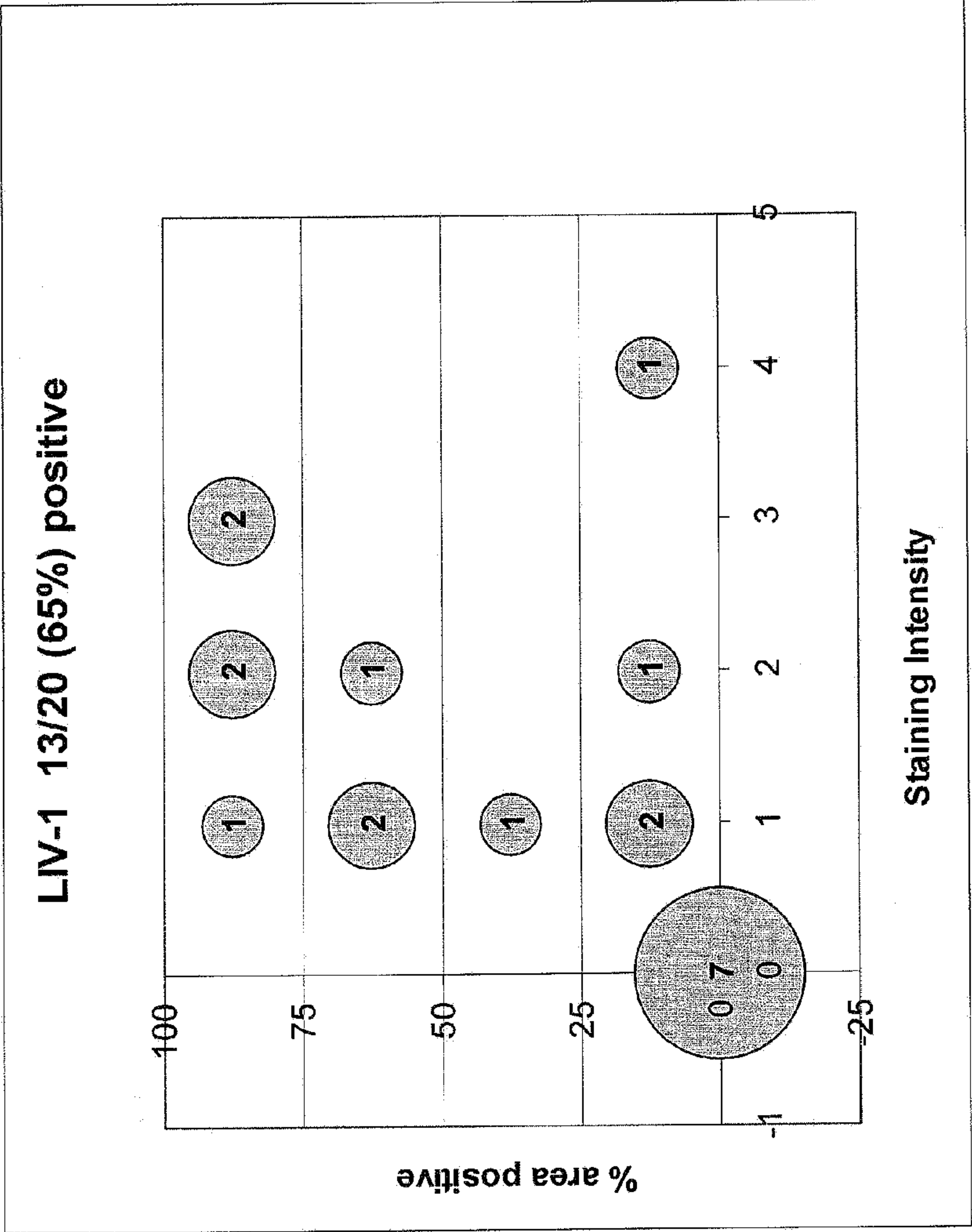
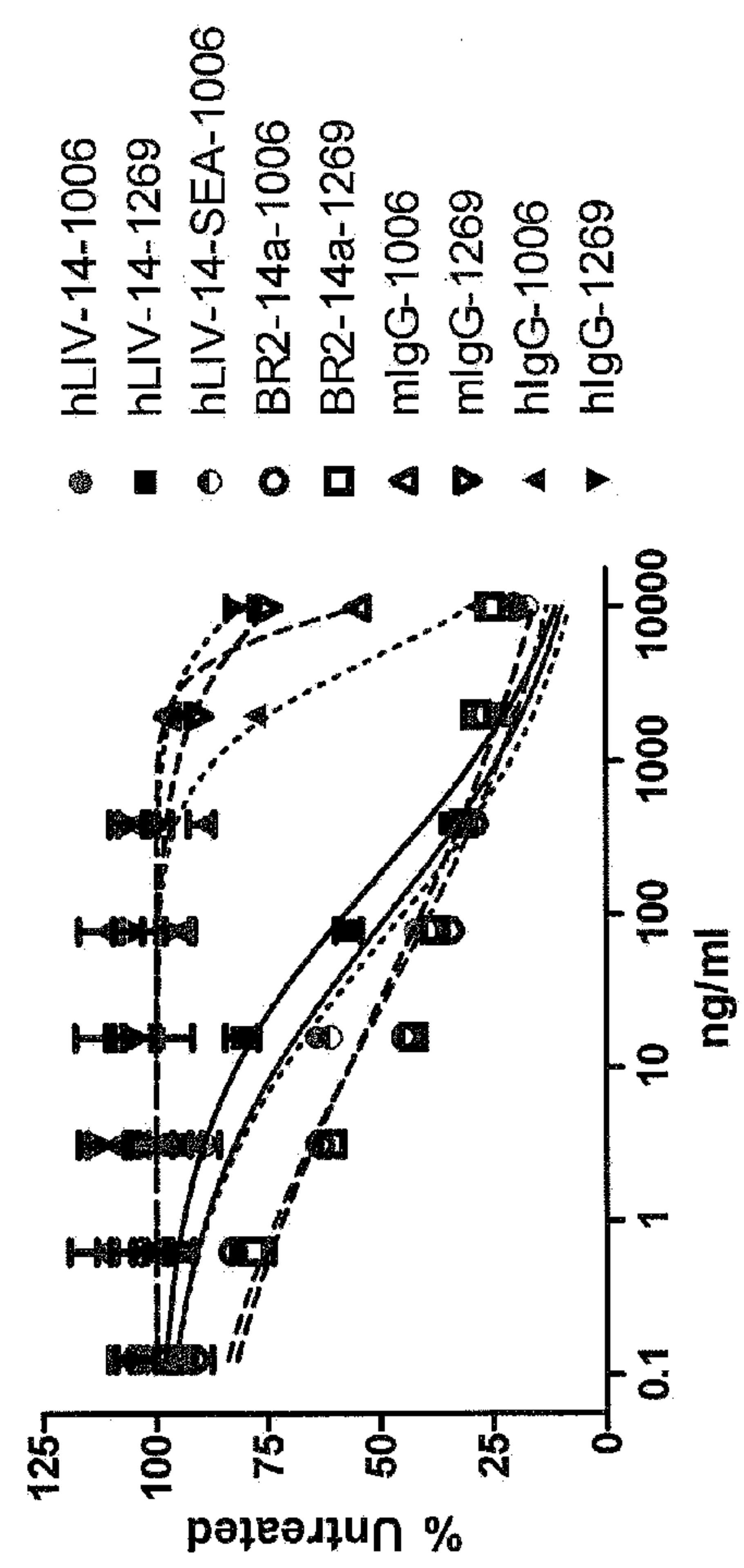


FIGURE 8

Cytotoxic Activity of hLIV-14 ADC
MCF-7 (ATCC) Cells. 144 h Treatment

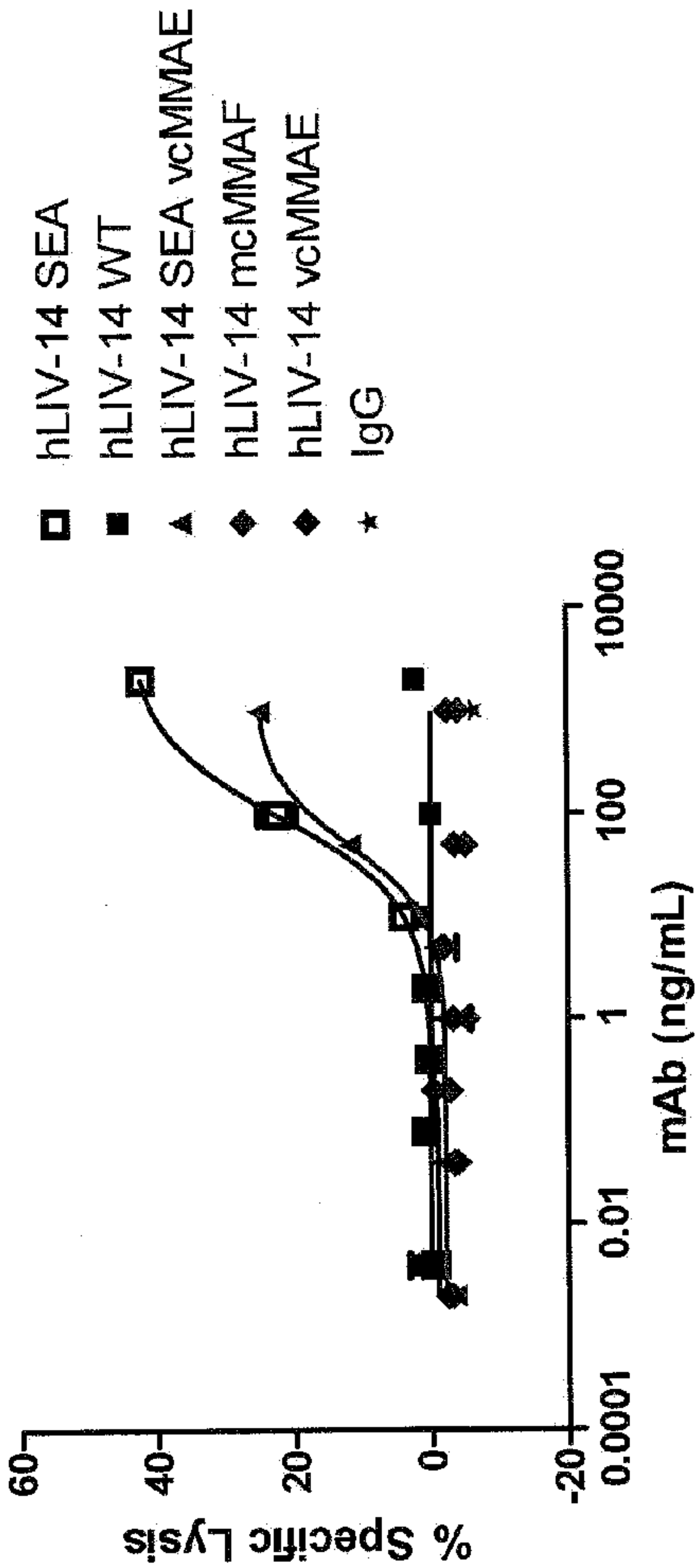


	hLIV-14-1006	hLIV-14-SEA-1006	BR2-14a-1-1006	hlgG-1006	mlgG-1006	hLIV-14-1269	BR2-14a-1269	hlgG-1269	mlgG-1269
IC50 ng/ml	91	91	20	>10 000	4786	195	20	>10 000	>10 000

FIGURE 9

ADCC Activity: hLIV14 SEA and ADC (Donor 1)

Target: MCF-7(ATCC) Donor: 2076181 (158V/V)

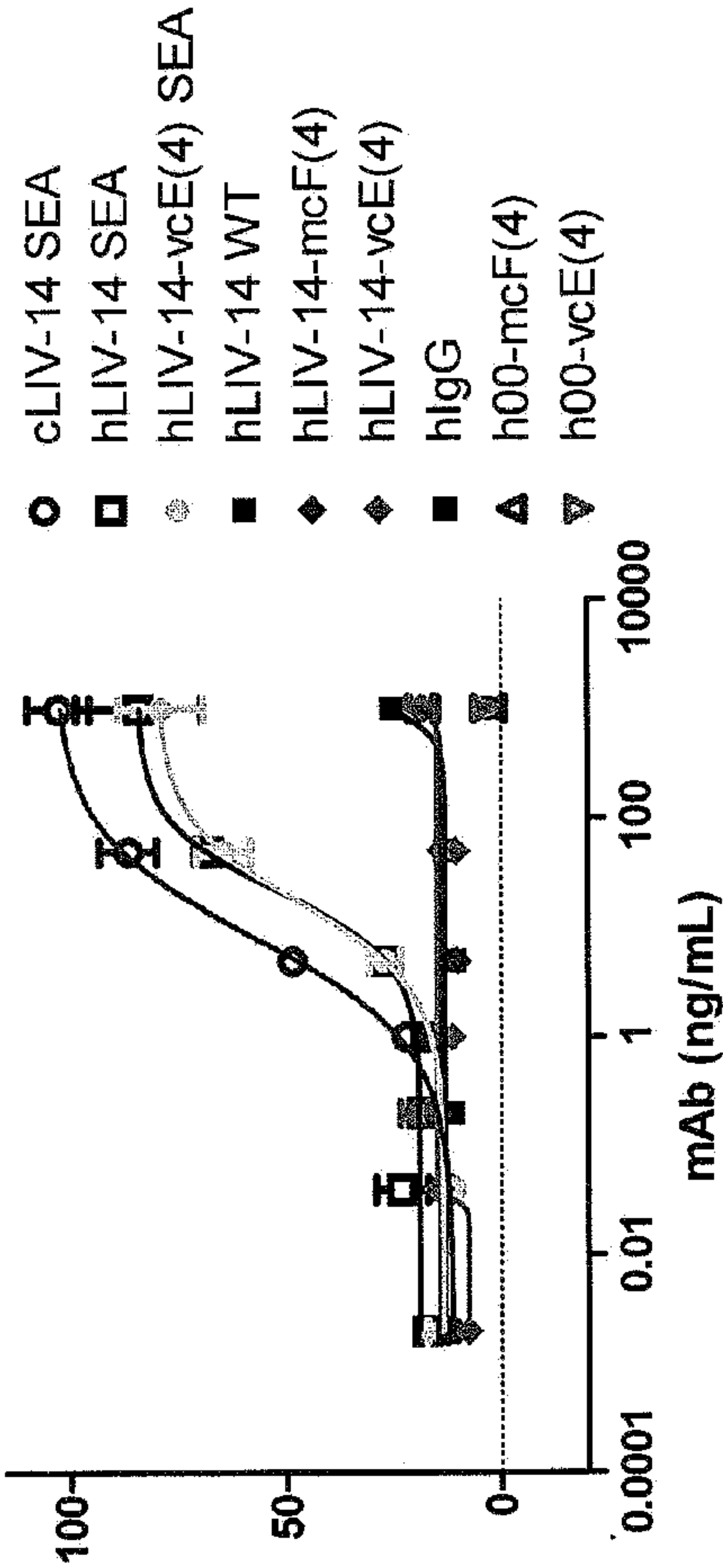


	IC50 (ng/mL)	Max Lysis
hLIV-14 SEA	97.1	44%
hLIV-14 SEA vcMMAE	49.9	26%

FIGURE 10

ADCC Activity: hLIV-14 SEA and ADC (Donor 2)

Target: MCF-7(ATCC) Donor: PO16 (158VVV)



	IC50 (ng/mL)	Max Lysis
hLIV-14 SEA	21.8	85%
hLIV-14 SEA vcMMAE	17.0	80%

FIGURE 11

Anti-tumor Activity of chimeric LIV-14 ADC on MCF7 Breast Carcinoma in Nude Mice

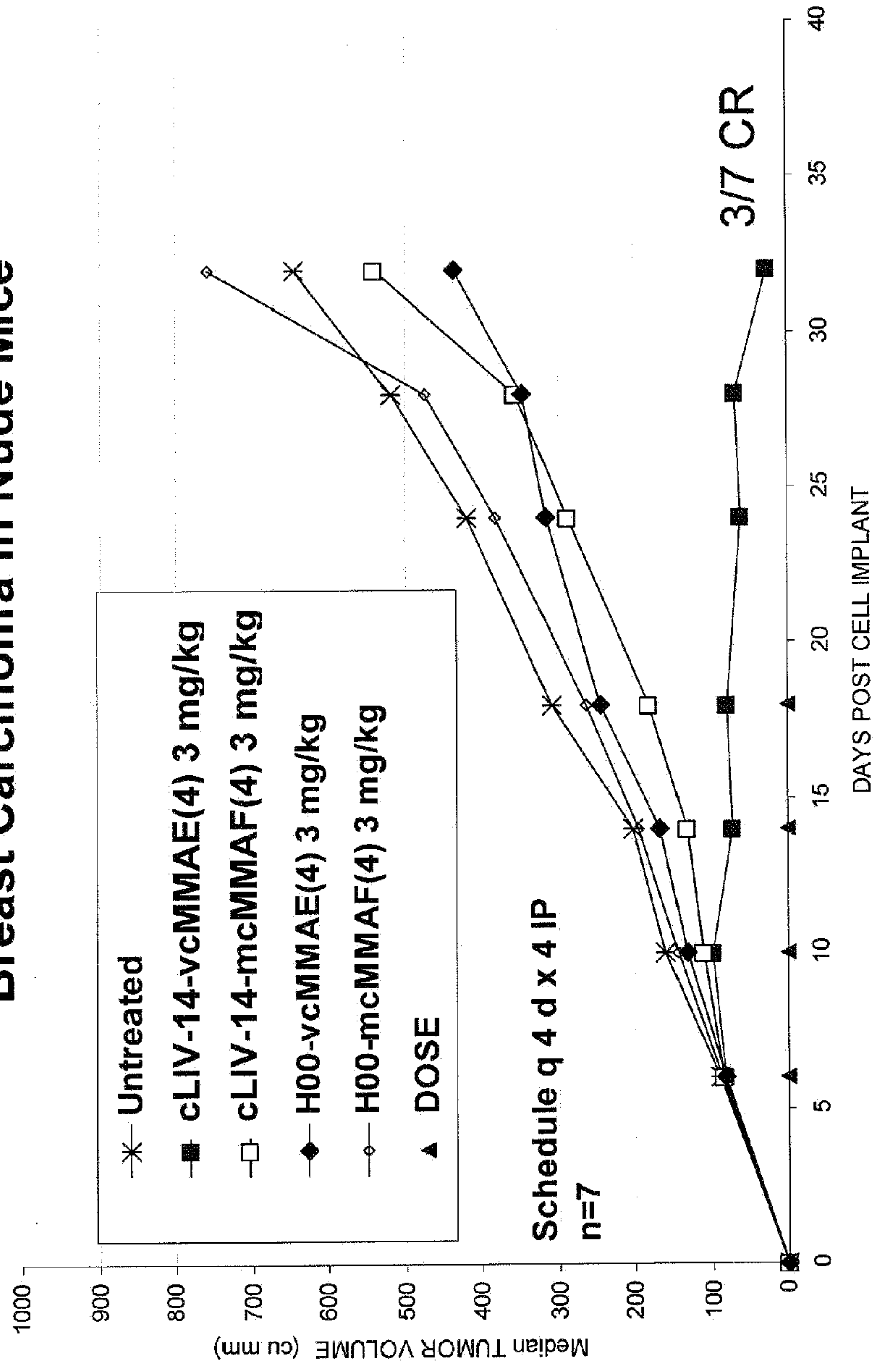


FIGURE 12

Anti-tumor Activity of chimeric LIV-14-vcMMAE on PC3
(ATCC) Prostate Carcinoma in Male Nude Mice

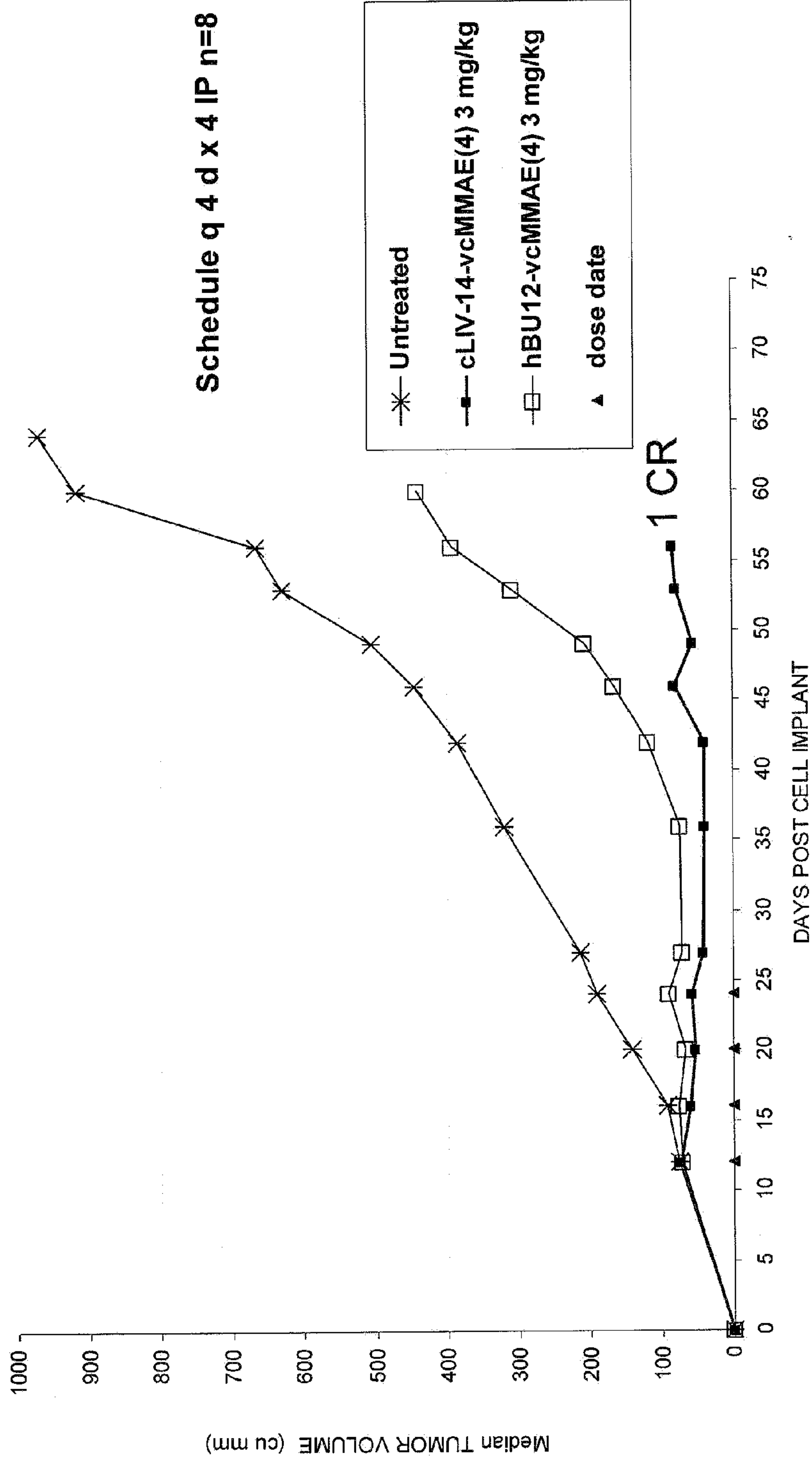


FIGURE 13

FIGURE 14

Activity of Humanized LIV-14 ADC on MCF7 (NCI) Breast Carcinoma Tumors in Nude Mice

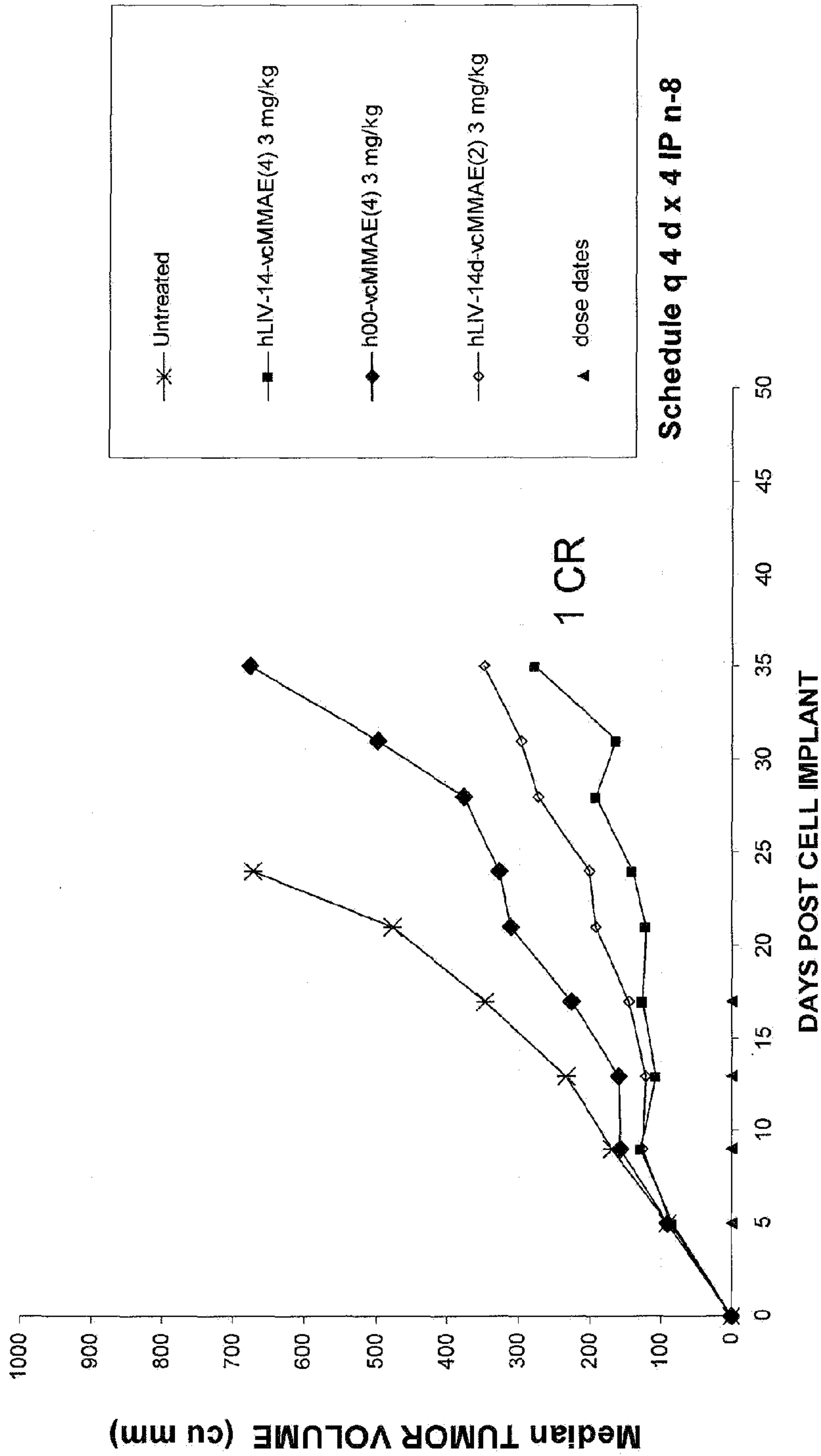
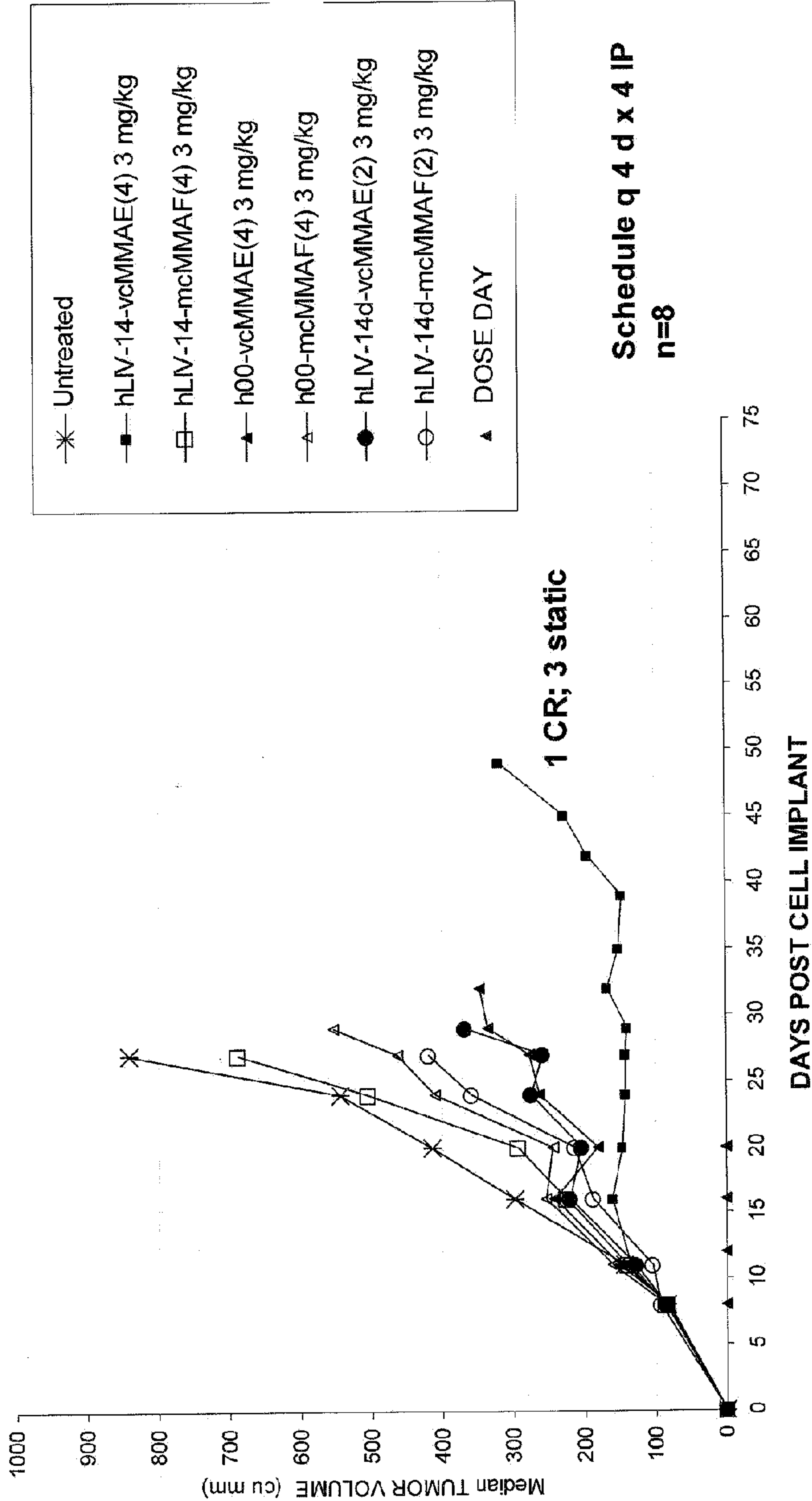


FIGURE 15

Activity of Humanized LIV-14 ADC On PC3 (DSMZ)
Prostate Carcinoma Tumors in Nude Female Mice



		10	20	30	40	50	
hLiv22 HA	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYIMHWVRQAPGQGLEWMGWIDPENGDT						
hLiv22 HB	IE.....						
hLiv22 HC							
hLiv22 HD	L.....						
hLiv22 HE	LNIE.....I.....						
hLiv22 HF	L.IE.....						
hLiv22 HG	L.IE.....						
mLiv22 H	E...Q.....LVRS.....L..T...LNIE.....K.R.E.....I.....						
CDRs	*****						*****

		60	70	80	90	100	110
hLiv22 HA	GPKFQGRVTMTDTSISTAYMELSRRLSDDTAVYYCARHNAHYGTWTFAYWGQGLTVTVSS						
hLiv22 HB	N.....						
hLiv22 HC	KA...A.....						
hLiv22 HD	TV.....						
hLiv22 HE	KA...A...N.....TV.....						
hLiv22 HF	V.....						
hLiv22 HG	N.....V.....						
mLiv22 H	KA...A...SN...LQ..S.T.G.....TV.....A						
CDRs	*****						*****

hLiv22 HA	
hLiv22 HB	
hLiv22 HC	
hLiv22 HD	
hLiv22 HE	
hLiv22 HF	
hLiv22 HG	
hLiv22 H	

FIGURE 16A

		10	20	30	40	50
hLiv22 LA	DV	MTQSPLSLPVT	LGQPASISCRSSQ	SLHSSGNTYLEWF	QORPGQSPRRLIYK	ISTRF
hLiv22 LB				Y.....		
hLiv22 LC				L.....		
hLiv22 LD					K.....	
hLiv22 LE					P.....	
hLiv22 LF				YL.....	KP.....	
hLiv22 LG				Y.....	P.....	
mLiv22 L	..L...	T.....S..DQ.....		YL.....	KP.....	
CDRs			*****			*****

		60	70	80	90	100
hLiv22 LA	SG	VPDRFSGSGSGT	DFTLKISRVEAED	VG	VYYCFQGS	HVPYTFGGG
hLiv22 LB						
hLiv22 LC						
hLiv22 LD						
hLiv22 LE						
hLiv22 LF						
hLiv22 LG						
mLiv22 L			L.L.....			L.....
CDRs	*			*****		

FIGURE 16B

hLIV22 Antigen Binding humanization round 2
grouped by H-Chain

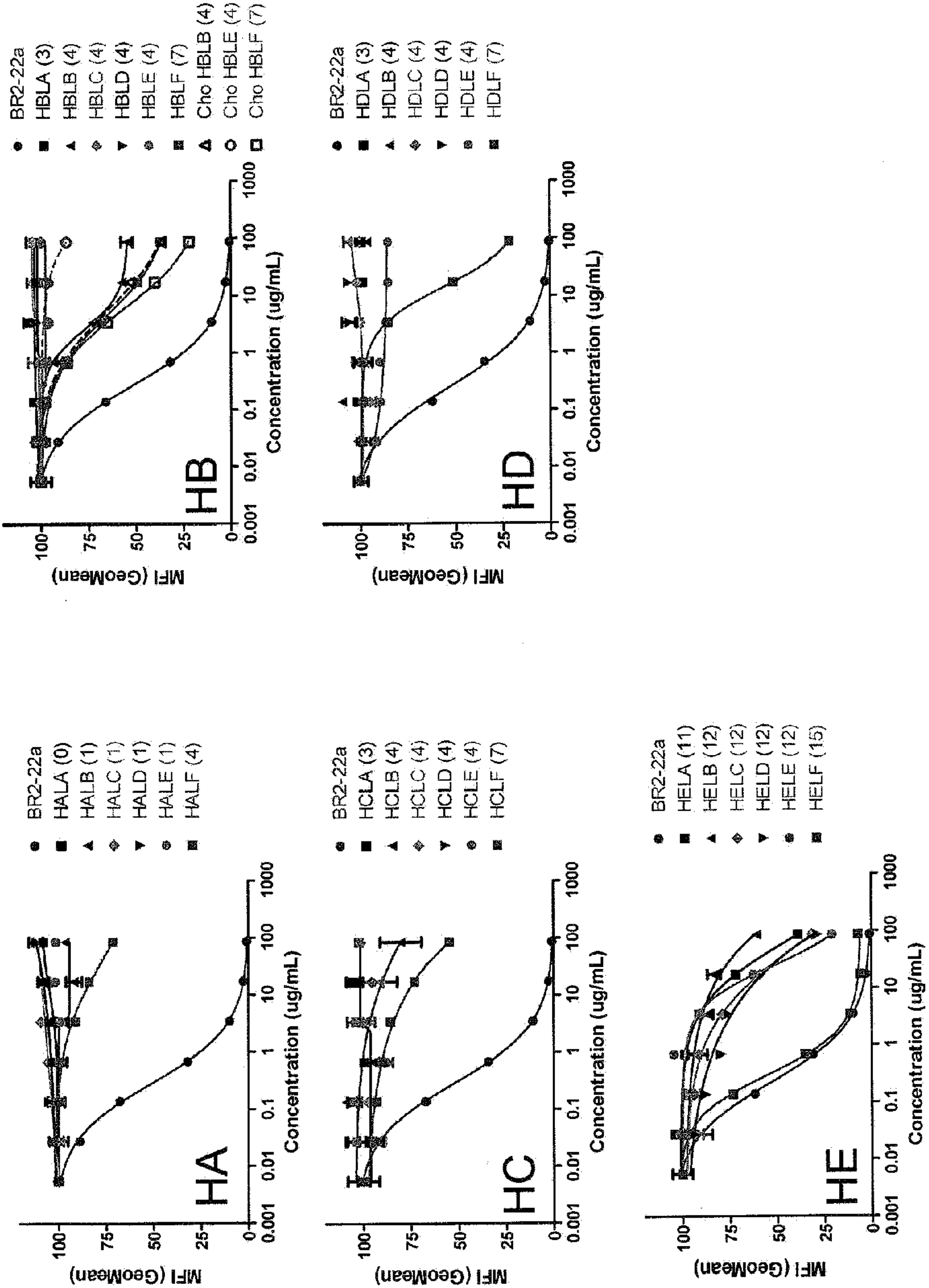


FIGURE 17

LIV22 Back Mutations (round2)

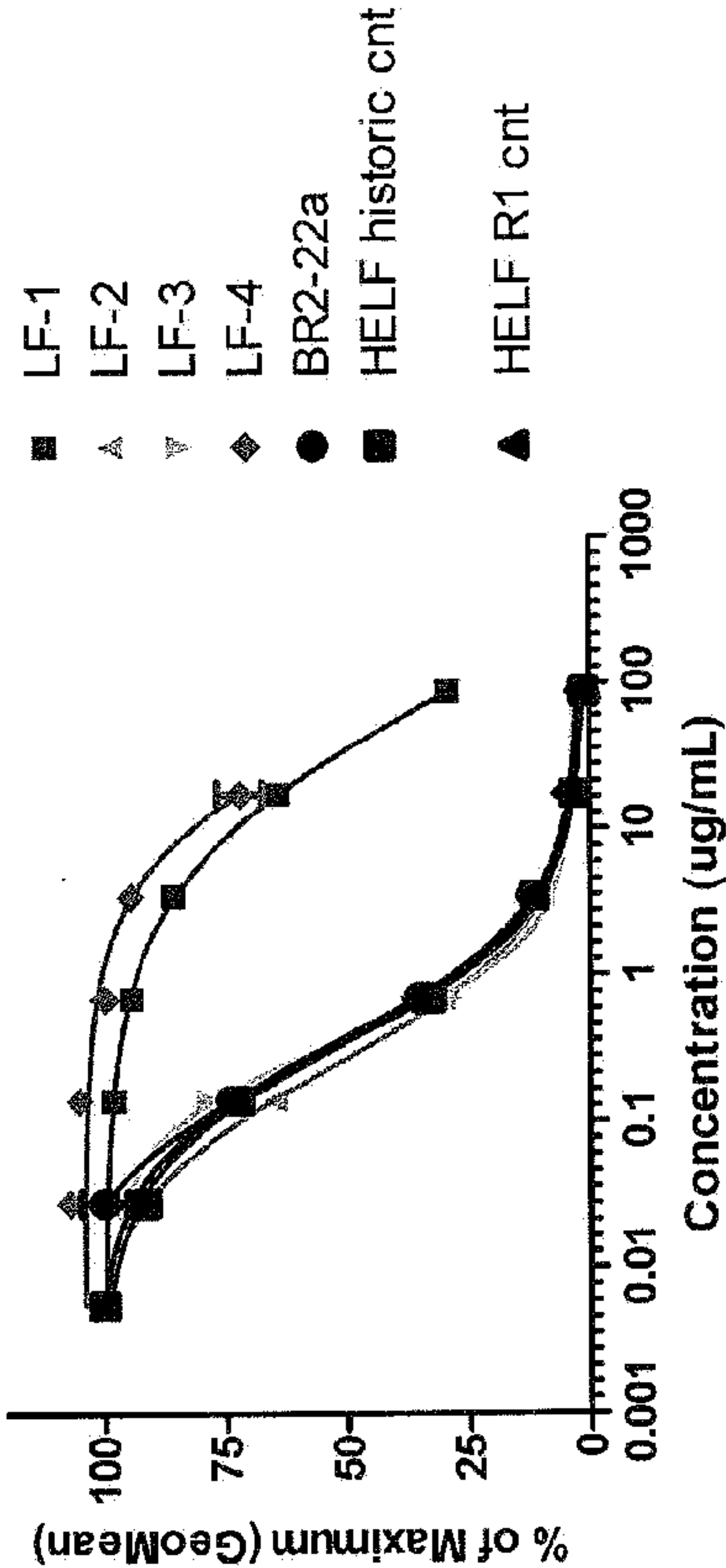
	# back mutations					# back mutations											
	L36	L37	L45	L46		H27	H28	H29	H30	H48	H66	H67	H71	H76	H93	H94	
LA	F	Q	R	R	HA	Y	T	F	T	M	R	V	R	S	A	R	0
LB	Y	Q	R	R	HB	Y	T	I	(E)	M	R	V	R	N	A	R	3
LC	F	L	R	R	HC	Y	T	F	T	M	K	A	A	S	A	R	3
LD	F	Q	K	R	HD	L	T	F	T	M	R	V	R	S	T	V	3
LE	F	Q	R	P	HE	L	N	I	(E)	I	K	A	A	N	T	V	11
LF	Y	L	K	P													

Additional LIV22 Mutations (round3)

	# back mutations					H27	H28	H29	H30	H48	H66	H67	H71	H76	H93	H94	# back mutations
LF	Y	L	K	P	HE	L	N	I	(E)	I	K	A	A	N	I	V	11
LF-1	F	L	K	P	HE-1	Y	N	I	(E)	I	K	A	A	N	I	V	10
LF-2	Y	Q	K	P	HE-2	L	T	I	(E)	I	K	A	A	N	I	V	10
LF-3	Y	L	R	P	HE-3	L	N	F	(E)	I	K	A	A	N	I	V	10
LF-4	Y	L	K	R	HE-4	L	N	I	T	I	K	A	A	N	I	V	10
					HE-5	L	N	I	(E)	M	K	A	A	N	I	V	10
					HE-6	L	N	I	(E)	I	R	A	A	N	I	V	10
					HE-7	L	N	I	(E)	I	K	V	A	N	I	V	10
					HE-8	L	N	I	(E)	I	K	A	R	N	I	V	10
					HE-9	L	N	I	(E)	I	K	A	A	S	I	V	10
					HE-10	L	N	I	(E)	I	K	A	A	N	A	V	10
					HE-11	L	N	I	(E)	I	K	A	A	N	I	R	10

FIGURE 18

hLIV22 Competition Binding: LF (round3)



Additional LIV22 Back Mutations: LF

LF	L36	L37	L45	L46	# back mutations	residues to maintain antigen binding:
LF	<u>Y</u>	<u>L</u>	<u>K</u>	<u>P</u>	4	Y36 (Tyrosine) & P46 (Proline)
LF-1	F	L	K	P	3	
LF-2	Y	Q	K	P	3	
LF-3	Y	L	R	P	3	
LF-4	Y	L	K	R	3	

FIGURE 19

hLIV22 Competition Binding: HE (round3)

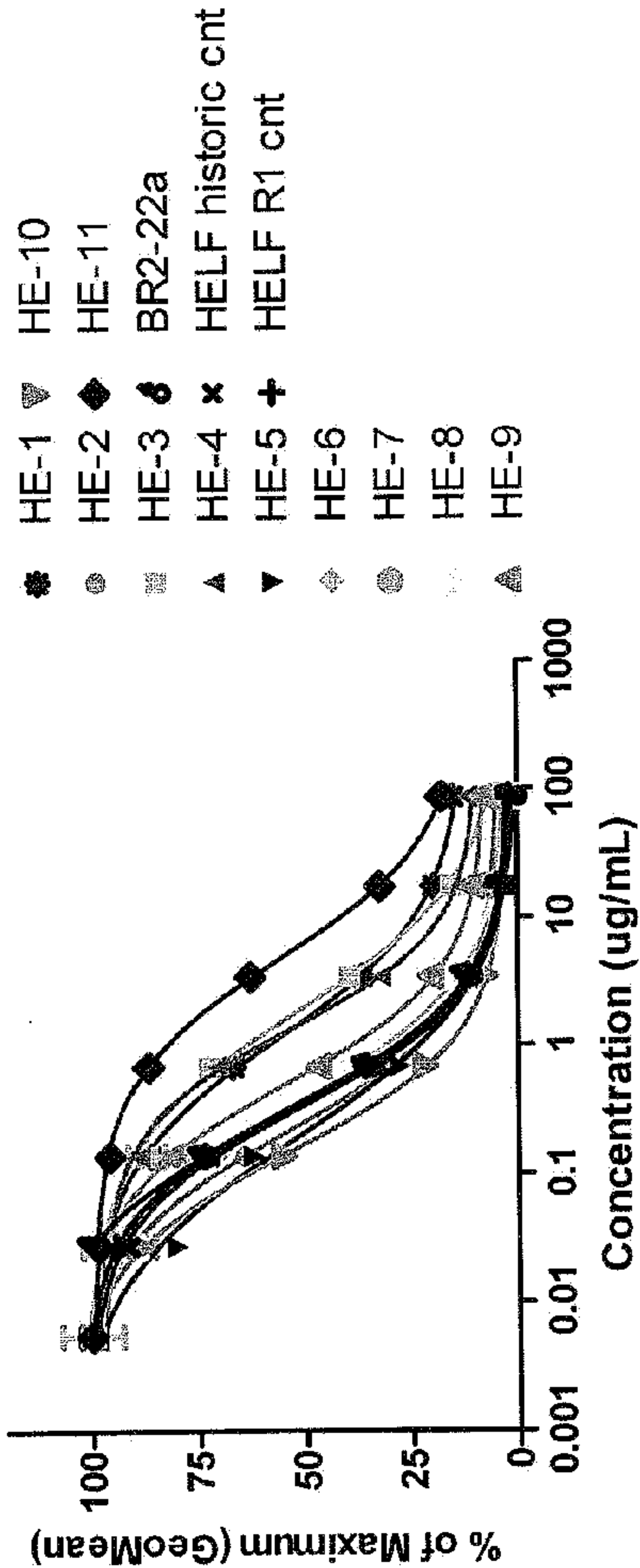


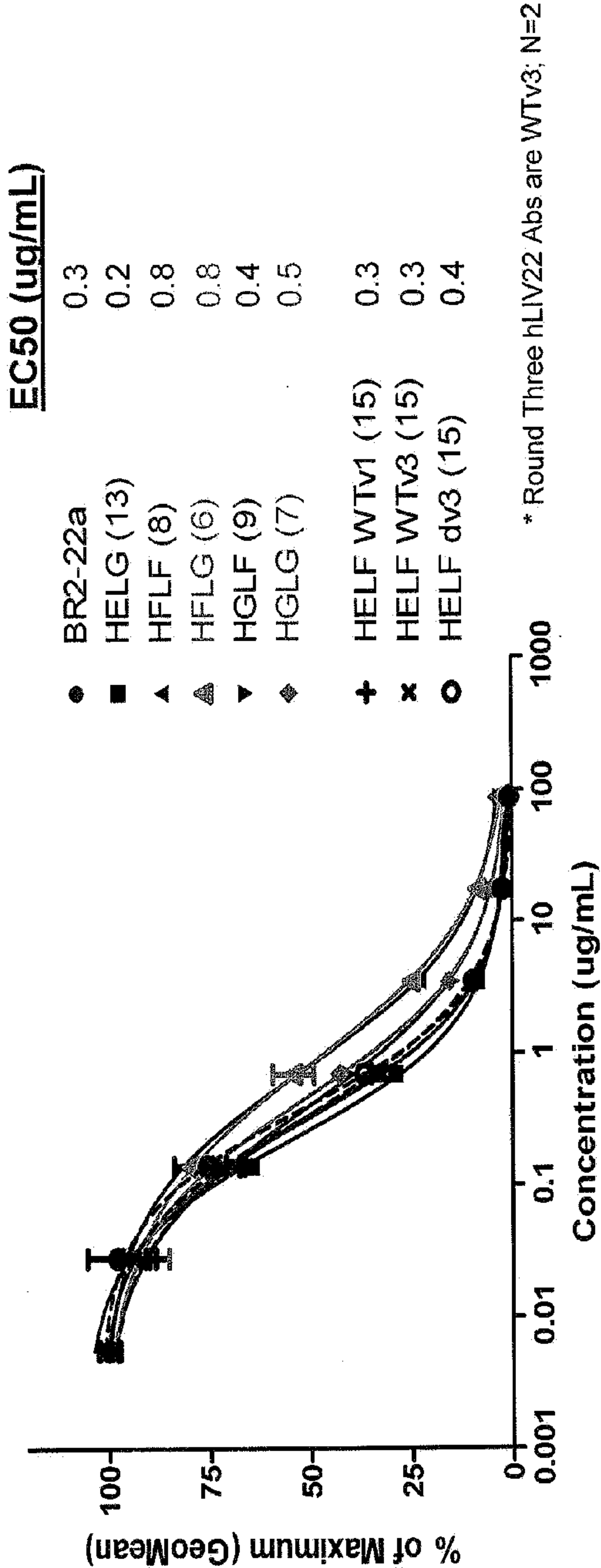
FIGURE 20

Additional hLIV22 Back Mutations: HE

	H27	H28	H29	H30	H48	H66	H67	H71	H76	H93	H94	# back mutations
HE	L	N	I	E	I	K	A	A	N	I	V	11
HE-1	Y	N	I	E	I	K	A	A	N	I	V	10
HE-2	L	T	I	E	I	K	A	A	N	I	V	10
HE-3	L	N	F	E	I	K	A	A	N	I	V	10
HE-4	L	N	I	T	I	K	A	A	N	I	V	10
HE-5	L	N	I	E	M	K	A	A	N	I	V	10
HE-6	L	N	I	E	I	R	A	A	N	I	V	10
HE-7	L	N	I	E	I	K	V	A	N	I	V	10
HE-8	L	N	I	E	I	K	A	A	N	I	V	10
HE-9	L	N	I	E	I	K	A	R	N	I	V	10
HE-10	L	N	I	E	I	K	A	A	S	A	V	10
HE-11	L	N	I	E	I	K	A	A	N	I	R	10

residues to maintain
antigen binding:
L27 (Leucine)
I29 (Isoleucine)
E30 (Glutamic Acid)
N76 (Asparagine)
V94 (Valine)

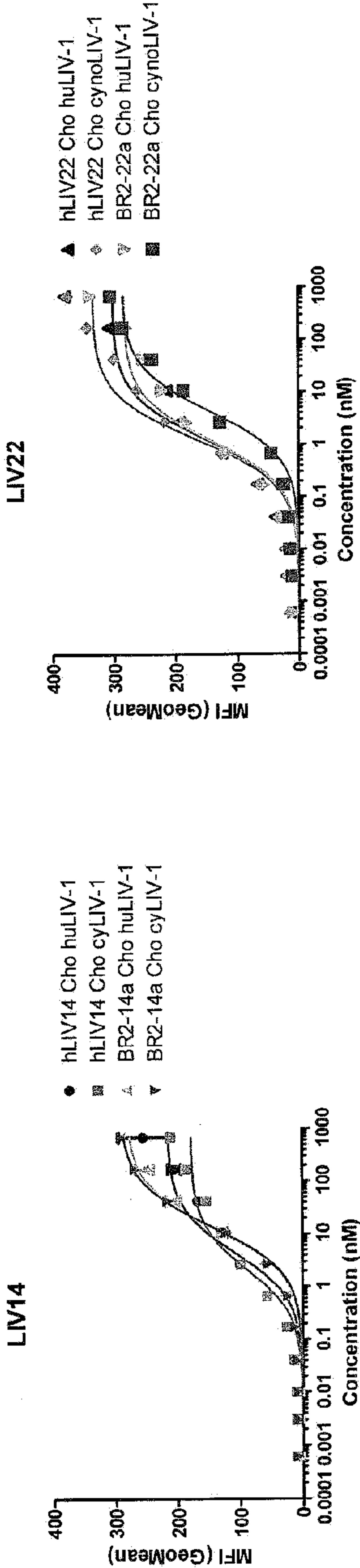
LIV22 Humanization: Round 4



	# Back mutations	EC50 (ug/mL)
HGLG	7	0.5
HGLF	9	0.4
HFLG	6	0.8
HFLF	8	0.8

FIGURE 21

Saturation Binding
AF-647



Apparent Kd (nM) of BR2-14a, BR2-22a, hLIV14 and hLIV14 for human and cyno LIV-1

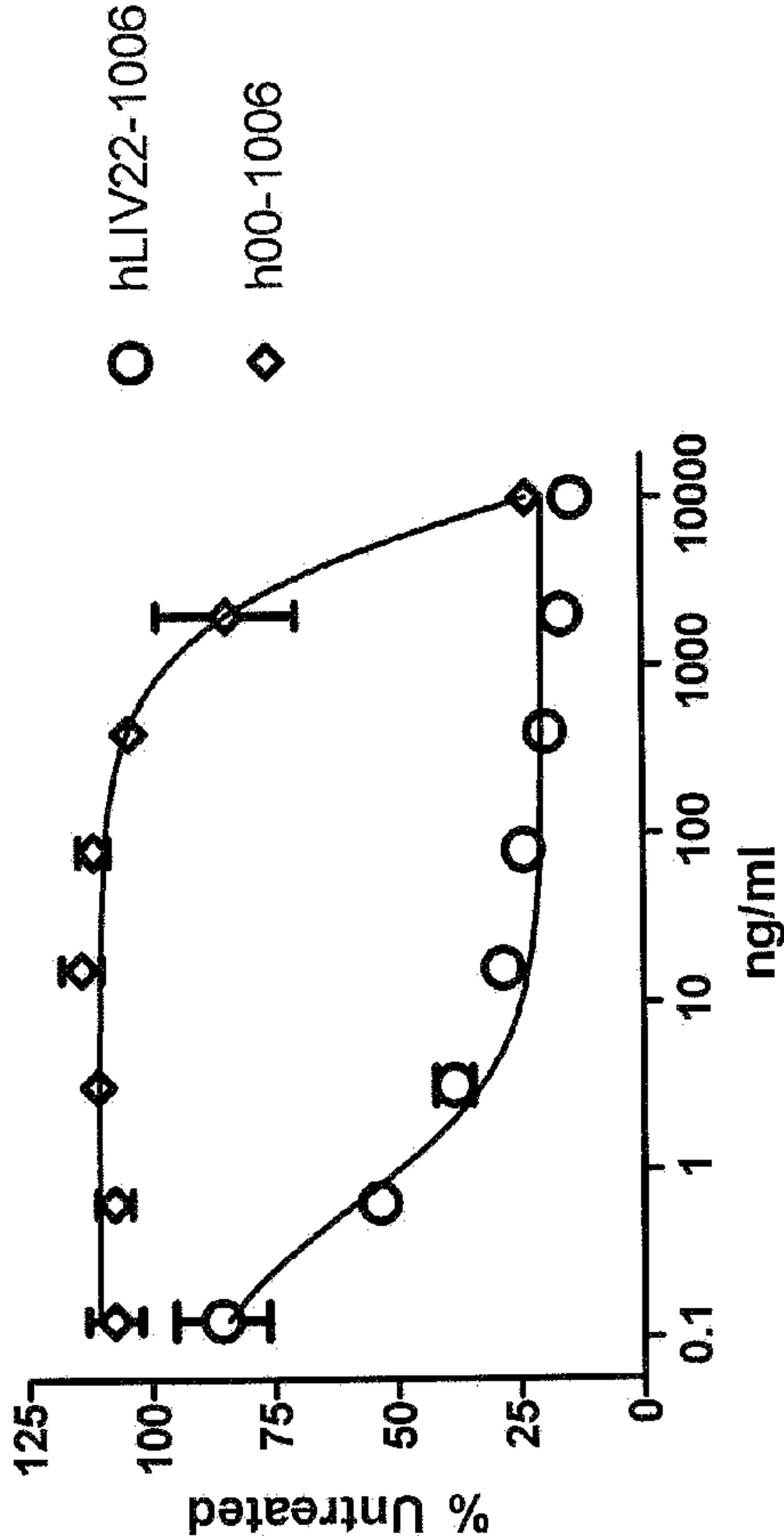
antigen	BR-14a	hLIV14	BR-22a	hLIV22
Human LIV1	12.5	4.3	1.2	1.2
Cyno LIV1	14.0	2.1	4.2	1.3

Negative control: m2H12

FIGURE 22

Cytotoxic Activity of hLIV22-vcMMAE

MCF-7 (ATCC) cells. 144 hour treatment

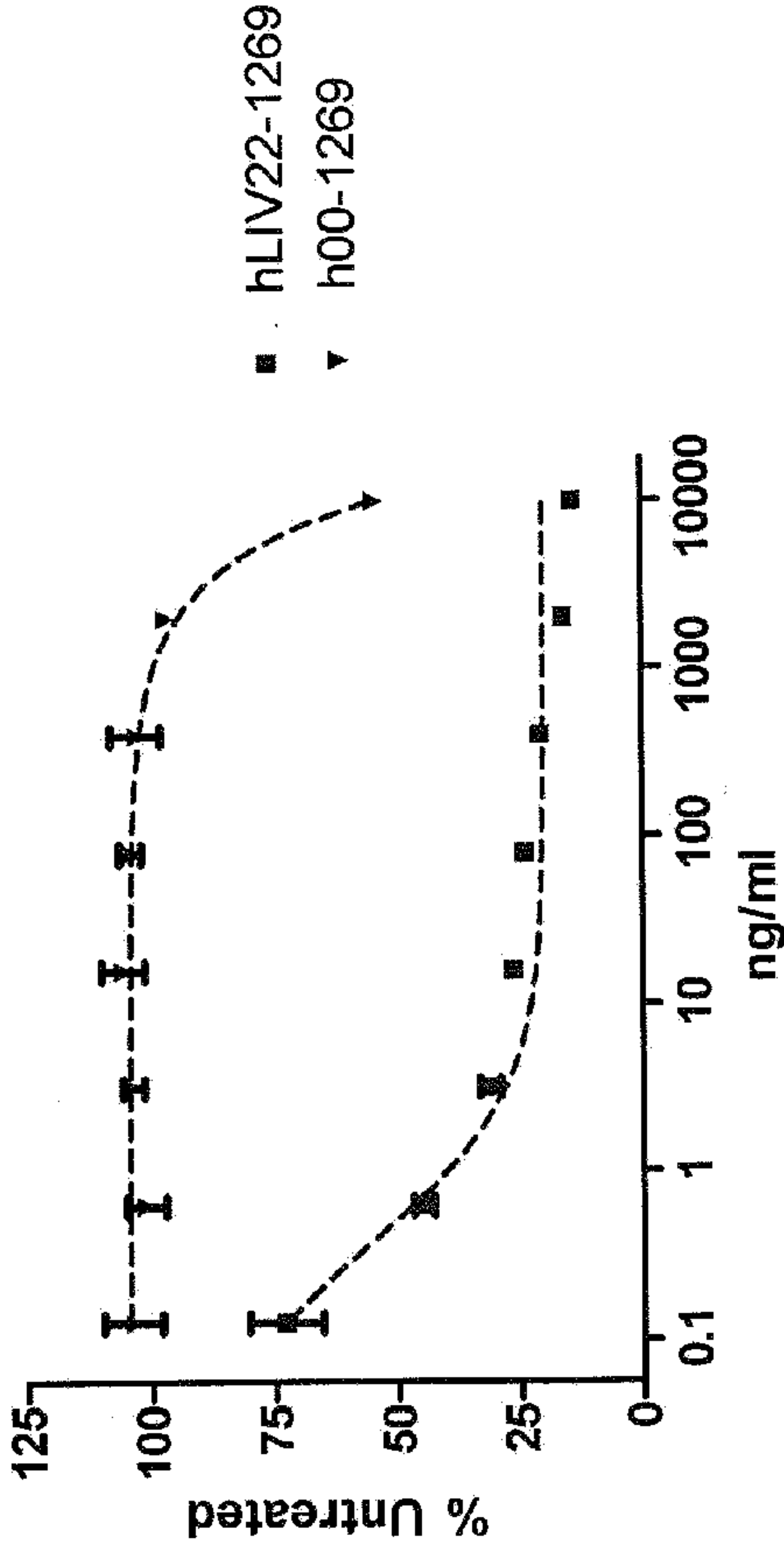


EC50 table. Average from 2 experiments

	EC50 (ng/ml)
hLIV22 -1006	0.9
h00 -1006	4981

FIGURE 23

Cytotoxic Activity of hLIV22-mcMMAF
MCF-7 (ATCC) cells. 144 hour treatment



EC50 table. Average from 2 experiments

-1269 ADC	EC50 (ng/ml)
hLIV22	0.7
h00	>10000

FIGURE 24

Activity of hLIV22 ADC on PC3(dsmz) Prostate Carcinoma
Model in Nude Female Mice

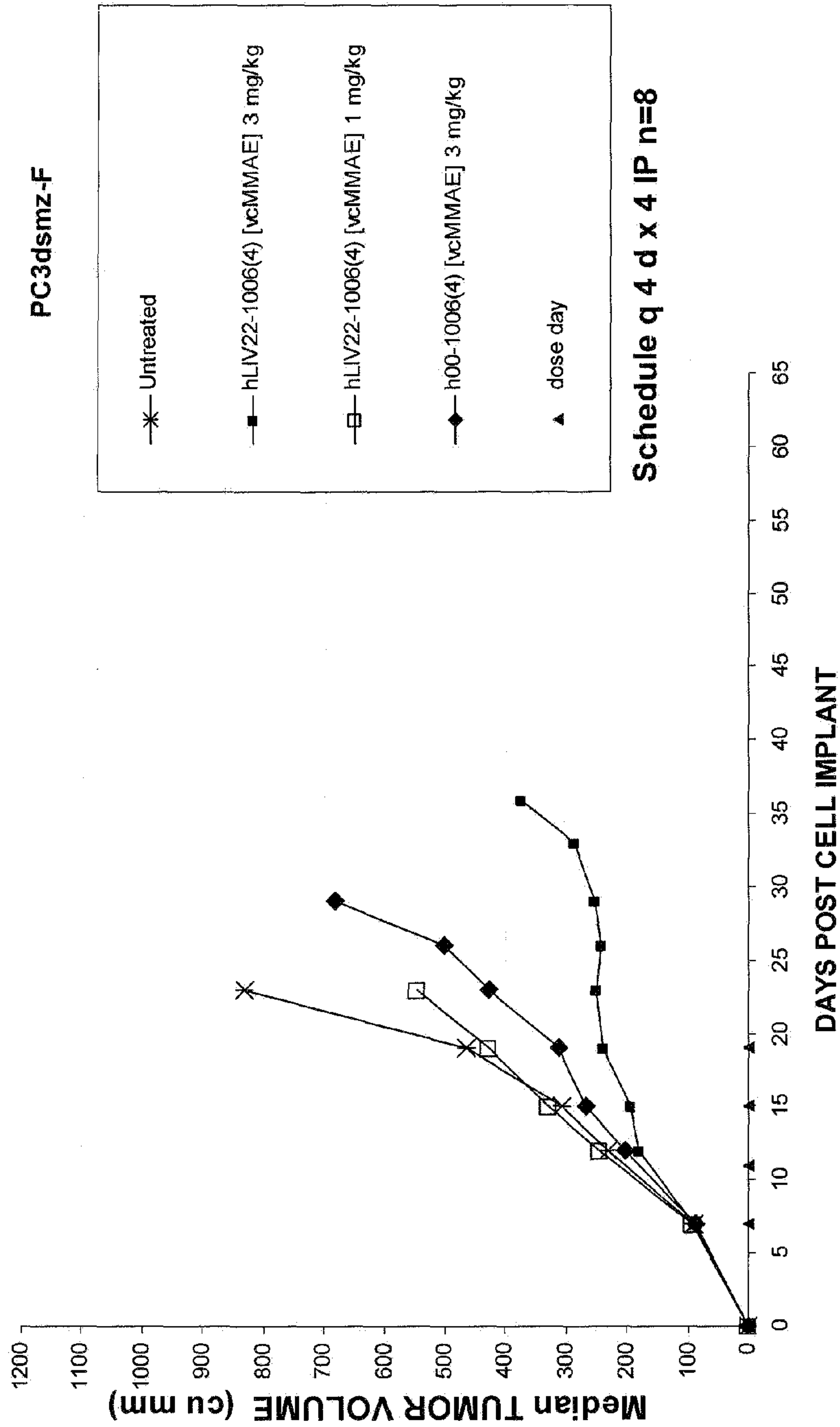


FIGURE 25

Activity hLIV22 on MCF7 (NCI) Breast Carcinoma Tumors in Nude Mice

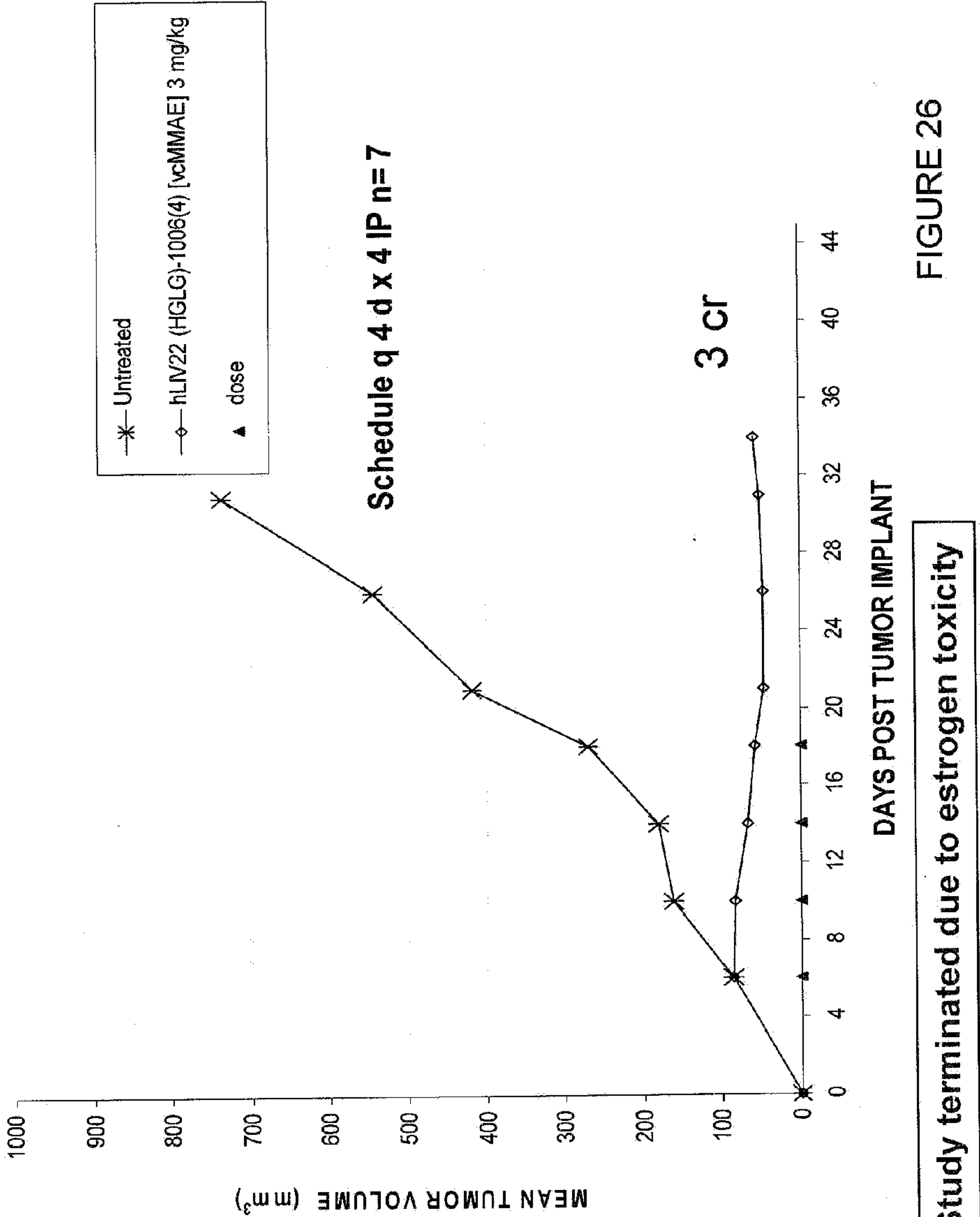


FIGURE 26

Activity of hLIV22 (HGLG) vcMMAE vs hLIV-14vcE

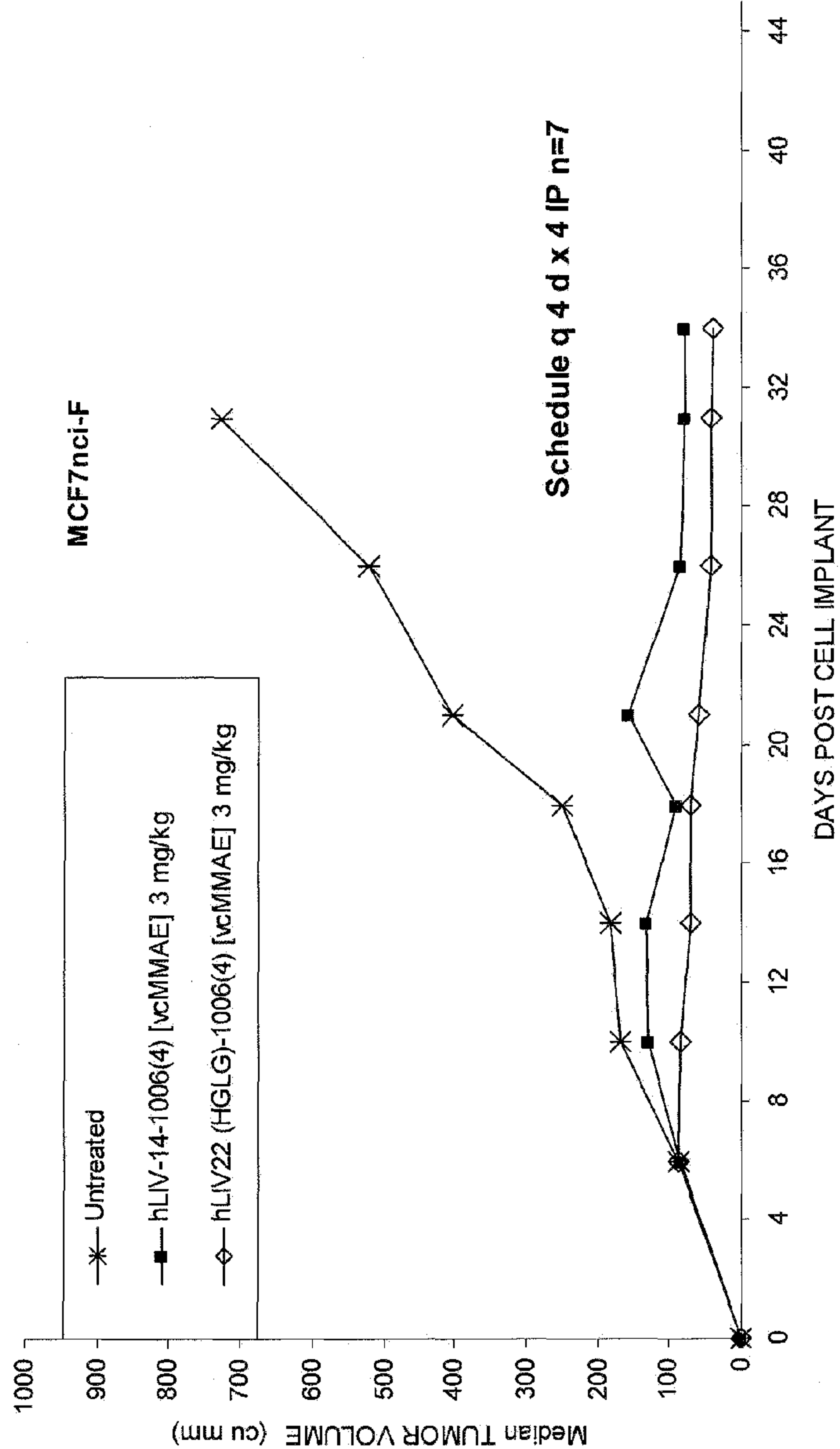


FIGURE 27

LIV-1 expression in melanoma biopsies

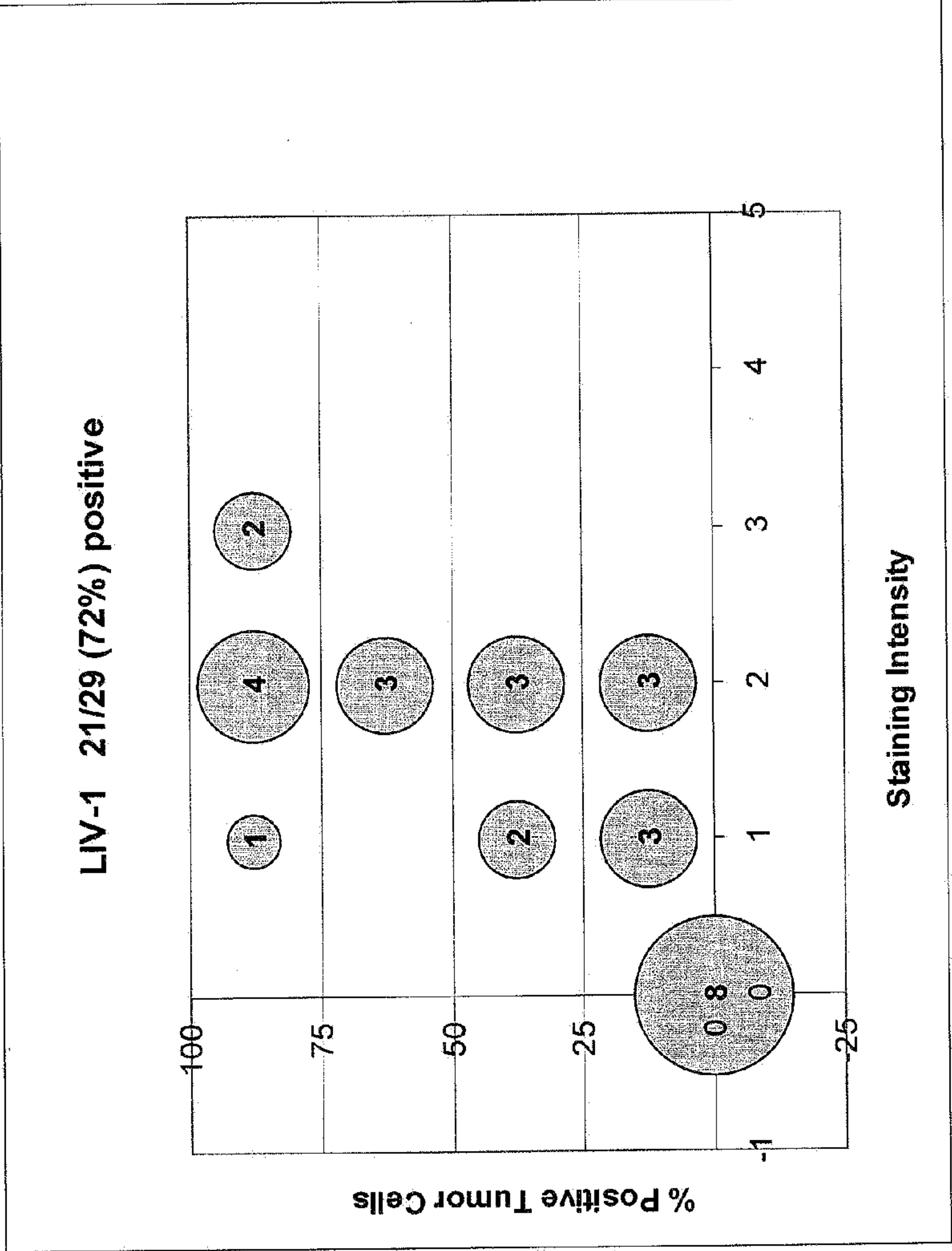


FIGURE 28