HUMANIZED ANTI-LT-β-R ANTIBODIES

RELATED APPLICATIONS

This is a continuation-in-part of U.S.S.N. 60/299,987, filed on June 21, 2001 which is a continuation-in-part of U.S.S.N. 60/275,289, filed on March 13, 2001 which is a continuation-in-part of U.S.S.N. 60/240,285, filed on October 13, 2000. The entire disclosure of each of the aforesaid patent applications are incorporated herein by reference.

FIELD OF THE INVENTION

This invention relates generally to humanized antibodies specific for the lymphotoxin beta receptor (LT-β-R).

BACKGROUND OF THE INVENTION

Lymphotoxin beta receptor (referred to herein as LT-β-R) is a member of the tumor necrosis factor family which has a well-described role both in the development of the immune system and in the functional maintenance of a number of cells in the immune system including follicular dendritic cells and a number of stromal cell types (Matsumoto et al., *Immunol. Rev.* 156:137 (1997). Known ligands to the LT-β-R include LTα1/β2 and a second ligand called LIGHT (Mauri et al. *Immunity* 8:21 (1998)). Activation of LT-β-R has been shown to induce the apoptotic death of certain cancer cell lines *in vivo* (PCT/US96/01386). Treatment with agonist LT-β-R activating agents, such as specific humanized anti-LT-β-R antibodies, would thus be useful for treating or reducing the advancement, severity or effects of neoplasia in subjects (e.g., humans).

SUMMARY OF THE INVENTION

The present invention provides humanized anti-lymphotoxin beta receptor (LT-β-R) antibodies and methods of using these antibodies to treat or reduce the advancement, severity or effects of neoplasia in subjects (e.g., humans).

Specifically, the invention embraces a humanized antibody that specifically binds to LT-β-R (e.g., human LT-β-R). This antibody comprises light chain complementary determining regions defined by amino acid residues 24 to 34, 50 to 56 and 89 to 97 of SEQ ID NO: 1, and/or heavy chain complementary determining regions defined by amino acid residues 31 to 35, 50 to 66 and 99 to 109 of SEQ ID NO: 2 and in addition at least one (e.g., 1, 2, 3, 4, or 5) of the following residues in its light chain:

K3, W41, I46, Q69 and Y71; or at least one (e.g. 1, 2, 3, 4, or 5) of the following residues in its heavy chain: F37, T40, A49, M89 and V93 (Kabat numbering convention).

The humanized antibody of this invention may comprise a light chain variable domain sequence defined by amino acid residues 1 to 107 of SEQ ID NO:8 and/or a heavy chain variable domain sequence defined by amino acid residues 1 to 120 of SEQ ID NO:16. The humanized antibody may also comprise the same heavy and/or light chain polypeptide sequences as an antibody produced by cell line E46.4 (ATCC patent deposit designation PTA-3357) or cell line E77.4 (ATCC patent deposit designation 3765).

In another embodiment, the humanized antibody of this invention substantially retains the binding properties of the parent antibody. In one embodiment the humanized antibody of this invention binds to LT-β-R with a functional affinity, for instance, of about 1 pM to about 10 pM, alternatively, about 10 pM to about 20 pM, alternatively, about 20 pM to about 30 pM, alternatively, about 30 pM to about 40 pM alternatively, about 40 pM to about 50 pM, alternatively, about 50 pM to about 60 pM, alternatively, about 60 pM to about 70 pM, alternatively, about 70 pM to about 80 pM, and alternatively, about 80 pM to about 90 pM, wherein the functional affinity is measured by FACS in accordance with Example 8.

In another embodiment, the humanized antibody of this invention is linked to an immunotoxin (e.g., ricin A chain and *Pseudonomas toxin*). The humanized antibody of this invention can also be linked to a chemotherapeutic drug (e.g., Adriamycin, 5FU, Vinblastine, Actinomycin D, Etoposide, Cisplatin, Methotrexate and Doxorubicin) or to a radioisotype. The present invention also embraces a combination therapy in which for instance, the humanized antibody of the present invention which is linked to an immunotoxin is used in combination with a humanized antibody of the present invention which is linked to a chemotherapeutic drug. The present invention further embraces a composition suitaible for administration to a mammal (ie human) having a tumor that overexpresses LTβR comprising a) a humanized anti- LTβR antibody either alone or linked to a immunotoxin or a chemotherapeutic drug and b) a cytotoxic factor, each present in amounts effective to reduce tumor volume upon administration to the mammal. The cytotoxic factor may include for instance, TNF-α, TNF-β, IL-1, INF-γ,

IL-2. Alternatively, the cytotoxic factor may by a chemotherapeutic drug. The chemotherapeutic drug may include for instance, Adriamycin, 5FU, Vinblastine, Actinomycin D, Etoposide, Cisplatin, Methotrexate and Doxorubicin.

The antibody of this invention can be, for instance, a whole antibody (i.e. with two full length light chains and two full length heavy chains) of any isotype and subtypes (e.g. IgM, IgD, IgG1, IgG2, IgG3, IgG4, IgE, IgA1 and IgA2); alternatively, it can be an antigen-binding fragment (e.g., Fab, $F(ab')_2$, and Fv) of a whole antibody. Embraced in this invention are also a composition comprising a pharmaceutically acceptable carrier; an isolated nucleic acid comprising a coding sequence for SEQ ID NO:8; an isolated nucleic acid comprising a coding sequence for SEQ ID NO:16; an isolated nucleic acid comprising a coding sequence for the light chain of an antibody produced by cell line E46.4 (ATCC patent deposit designation PTA-3357) or cell line E77.4 (ATCC patent deposit designation 3765); an isolated nucleic acid comprising a coding sequence for the heavy chain of an antibody produced by cell line E46.4 (ATCC patent deposit designation PTA-3357) or cell line E77.4 (ATCC patent deposit designation 3765); an isolated nucleic acid comprising a coding sequence for residues 1-107 of SEQ ID NO:8; and an isolated nucleic acid comprising a coding sequence for residues 1-120 of SEQ ID NO:16.

Embraced within the present invention are also cells from cell lines that produce humanized anti-LTβR antibody, included, for instance, cell line E46.4 (ATCC patent deposit designation PTA-3357) and cell line E77.4 (ATCC patent deposit designation 3765). In one embodiment the cell line produces from about 250mg/L to about 300 mg/L of said antibody, alternatively, the cell line produces from about 300mg/L to about 350 mg/L of said antibody, alternatively, the cell line produces from about 350mg/L to about 400 mg/L of said antibody, alternatively, the cell line produces from about 400mg/L to about 450 mg/L of said antibody, alternatively, the cell line produces from about 450mg/L to about 500 mg/L of said antibody, alternatively, the cell line produces from about 500mg/L to about 550 mg/L of said antibody and alternatively, the cell line produces from about 550mg/L to about 600 mg/L of said antibody. The concentration of the antibody produced by the cell lines is measures as a harvest titer from a 10 day fed batch culture.

The present invention also provides a method of treating or reducing the advancement, severity or effects of neoplasia in a subject (e.g., human) comprising

administering to the subject an effective amount of an antibody of this invention. An effective amount of the composition can be administered in one or more dosages. In another embodiment the present invention provides a method of treating or reducing the advancement, severity or effects of neoplasia in a subject (e.g., human) comprising administering to the subject an effective amount of an antibody of this invention and a cytotoxic factor. The cytotoxic factor may include for instance, TNF-α, TNF-β, IL-1, INF-γ, IL-2. Alternatively, the cytotoxic factor may by a chemotherapeutic drug. The chemotherapeutic drug may include for instance, Adriamycin, 5FU, Vinblastine, Actinomycin D, Etoposide, Cisplatin, Methotrexate and Doxorubicin.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A (captured) and Figure 1B (soluble) shows a graph of cytotoxicity on WiDr cells. mCBE11 (murine) (diamond), huCBE11#2 (humanized anti-LT-β-R antibody comprising version 2 of the light chain (VL#2) and version 2 of the heavy chain(VH#2)) (circle), huCBE11#4 (humanized anti-LT-β-R antibody comprising version 3 of the light chain (VL#3) and version 4 of the heavy chain (VH#4)) (star).

Figure 2A (captured) and Figure 2B (soluble) shows a graph of IL-8 agonism on A375 cells. mCBE11 (diamonds), huCBE11#2 (humanized anti-LT-β-R antibody comprising version 2 of the light chain (VL#2) and version 2 of the heavy chain(VH#2)) (circle), huCBE11#4 (humanized anti-LT-β-R antibody comprising version 3 of the light chain (VL#3) and version 4 of the heavy chain (VH#4)) (stars).

Figure 3 shows a graph of tumor volume versus days of administration. mCBE11 (triangles), hu CBE11#4 (humanized anti-LT-β-R antibody comprising version 3 of the light chain (VL#3) and version 4 of the heavy chain (VH#4)) (circles), no treatment (squares).

Figure 4 shows a graph of percent survival of animals versus days of post tumor injection. mCBE11 (triangles), huCBE11#4 (humanized anti-LT-β-R antibody comprising version 3 of the light chain and version 4 of the heavy chain) (circles), no treatment (squares).

Figure 5 shows a graph of tumor volume versus days post-injection. HuCBE11#4 (humanized anti-LT-β-R antibody comprising version 3 of the light chain

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(VL#3) and version 4 of the heavy chain (VH#4)) (circles) and control with no treatment (squares).

Figure 6 shows a graph of pre-grown tumor volume versus days of post-treatment. Control (square); huCBE11#4 (humanized anti-LT-β-R antibody comprising version 3 of the light chain (VL#3) and version 4 of the heavy chain (VH#4)) at different dosages: 500ug (circles), 100ug (triangles), and 20ug (diamonds); mCBE11 (crosses).

Figure 7 shows a graph of percent survival of animals with pre-grown tumors versus days of post-treatment. Control (square); huCBE11#4 (humanized anti-LT-β-R antibody comprising version 3 of the light chain (VL#3) and version 4 of the heavy chain (VH#4)) at different dosages: 500ug (circles), 100ug (triangles), and 20ug (diamonds); mCBE11 (crosses).

Figure 8 shows a graph of mean fluorescence intensity versus huCBE11#4 concentrations on a log scale.

DETAILED DESCRIPTION

Sequence Identification Numbers

Nucleotide and amino acid sequences referred to in the specification have been given the following sequence identification numbers:

SEQ ID NO:1 – Amino acid sequence of mCBE11 heavy chain variable region.

SEQ ID NO:2 – Amino acid sequence of mCBE11 light chain variable domain.

SEQ ID NO:3 – Nucleic acid sequence of humanized CBE11 light chain variable region (version 1-VL#1).

SEQ ID NO:4 – Amino acid sequence of humanized CBE11 light chain variable region (version 1-VL#1).

SEQ ID NO:5 – Nucleic acid sequence of humanized CBE11 light chain variable region (version 2-VL#2).

SEQ ID NO:6 – Amino acid sequence of humanized CBE11 light chain variable region (version 2-VL#2)

SEQ ID NO:7 – Nucleic acid sequence of humanized CBE11 light chain variable region (version 3-VL#3).

SEQ ID NO:8 – Amino acid sequence of humanized CBE11 light chain variable region (version 3-VL#3)

SEQ ID NO:9 – Nucleic acid sequence of humanized CBE11 heavy chain variable region (version 1-VH#1)

SEQ ID NO:10 – Amino acid sequence of humanized CBE11 light chain variable region (version 1-VH#1)

SEQ ID NO:11 – Nucleic acid sequence of humanized CBE11 heavy chain variable region (version 2-VH#2)

SEQ ID NO:12 – Amino acid sequence of humanized CBE11 light chain variable region (version 2-VH#2)

SEQ ID NO:13 – Nucleic acid sequence of humanized CBE11 heavy chain variable region (version 3-VH#3)

SEQ ID NO:14 – Amino acid sequence of humanized CBE11 light chain variable region (version 3-VH#3)

SEQ ID NO:15 – Nucleic acid sequence of humanized CBE11 heavy chain variable region (version 4-VH#4)

SEQ ID NO:16 – Amino acid sequence of humanized CBE11 light chain variable region (version 4-VH#4)

SEQ ID NO:17 - FR1 primer to introduce a Bsu36I site.

SEQ ID NO:18 - FR2 primer to introduce NciI and HpaII sites.

SEQ ID NO:19 - FR3 primer to introduce Bsu36I and PstI sites.

SEQ ID NO:20 FR2 primer to introduce SmaI site.

SEQ ID NO:21 - FR3 primer to introduce PvuI site.

SEQ ID NO:22 - FR2 primer to introduce SmaI and HhaI sites.

SEQ ID NO:23 - FR3 primer to introduce PvuII and FspI sites.

SEQ ID NO:24 - FR1 primer to introduce HinfI and NsiI sites.

SEQ ID NO:25 - FR2 primer to introduce HaeII and HhaI sites.

SEQ ID NO:26 - FR3 primer to introduce Bsu36I, DdeI and PstI sites.

SEQ ID NO:27 - FR1 primer to introduce EcoRV site.

SEQ ID NO:28 - FR3 primer to introduce RsaI site.

SEQ ID NO:29 - FR1 primer to introduce EcoRV site.

SEQ ID NO:30 - FR2 primer to introduce HindIII site.

SEQ ID NO:31 - FR3 primer to introduce RsaI site.

SEQ ID NO:32 - Full huCBE11 light chain (version 3) including constant domain.

SEQ ID NO:33 - Full huCBE11 heavy chain (version 4) including constant domain.

Definitions

The term humanized antibody, as used herein, refers to herein an antibody derived from a non-human antibody, typically murine, that retains or substantially retains the antigen-binding properties of the parent antibody but which is less immunogenic in humans.

The term complementarity determining region (CDR), as used herein, refers to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site as delineated by Kabat et al (1991).

The term framework region (FR), as used herein, refers to amino acid sequences interposed between CDRs. These portions of the antibody serve to hold the CDRs in appropriate orientation (allows for CDRs to bind antigen).

The term constant region (CR) as used herein, refers to the portion of the antibody molecule which confers effector functions. In the present invention, murine constant regions are substituted by human constant regions. The constant regions of the subject chimeric or humanized antibodies are derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu. Further, heavy chains of various subclasses (such as the IgG subclasses of heavy chains) are responsible for different effector functions and thus, by choosing the desired heavy chain constant region, antibodies with desired effector function can be produced. Preferred constant regions are gamma 1 (IgG1), gamma 3 (IgG3) and gamma 4 (IgG4). More preferred is an Fc region of the gamma 1 (IgG1) isotype. The light chain constant region can be of the kappa or lambda type, preferably of the kappa type.

The term chimeric antibody as used herein refers to an antibody containing sequences derived from two different antibodies, which typically are of different species. Most typically chimeric antibodies comprise human and murine antibody fragments, generally human constant and murine variable region.

The term immunogenicity as used herein refers to a measure of the ability of a targeting protein or therapeutic moiety to elicit an immune response (humoral or cellular) when administered to a recipient. The present invention is concerned with the immunogenicity of the subject humanized antibodies.

Humanized antibody of reduced immunogenicity refers to a humanized antibody exhibiting reduced immunogenicity relative to the parent antibody, e.g., the murine antibody.

Humanized antibody substantially retaining the binding properties of the parent antibody refers to a humanized antibody which retains the ability to specifically bind the antigen recognized by the parent antibody used to produce such humanized antibody. Preferably the humanized antibody will exhibit the same or substantially the same antigen-binding affinity and avidity as the parent antibody. Ideally, the affinity of the antibody will not be less than 10% of the parent antibody affinity, more preferably not less than about 30%, and most preferably the affinity will not be less than 50% of the parent antibody. Methods for assaying antigen-binding affinity are well known in the art and include half-maximal binding assays, competition assays, and Scatchard analysis. Suitable antigen binding assays are described in this application.

The present invention is directed to humanized monoclonal antibodies which bind human LT-β-R and their use as therapeutic agents. The present invention is further directed toward nucleic acid sequences which encode said humanized antibodies, and their expression in recombinant host cells. More specifically, the present invention is directed toward humanized antibodies derived from murine CBE11 which specifically binds to human LT-β-R.

Murine CBE11 (mCBE11) is a murine IgG1, kappa antibody isolated from a mouse immunized with a human LT-β-R-Ig fusion protein (Browning et al., *J. Immunol.* 154: 33 (1995)). mCBE11 functionally activates LT-β-R both *in vitro* and *in vivo* (PCT/US96/01386) and its isolation and anti-tumor properties have been described (Browning et al. *J. Exp. Med.* 183:867 (1996). The hybridoma cell line which produces mCBE11 has been previously deposited with the American Type Culture Collection (ATCC) according to the provisions of the Budapest Treaty by the Applicants of the present invention and was assigned the ATCC accession number HB 11793. (PCT/US96/01386). Applicants have also shown that LT-β receptor cross-linking with various agonist anti-LT-β-R antibodies activate the LT-β receptor (i.e. can mimic the effects of the natural ligands). (PCT/US96/01386) Receptor activation in turn has been shown to inhibit tumor growth in a variety of *in vivo* tumor models for which LT-β receptor is expressed. LT-β receptor has been shown to be expressed on a number of

cancer cells including for example non small cell lung cancer cells (NSCLC), colorectal cancer cells (CRC), breast cancer cells, as well as on prostate, gastric, skin, stomach, esophagus and bladder cancer cells. Non-limiting examples of tumors that the agonist LT-β-R antibodies inhibit include the following solid tumors: HT29 colon adenocarcinoma, HT3 cervical carcinoma, A375 melanoma, MDA-231 breast carcinoma and primary colon tumors. Therefore, agonist LT-β-R antibodies possess properties which render it useful for treatment of diseases wherein LT-β-R activation and/or modulation of the LT-β-R / LT-β-R ligand interaction is desirable including for example the treating or reducing the advancement, severity or effects of neoplasia in a subject (e.g., human).

Humanizing the mCBE11 monoclonal antibody including the modeling analysis and back mutations required to substantially retain the binding properties of the mCBE11 monoclonal antibody is described herein.

Modeling Analysis Of The Mouse Variable Regions

The CDRs contain the residues most likely to bind antigen and must be retained in the reshaped antibody. CDRs are defined by sequence according to Kabat et al., Sequence of Proteins of Immunological Interest, 5th Edition, The United States Department of Health and Human Services, The United States Government Printing Office, 1991. CDRs fall into canonical classes (Chothia et al, 1989 Nature, 342, 877-883) where key residues determine to a large extent the structural conformation of the CDR loop. These residues are almost always retained in the reshaped antibody. The polypeptide sequence of the light chain variable domain of mCBE11 is shown below with the CDR's underlined and the residue position numbers are designated according with the Kabat numbering system:

1 DIKMTQSPSS MYASLGERVT ITCKAGQDIK SYLSWYQQKP
41 WKSPKILIYY ATRLADGVPS RFSGSGSGQD YSLTISSLES
81 DDTATYYCLQ HGESPWTFGG GTKLEIK
(SEQ ID NO:1)

The polypeptide sequence of the heavy chain variable domain of mCBE11 is shown below with the CDR's underlined and the residue position number are designated according with the Kabat numbering system:

1 EVQLVESGGG LVKPGGSLKL SCAASGFTFS DYYMYWFRQT
41 PEKRLEWVAT ISDGGSYTYY PDSVKGRFTI SRDNAKNNLY

81 LQMSSLKSED TAMYYCVREE NGNFYYFDYW GQGTTVTVSS
(SEQ ID NO:2)

The variable light and heavy chains of mCBE11 were compared with the consensus sequences for mouse and human subgroups (Johnson, G., Wu, T. T. Kabat Database and its applications: future directions Nucleic Acid Research, 29, 205-206, 2001; Wu and Kabat, J. Exp. Med. 132:211-250 (1970)) using the program FASTA. The mCBE11 variable light chain is a member of mouse subgroup V with a 74% identity in 110 amino acid overlap and the mCBE11 variable heavy chain is a member of mouse subgroup IIId with a 79% identity in 132 amino acid overlap. The variable light chain corresponds to human subgroup I with a 66% identity in 113 amino acid overlap. The variable heavy chain corresponds to human subgroup III with a 71% identity in 131 amino acid overlap.

The CDRs of the present invention were classified into canonical classes. The L1 loop fell into canonical class 2 (11 residue loop), L2 into class 1 (7 residues) and L3 into class 1 (9 residues). The H1 loop fell into class 1 (5 residues) and the H2 loop into class 3 (17) residues. The H3 loop did not belong to a canonical class.

The residues at the interface between the variable light and heavy chains have been defined (Chothia et al, 1985 J. Mol. Biol., 186, 651-663). These are usually retained in the reshaped antibody. In mCBE11 several of these residues are unusual at the interface, namely S34, I46, L89, H91 in VL and Y35, F37, V93, E95 in VH.

Unusual framework residues were determined by analysing all mouse and human variable chain sequences in the September 1999 version of the Kabat database [NCBI, NIH]. It is believed that mCBE11-specific differences might indicate somatic mutations that enhance binding activity if these differences were close to the binding site. Unusual mouse residues further away from the binding site and unusual human residues were removed in case they would create immunogenic epitopes in the reshaped antibody. Unusual framework residues found in the mCBE11 were K3, M11, Y12, W41, Q69, S72, D81, T83 in the light chain; and F37, T40, E42, A49, N77 in the heavy chain. While most of these residues were not retained in the humanized CBE11 antibodies some of these unusual framework residues were retained including for example F37 and A49 in the heavy chains.

Modeling The Structure Of The Variable Regions

The light and heavy chains of the present invention were aligned against the non-redundant database to determine structural frames to be used to construct three dimensional models of the mCBE11 light and heavy chains. Using BLAST the light chain was found to have 93% sequence identity to monoclonal murine antibody 5g9 (1AHW), and the heavy chain was found to have 81% sequence identity to murine IGGA2 Fab fragment (Fab 17/9) (1IFH). Using the molecular modeling package Sybyl (Tripos Inc.) the three dimensional structures of the light and heavy chains were built using the light chain of 5g9 and the heavy chain of IGGA2 Fab fragment, respectively. The structural integrity of the models was assessed at the console and were found to be reasonable.

Design Of The Reshaped Variable Regions

Homology matching was used to choose human acceptor frameworks to "accept" mCBE11 CDRs. Both the Kabat database and the non-redundant database from NCBI, ENTRZ (The National Institutes of Health) were searched using the software program BLAST. The choice of human acceptor frameworks was made based on sequence identity between mCBE11 frameworks and human frameworks (excluding frameworks from previously humanized antibodies).

The eventual choice of human frameworks was from antibody TNF-A1'CL (kabat ID 004770) against human tumor necrosis factor alpha (Griffiths et al, 1993 EMBO J. 12:725-734) for the variable light (VL) chain (human kappa subgroup I) and antibody FLA-IgG'CL (kabat id 040003) of unknown specificity (Malisan et al, 1996 Blood 87:717-724) for the variable heavy (VH) chain (human subgroup III). The human VL and VH frameworks have 15 and 11 residues differences compared to the murine sequences.

Back Mutations of the human frameworks

The most unpredictable procedure in the humanization of monoclonal antibodies is the identification of critical framework residues from the parent antibody (i.e. in the present case, the parent antibody is of mouse origin) that need to be retained in order to substantially retain the binding properties of the parent antibody while at the same time minimizing the potential immunogenicity of the resultant antibody. It is especially important to retain canonical residues, interface packing residues and unusual murine residues which are close to the binding site. In addition, residues in the 'Vernier Zone' (which forms a platform on which the CDRs rest) (Foote & Winter, 1992 J. Mol.

Biol. 224, 487-499) and those close to CDR H3 are considered. Mutations back to the parent antibody (i.e. back mutating from human framework residues to mouse) are referred to herein as back mutations.

Three versions of the variable light reshaped chain (hu-CBE11 VL) and four versions of the variable heavy reshaped chain (hu-CBE11 VH) have been made. In general, the first version contains the most back mutations and the third version contains the fewest (ie the most "humanized") except for the fourth version of the hu-CBE11 VH. The present invention contemplates humanized antibodies derived from mCBE11 which possess a variable light chain selected from the variable light chains described below (i.e. VL#1, VL#2 or VL#3) and a variable heavy chain selected from the variable heavy chains described below (i.e. VH#1, VH#2, VH#2, or VH#4) in any combination.

(A) Light chain:

3 Q (glutamine)->K (lysine) It is retained in the first version since previous reshaping experiments have shown (e.g. Kolbinger et al, 1993 Prot. Eng., 6, 971-980) it might be important for antigen binding or CDR conformation.

41 G (glycine)->W (tryptophan) It was retained in the first and second versions.

46 L (leucine)->I (isoleucine) It was retained in the first and second versions since it is both an interface residue and in the vernier zone. In addition, it is an unusual reside occurring 9 times in mouse sequences and once in human. It is likely to affect the packing of the variable chains and may contact CDRs.

69 T (threonine)->Q (glutamine) This residue is in the vernier zone and may influence CDR conformation. The change from a short T to a longer Q may also mean that it contacts antigen. The Q is unusual occurring 58 times in mouse and twice in human. It was retained in the first version.

71 F (phenylalanine)->Y (tyrosine) This residue is a canonical position and was retained in the all versions. It is also relatively unusual in human sequences only occurring 25 times.

(B) Heavy Chain

37 V (valine)->F (phenylalanine) It was retained in the first, second and fourth versions. The F at this position is unusual only occurring 15 times in mouse and 18 times in human. It is also an interface residue.

40 A (alanine)->T (threonine) It was retained in the first version. Mutation at this position has been tried in 5 previous humanization experiments although never the change from A to T. One example is the change from A to S in the veneering of BrE-3 (Couto et al, 1994 Hybridoma, 13, 215-219) in which binding affinity was increased, although the reason was never determined. In this case, the heavy chain was also human subgroup III.

49 S (serine)->A (alanine) This residue is under the CDRs and in the vernier zone and was retained in all versions.

89 V (valine)->M (methionine) It was retained the first version. This position has been back-mutated in several humanization experiments. It was retained the first version.

93 A (alanine)->V (valine) This position is both an interface residue and in the vernier zone. It was retained in the first and second versions.

The amino acid and nucleic acid sequences of each of the different versions of the variable light and heavy chains made are as follows:

Reshaped Variable Light Chains

Reshaped variable light chain of CBE11 - version 1 light chain (VL#1) (Plasmid pAND066)

```
   1  GATATTAAGATGACCCAGTCTCCATCATCCTTGTCTGCATCGGTGGGAGACAGGGTCACT 60
      D  I  K  M  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T
            aa3
  61  ATCACTTGCAAGGCGGGTCAGGACATTAAAAGCTATTTAAGCTGGTACCAGCAGAAACCA 120
      I  T  C  K  A  G  Q  D  I  K  S  Y  L  S  W  Y  Q  Q  K  P

 121  TGGAAAGCGCCTAAGATCCTGATCTATTATGCAACAAGGTTGGCAGATGGGGTCCCATCA 180
      W  K  A  P  K  I  L  I  Y  Y  A  T  R  L  A  D  G  V  P  S
     aa41          aa46
 181  AGATTCAGTGGCAGTGGATCTGGGCAAGATTATACTCTAACCATCAGCAGCCTGCAGCCT 240
      R  F  S  G  S  G  S  G  Q  D  Y  T  L  T  I  S  S  L  Q  P
                              aa69  aa71
 241  GAGGATTTCGCAACTTATTACTGTCTACAGCATGGTGAGAGCCCGTGGACGTTCGGTGGA 300
      E  D  F  A  T  Y  Y  C  L  Q  H  G  E  S  P  W  T  F  G  G

 301  GGCACCAAGCTGGAGATCAAA 321
      G  T  K  L  E  I  K
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SEQ ID NO:3-represents the nucleic acid sequence of the reshaped VL#1 above.

SEQ ID NO:4-represents the amino acid sequence of the reshaped VL#1 above.

Reshaped variable light chain of CBE11 - version 2 light chain (VL#2) (Plasmid pAND070)

```
   1  GATATCCAGATGACCCAGTCTCCATCATCCTTGTCTGCATCGGTGGGAGACAGGGTCACT 60
      D  I  Q  M  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T

  61  ATCACTTGCAAGGCGGGTCAGGACATTAAAAGCTATTTAAGCTGGTACCAGCAGAAACCA 120
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        I  T  C  K  A  G  Q  D  I  K  S  Y  L  S  W  Y  Q  Q  K  P

121 TGGAAAGCGCCTAAGATCCTGATCTATTATGCAACAAGGTTGGCAGATGGGGTCCCATCA 180
    W  K  A  P  K  I  L  I  Y  Y  A  T  R  L  A  D  G  V  P  S
aa41                aa46
181 AGATTCAGTGGCAGTGGATCTGGTACAGATTATACTCTAACCATCAGCAGCCTGCAGCCT 240
    R  F  S  G  S  G  S  G  T  D  Y  T  L  T  I  S  S  L  Q  P
                                   aa71
241 GAGGATTTCGCAACTTATTACTGTCTACAGCATGGTGAGAGCCCGTGGACGTTCGGTGGA 300
    E  D  F  A  T  Y  Y  C  L  Q  H  G  E  S  P  W  T  F  G  G

301 GGCACCAAGCTGGAGATCAAA 321
    G  T  K  L  E  I  K
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SEQ ID NO:5-represents the nucleic acid sequence of the reshaped VL#2 above.

SEQ ID NO:6-represents the amino acid sequence of the reshaped VL#2 above.

Reshaped variable light chain of CBE11 - version 3 light chain (VL#3) (Plasmid pAND074)

```
  1 GATATCCAGATGACCCAGTCTCCATCATCCTTGTCTGCATCGGTGGGAGACAGGGTCACT 60
    D  I  Q  M  T  Q  S  P  S  S  L  S  A  S  V  G  D  R  V  T

 61 ATCACTTGCAAGGCGGGTCAGGACATTAAAAGCTATTTAAGCTGGTACCAGCAGAAACCA 120
    I  T  C  K  A  G  Q  D  I  K  S  Y  L  S  W  Y  Q  Q  K  P

121 GGGAAAGCGCCTAAGCCTCTGATCTATTATGCAACAAGGTTGGCAGATGGGGTCCCATCA 180
    G  K  A  P  K  L  L  I  Y  Y  A  T  R  L  A  D  G  V  P  S

181 AGATTCAGTGGCAGTGGATCTGGTACAGATTATACTCTAACCATCAGCAGCCTGCAGCCT 240
    R  F  S  G  S  G  S  G  T  D  Y  T  L  T  I  S  S  L  Q  P
                                   aa71
241 GAGGATTTCGCAACTTATTACTGTCTACAGCATGGTGAGAGCCCGTGGACGTTCGGTGGA 300
    E  D  F  A  T  Y  Y  C  L  Q  H  G  E  S  P  W  T  F  G  G

301 GGCACCAAGCTGGAGATCAAA 321
    G  T  K  L  E  I  K
```

SEQ ID NO:7-represents the nucleic acid sequence of the reshaped VL#3 above.

SEQ ID NO:8-represents the amino acid sequence of the reshaped VL#3 above.

Reshaped Variable Heavy Chains:

Reshaped variable heavy chain of CBE11 - version 1 heavy chain (VH#1) (Plasmid pAND067)

```
  1 GAGGTACAACTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAGGCTC 60
    E  V  Q  L  V  E  S  G  G  G  L  V  K  P  G  G  S  L  R  L

 61 TCCTGTGCAGCCTCTGGATTCACTTTCAGTGACTATTACATGTATTGGTTTCGCCAGACT 120
    S  C  A  A  S  G  F  T  F  S  D  Y  Y  M  Y  W  F  R  Q  T
                                                  aa37    aa40
121 CCGGGAAAGGGGCTGGAGTGGGTCGCAACCATTAGTGATGGTGGTAGTTACACCTACTAT 180
    P  G  K  G  L  E  W  V  A  T  I  S  D  G  G  S  Y  T  Y  Y
                      aa49
181 CCAGACAGTGTGAAGGGGCGATTCACCATCTCCAGAGACAATGCCAAGAACAGCCTCTAC 240
    P  D  S  V  K  G  R  F  T  I  S  R  D  N  A  K  N  S  L  Y

241 CTGCAGATGAGCAGCCTGAGGGCTGAGGACACAGCCATGTATTACTGTGTAAGAGAGGAG 300
    L  Q  M  S  S  L  R  A  E  D  T  A  M  Y  Y  C  V  R  E  E
                                        aa89        aa93
301 AATGGTAACTTTTACTACTTTGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA 360
    N  G  N  F  Y  Y  F  D  Y  W  G  Q  G  T  T  V  T  V  S  S
```

SEQ ID NO:9-represents the nucleic acid sequence of the reshaped VH#1 above.

SEQ ID NO:10-represents the amino acid sequence of the reshaped VH#1 above.

Reshaped variable heavy chain of CBE11 - version 2 heavy chain (VH#2) (Plasmid pAND071)

```
   1  GAGGTACAACTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAGGCTC 60
      E  V  Q  L  V  E  S  G  G  G  L  V  K  P  G  G  S  L  R  L

  61  TCCTGTGCAGCCTCTGGATTCACTTTCAGTGACTATTACATGTATTGGTTTCGCCAGGCC 120
      S  C  A  A  S  G  F  T  F  S  D  Y  Y  M  Y  W  F  R  Q  A
                                                   aa37
 121  CCGGGAAAGGGGCTGGAGTGGGTCGCAACCATTAGTGATGGTGGTAGTTACACCTACTAT 180
      P  G  K  G  L  E  W  V  A  T  I  S  D  G  G  S  Y  T  Y  Y
                        aa49
 181  CCAGACAGTGTGAAGGGGCGATTCACCATCTCCAGAGACAATGCCAAGAACAGCCTCTAC 240
      P  D  S  V  K  G  R  F  T  I  S  R  D  N  A  K  N  S  L  Y

 241  CTGCAGATGAGCAGCCTGAGGGCTGAGGACACAGCTGTGTATTACTGTGTAAGAGAGGAG 300
      L  Q  M  S  S  L  R  A  E  D  T  A  V  Y  Y  C  V  R  E  E
                                                   aa93
 301  AATGGTAACTTTTACTACTTTGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA 360
      N  G  N  F  Y  Y  F  D  Y  W  G  Q  G  T  T  V  T  V  S  S
```

SEQ ID NO:11-represents the nucleic acid sequence of the reshaped VH#2 above.

SEQ ID NO:12-represents the amino acid sequence of the reshaped VH#2 above.

Reshaped variable heavy chain of CBE11 - version 3 heavy chain (VH#3) (Plasmid pAND075)

```
   1  GAGGTACAACTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAGGCTC 60
      E  V  Q  L  V  E  S  G  G  G  L  V  K  P  G  G  S  L  R  L

  61  TCCTGTGCAGCCTCTGGATTCACTTTCAGTGACTATTACATGTATTGGGTGCGCCAGGCC 120
      S  C  A  A  S  G  F  T  F  S  D  Y  Y  M  Y  W  V  R  Q  A

 121  CCGGGAAAGGGGCTGGAGTGGGTCGCAACCATTAGTGATGGTGGTAGTTACACCTACTAT 180
      P  G  K  G  L  E  W  V  A  T  I  S  D  G  G  S  Y  T  Y  Y
                        aa49
 181  CCAGACAGTGTGAAGGGGCGATTCACCATCTCCAGAGACAATGCCAAGAACAGCCTCTAC 240
      P  D  S  V  K  G  R  F  T  I  S  R  D  N  A  K  N  S  L  Y

 241  CTGCAGATGAGCAGCCTGAGGGCTGAGGACACAGCTGTGTATTACTGCGCAAGAGAGGAG 300
      L  Q  M  S  S  L  R  A  E  D  T  A  V  Y  Y  C  A  R  E  E

 301  AATGGTAACTTTTACTACTTTGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA 360
      N  G  N  F  Y  Y  F  D  Y  W  G  Q  G  T  T  V  T  V  S  S
```

SEQ ID NO:13-represents the nucleic acid sequence of the reshaped VH#3 above.

SEQ ID NO:14-represents the amino acid sequence of the reshaped VH#3 above.

Reshaped variable heavy chain of CBE11 - version 4 heavy chain (VH#4) (Plasmid pAND090)

```
   1  GAGGTACAACTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTGAGGCTC 60
      E  V  Q  L  V  E  S  G  G  G  L  V  K  P  G  G  S  L  R  L
```

```
 61  TCCTGTGCAGCCTCTGGATTCACTTTCAGTGACTATTACATGTATTGGTTTCGCCAGGCC 120
     S  C  A  A  S  G  F  T  F  S  D  Y  Y  M  Y  W  F  R  Q  A
                                                   aa37
121  CCGGGAAAGGGGCTGGAGTGGGTCGCAACCATTAGTGATGGTGGTAGTTACACCTACTAT 180
     P  G  K  G  L  E  W  V  A  T  I  S  D  G  G  S  Y  T  Y  Y
                          aa49
181  CCAGACAGTGTGAAGGGGCGATTCACCATCTCCAGAGACAATGCCAAGAACAGCCTCTAC 240
     P  D  S  V  K  G  R  F  T  I  S  R  D  N  A  K  N  S  L  Y

241  CTGCAGATGAGCAGCCTGAGGGCTGAGGACACAGCTGTGTATTACTGCGCAAGAGAGGAG 300
     L  Q  M  S  S  L  R  A  E  D  T  A  V  Y  Y  C  A  R  E  E

301  AATGGTAACTTTTACTACTTTGACTACTGGGGCCAAGGGACCACGGTCACCGTCTCCTCA 360
     N  G  N  F  Y  Y  F  D  Y  W  G  Q  G  T  T  V  T  V  S  S
```

SEQ ID NO:15-represents the nucleic acid sequence of the reshaped VH#4 above.

SEQ ID NO:16-represents the amino acid sequence of the reshaped VH#4 above.

Antibodies consisting of different versions of the light and heavy chains were made and used for further studies. For instance, the antibody consisting of reshaped huCBE11 version 3 light variable chain (VL#3) and reshaped huCBE11 version 4 heavy variable chain (VH#4), named huCBE11#4 or hCBE11, was made and cell lines, E46.4 and E77.4, producing the antibody were deposited with the A.T.C.C. depository (ATCC patent deposit designation PTA-3357 and 3765, respectively).

The invention further contemplates equivalents and variants of the reshaped VH and VL sequences, i.e. those containing one or more conservative amino acid substitution which do not substantially affect LT-β-R binding. Humanized LT-β-R antibodies containing these humanized variable heavy and light sequences may be obtained by recombinant methods as described in the Examples.

Uses

The humanized anti- LT-β-R antibodies of the present invention have use in treating disease conditions wherein LT-β-R activation is therapeutically beneficial. Such conditions include treating, preventing or reducing the advancement, severity or effects of neoplasia.

In one embodiment of the invention is a method of treating a mammal (i.e. human) for a condition associated with undesired cell proliferation by administering to the mammal a therapeutically effective amount of a composition comprising humanized LT-β-R antibodies of the present invention.

In another embodiment of the invention is a method of treating a mammal (i.e. human) having a solid tumor (i.e. a carcinoma) that overexpresses LT-β-R comprising administering to said mammal a humanized LT-β-R antibody that binds to LT-β-R in

an amount effective to reduce the tumor volume. Examples of cancers whose cell proliferation is modulated by LT-β-R may be screened by measuring in vitro the level of LT-β-R and/or LT-β-R ligand (ie LTα1β2 or LIGHT) message expressed in tumor tissue libraries. Tumor tissue libraries in which of LT-β-R and/or LT-β-R ligand (ie LTα1β2 or LIGHT) message is highly expressed would be candidates. Tumor types contemplated in the present invention include solid tumors including but not limited to non small cell lung cancer (NSCLC), colorectal cancer (CRC), breast cancer, as well as on prostate, gastric, skin, stomach, esophagus and bladder cancer.

The humanized antibodies of the subject invention which are used in treating conditions associated with undesired cell proliferation, in particular tumor therapy, advantageously inhibit tumor cell growth, as measured for example by a decrease in the tumor volume, greater than about 10%, 20%, 30% or 40% and most advantageously greater than about 50%. The humanized antibodies are obtained through screening (see, for example, the discussion in Example 3). For example, humanized antibodies for use in the present invention can be selected on the basis of decreased tumor volume versus untreated cancer cells (e.g., greater than about 10%, 20%, 30%, 40% or 50%).

The present invention also provides pharmaceutical compositions comprising a humanized antibody of the present invention and a pharmaceutically acceptable excipient. Suitable carriers, for instance, and their formulations, are described in Remington' Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Co., edited by Oslo et al. Typically an appropriate amount of a pharmaceutically acceptable salt is used in the formulation to render the formulation isotonic. Examples of the carrier include buffers such as saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7.4 to about 7.8. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers, which matrices are in the form of shaped articles, e.g. liposomes, films or microparticles. It will be apparent to those of skill in the art that certain carriers may be more preferable depending upon for instance the route of administration and concentration of the pharmaceutical composition being administered.

Administration may be accomplished by injection (eg intravenous, intraperitoneal, subcutaneous, intramuscular) or by other methods such as infusion that ensure delivery to the bloodstream in an effective form.

The humanized antibodies of the present invention can be administered at an effective dose to treat the particular clinical condition addressed. Determination of a preferred pharmaceutical formulation and a therapeutically efficient dose regiment for a given application is well within the skill of the art taking into consideration, for example, the weight and condition of the patient, the extent of the desired treatment and the tolerance of the patient for the treatment. For example, an effective dosage will be in the range of about 0.05 to about 100 milligrams per kilogram of body weight per day. More particularly, about 0.05mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1mg, 2 mg, 3 mg, 4mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 15 mg, 20 mg, or 25 mg, per kilogram body weight per day. Alternatively about 0.05 to about 100 milligrams, more particularly, about 0.05mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1mg, 2 mg, 3 mg, 4mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 15 mg, 20 mg, or 25 mg, per kilogram body weight per week. Alternatively about 0.05 to about 100 milligrams, more particularly, about 0.05mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1mg, 2 mg, 3 mg, 4mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 15 mg, 20 mg, or 25 mg, per kilogram body weight per two weeks. Alternatively about 0.05 to about 100 milligrams, more particularly, about 0.05mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1mg, 2 mg, 3 mg, 4mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 15 mg, 20 mg, or 25 mg, per kilogram body weight per three weeks. Alternatively about 0.05 to about 100 milligrams, more particularly, about 0.05mg, 0.1 mg, 0.2 mg, 0.3 mg, 0.4mg, 0.5 mg, 0.6 mg, 0.7 mg, 0.8 mg, 0.9 mg, 1mg, 2 mg, 3 mg, 4mg, 5 mg, 6 mg, 7 mg, 8 mg, 9 mg, 10 mg, 15 mg, 20 mg, or 25 mg, per kilogram body weight per four weeks.

Practice of the present invention will employ, unless indicated otherwise, conventional techniques of cell biology, cell culture, molecular biology, microbiology, recombinant DNA, protein chemistry, and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, **Molecular Cloning: A Laboratory Manual,** 2nd edition. (Sambrook, Fritsch and Maniatis, eds.), Cold Spring Harbor Laboratory Press, 1989; **DNA Cloning**, Volumes I and II (D.N. Glover, ed), 1985; **Oligonucleotide Synthesis**, (M.J. Gait, ed.), 1984; U.S. Patent No.

4,683,195 (Mullis et al.,); **Nucleic Acid Hybridization** (B.D. Hames and S.J. Higgins, eds.), 1984; Transcription and Translation (B.D. Hames and S.J. Higgins, eds.), 1984; **Culture of Animal Cells** (R.I. Freshney, ed). Alan R. Liss, Inc., 1987; **Immobilized Cells and Enzymes,** IRL Press, 1986; **A Practical Guide to Molecular Cloning** (B. Perbal), 1984; **Methods in Enzymology,** Volumes 154 and 155 (Wu et al., eds), Academic Press, New York; **Gene Transfer Vectors for Mammalian Cells** (J.H. Miller and M.P. Calos, eds.), 1987, Cold Spring Harbor Laboratory; **Immunochemical Methods in Cell and Molecular Biology** (Mayer and Walker, eds.), Academic Press, London, 1987; **Handbook of Experiment Immunology,** Volumes I-IV (D.M. Weir and C.C. Blackwell, eds.), 1986; **Manipulating the Mouse Embryo,** Cold Spring Harbor Laboratory Press, 1986.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof.

EXAMPLES:

Example 1

Construction And Expression Of chCBE11

cDNAs encoding the murine CBE11 variable regions of the heavy and light chains were used to construct vectors for expression of murine-human chimeras (chCBE11) in which the muCBE11 variable regions were linked to human IgG1 and kappa constant regions. For construction of the heavy chain chimera, a 0.36 kb PstI-BstEII fragment from the CBE11 heavy chain subclone pEAG970 was subcloned into the phosphatased 2.82 kb PstI-BstEII vector fragment from the 5a8 heavy chain plasmid pLCB7 (5a8 is a molecularly cloned CD4-specific mAb previously characterized at Biogen), to add a murine heavy chain signal sequence and splice donor site to the muCBE11 heavy chain variable region. In this plasmid, called pEAG979, the heavy chain mature N-terminus differs by two residues from the N-terminal sequence of purified authentic CBE11 heavy chain derived from Edman degradation, since it was primer-determined during PCR. To correct the heavy chain N-terminus, pEAG979 was subjected to unique site elimination (USE) mutagenesis using an Amersham Pharmacia Biotech USE mutagenesis kit following the manufacturer's recommended protocol. Mutated plasmids were identified by screening for introduced AvaII, PstI, and RsaI changes. The heavy chain sequence in the resultant plasmid pEAG981 was

confirmed by DNA sequencing. The 0.44 kb NotI-HindIII heavy chain variable domain fragment from pEAG981 and the 1.21 kb HindIII-NotI fragment from the plasmid pEAG964, containing a human IgG1 constant region, were subcloned into the NotI site of the pCEP4 (Invitrogen) EBV expression vector-derived plasmid pCH269, producing plasmid pEAG983.

For construction of the light chain chimera, a 0.11 kb NotI-EcoRV fragment from the plasmid pMDR985 and a 0.37 kb EcoRV-BamHI fragment from the CBE11 light chain variable domain plasmid pEAG967 were subcloned into the phosphatased 2.94 kb NotI-BamHI vector fragment from Stratagene's pBluescriptIISK+ cloning vector, to add a murine light chain signal sequence and a 5' NotI site in the resulting plasmid pEAG978. This plasmid was subjected to USE mutagenesis using an Amersham Pharmacia Biotech USE mutagenesis kit following the manufacturer's recommended protocol, with mutagenic primers which encoded a V3K substitution to match the authentic CBE11 light chain N-terminus, and which introduced a BglII site at the 3' end of the light chain variable domain. Mutated plasmids were identified by screening for introduced BglII, EcoRV, and MseI site changes. The light chain sequence in the resultant plasmid pEAG980 was confirmed by DNA sequencing. The 0.41 kb NotI-BglII light chain variable domain fragment from pEAG980 and the 0.68 kb BclI-NotI fragment from the plasmid pEAG963, containing a human kappa light chain constant domain, were subcloned into the NotI site of the pCEP4 (Invitrogen) EBV expression vector-derived plasmid pCH269, producing plasmid pEAG982.

Expression vectors (chCBE11 heavy chain vector pEAG983 and chCBE11 light chain vector pEAG982) were co-transfected into 293-EBNA cells and transfected cells were tested for antibody secretion and specificity (empty vector- and ch5c8 (a molecularly cloned CD154-specific mAb previously characterized at Biogen)-transfected cells served as controls). Western blot analysis (developed with anti-human heavy and light chain antibodies) of protein A immunoprecipitates from whole cell lysates and conditioned medium indicated that chCBE11-transfected cells synthesized and efficiently secreted heavy and light chains at levels similar to ch5c8-transfected cells. FACS analysis of LT-β-R -expressing HT-29 cells stained with conditioned medium from transfected cells indicated that the chCBE11 antibody bound and produced staining patterns similar to those of muCBE11, while conditioned medium

from mock- and ch5c8-transfected cells failed to stain LT-β-R on HT-29 cells. Chimeric CBE11 produced from transient transfection was purified and demonstrated to induce IL-8 secretion by LT-β-R -expressing A375 melanoma cells and to inhibit growth of WiDRr adenocarcinoma cells in nude mice.

Example 2

Construction And Expression Of huCBE11

Design of the reshaped variable domains to produce humanized CBE11 (huCBE11) was carried out as described supra. The choice of the human acceptor frameworks was by homology matching: human kappa subgroup I mAb TNF-A1 for the light chain (Griffiths et al., 1993), and human subgroup III mAb FLA-IgG for the heavy chain (Malisan et al., 1996). Three versions of each of the variable light and four versions of the variable heavy reshaped chains were designed. In general the first version contains the most back mutations to the murine donor sequences, while the last version contains the fewest (i.e., the most "humanized").

The huCBE11 variable regions were made by unique site elimination (USE) mutagenesis using an Amersham Pharmacia Biotech USE mutagenesis kit following the manufacturer's recommended protocol, using the chCBE11 variable domain plasmids as starting templates. The mutagenic primers for the framework (FR) changes are described below. The cDNA sequence of the human acceptor frameworks (Kabat database #004770 for the light chain and Kabat #040003 for the heavy chain) were used, with silent mutations introduced to produce restriction site changes to facilitate identification of mutated plasmids. Mutated plasmids were identified by identified by screening for the introduced restriction site changes. The variable region cDNA sequences in the resultant plasmids were confirmed by DNA sequencing.

VH#1 used pEAG981 template with the following primers: FR1 primer 5' GCC TGG AGG GTC CCT GAG GCT CTC CTG TGC AGC CTC 3' (SEQ ID NO:17), which introduced a Bsu36I site; FR2 primer 5' GTT TCG CCA GAC TCC GGG AAA GGG GCT GGA GTG GGT CGC AAC 3' (SEQ ID NO:18), which introduced NciI and HpaII sites; and FR3 primer 5' CAG AGA CAA TGC CAA GAA CAG CCT CTA CCT GCA GAT GAG CAG CCT GAG GGC TGA GGA CAC AGC CAT G 3' (SEQ ID NO:19), which introduced Bsu36I and PstI sites and removes an RsaI site. The resultant VH#1 plasmid was designated pAND067.

VH#2 used pAND067 template with the following primers: FR2 primer 5' CAT GTA TTG GTT TCG CCA GGC CCC GGG AAA GGG GCT GG 3' (SEQ ID NO:20), which introduced a SmaI site; and FR3 primer 5' GGG CTG AGG ACA CAG CTG TGT ATT ACT GTG TAA GAG 3' (SEQ ID NO:21), which introduced a PvuII site. The resultant VH#2 plasmid was designated pAND071.

VH#3 used plasmid pAND067 template with the following primers: FR2 primer 5' GTG ACT ATT ACA TGT ATT GGG TGC GCC AGG CCC CGG GAA AGG GGC TGG AG 3' (SEQ ID NO:22), which introduced SmaI and HhaI sites; and FR3 primer 5' GAG GGC TGA GGA CAC AGC TGT GTA TTA CTG CGC AAG AGA GGA GAA TGG TAA C 3' (SEQ ID NO:23), which introduced PvuII and FspI sites. The resultant VH#3 plasmid was designated pAND075.

Expression vectors for the huCBE11 heavy chains were made by subcloning the 0.44 kb NotI-HindIII heavy chain variable domain fragments from pAND067, pAND071, or pAND075, and the 1.21 kb HindIII-NotI fragment from the plasmid pEAG964, containing a human IgG1 constant region, were subcloned into the NotI site of the pCEP4 EBV expression vector-derived plasmid pCH269, producing heavy chain expression vectors pAND069 (VH#1), pAND073 (VH#2), and pAND077 (VH#3).

VL#1 used plasmid pEAG980 template with the following primers: FR1 primer 5' CTT GCA AGT GAT AGT GAC CCT GTC TCC CAC CGA TGC AGA CAA GGA TGA TGG AGA CTG GGT CAT C 3' (SEQ ID NO:24), which removed HinfI and NsiI sites; FR2 primer 5' CAT AAT AGA TCA GGA TCT TAG GCG CTT TCC ATG GTT TCT GCT G 3' (SEQ ID NO:25), which introduced HaeII and HhaI sites; and FR3 primer 5' GTA GAC AGT AAT AAG TTG CGA AAT CCT CAG GCT GCA GGC TGC TGA TGG TTA GAG TAT AAT CTT GCC CAG ATC 3' (SEQ ID NO:26), which introduced Bsu36I, DdeI, and PstI sites. The resultant VL#1 plasmid was designated pAND066.

VL#2 used plasmid pAND066 template with the following primers: FR1 primer 5' GAT GGA GAC TGG GTC ATC TGG ATA TCA CCT CTG GCA CCT G 3' (SEQ ID NO:27), which introduced an EcoRV site; and FR3 primer 5' GAT GGT TAG AGT ATA ATC TGT ACC AGA TCC ACT GCC ACT G 3' (SEQ ID NO:28), which introduced an RsaI site. The resultant VL#2 plasmid was designated pAND070.

VL#3 used plasmid pAND066 template with the following primers: FR1 primer 5' GAT GGA GAC TGG GTC ATC TGG ATA TCA CCT CTG GCA CCT G 3' (SEQ

ID NO:29), which introduced an EcoRV site; FR2 primer 5' CAA CCT TGT TGC ATA ATA GAT CAG AAG CTT AGG CGC TTT CCC TGG TTT CTG CTG GTA CC 3' (SEQ ID NO:30), which introduced a HindIII site and removed NcoI and StyI sites; and FR3 primer 5' GAT GGT TAG AGT ATA ATC TGT ACC AGA TCC ACT GCC ACT G 3' (SEQ ID NO:31), which introduced an RsaI site. The resultant VL#3 plasmid was designated pAND074.

Expression vectors for the huCBE11 light chains were made by subcloning the 0.41 kb NotI-BglII light chain variable domain fragments from pAND066, pAND070, or pAND074 and the 0.68 kb BclI-NotI fragment from the plasmid pEAG963, containing a human kappa light chain constant domain, were subcloned into the NotI site of the pCEP4 EBV expression vector-derived plasmid pCH269, producing light chain expression vectors pAND068 (VL#1), pAND072 (VL#2), and pAND076 (VL#3).

Expression vectors were co-transfected into 293-EBNA cells and transfected cells were tested for antibody secretion and specificity (empty vector-transfected cells served as negative control). Western blot analysis (developed with anti-human heavy and light chain antibodies) of protein A immunoprecipitates from whole cell lysates and conditioned medium indicated that huCBE11-transfected cells synthesized and efficiently secreted heavy and light chains at levels similar to chCBE11-transfected cells. FACS analysis of LT-β-R -expressing HT-29 cells stained with conditioned medium from transfected cells indicated that the huCBE11#3 mAb bound less well than huCBE11#1 and huCBE11#2 mAbs relative to chCBE11 (Table 1 below) where huCBE11#1 (VL#1 with VH#1); huCBE#2 (VL#2 with VH#2) and huCBE11#3 (VL#3 with VH#3). Mix and match co-transfections suggested that the reduction could be attributed to the VH#3, which differed from VH#2 at two framework residues: FR2 F37V and FR3 V93A. To examine the individual contributions of each of these changes, new heavy chain expression vectors were constructed. Plasmid pAND089, the F37V variant of VH#2, was made by subcloning the 311 bp NotI-PstI fragment from pAND075, the 126 bp PstI-HindIII fragment from pAND071, and the 1.21 kb HindIII-NotI fragment from the plasmid pEAG964 into the NotI site of the pCEP4 EBV expression vector-derived plasmid pCH269. Plasmid pAND090, the V93A variant of VH#2, was made by subcloning the 311 bp NotI-PstI fragment from pAND071, the 126 bp PstI-HindIII fragment from pAND075, and the 1.21 kb HindIII-NotI fragment from the plasmid pEAG964 into the NotI site of the pCEP4 EBV expression vector-derived

plasmid pCH269. These H2/H3 chimeric heavy chains were co-transfected into 293-EBNA cells with VL#2 or VL#3. FACS analysis indicated that the V93A H2 variant restored LT-β-R binding when paired with the VL#3 (Table 1 supra). The pAND076 and pAND090 pairing was designated huCBE11#4 (Table 1 supra).

Co-transfections of 293-EBNA cells with chCBE11 and huCBE11 versions #1-4 were scaled up and conditioned medium was harvested. Antibody was purified on Protein A-Sepharose. Purified mAbs were assayed for activity.

Table 1. FACS staining of HT-29 cells by chCBE11 and huCBE11

	Light chain	Heavy chain	Relative MFI
ChCBE11	pEAG982	pEAG983	1.00
HuCBE11#1	pAND068	pAND069	1.00
HuCBE11#2	pAND072	pAND073	1.00
HuCBE11#3	pAND076	pAND077	0.62
L2/H3	pAND072	pAND077	0.42
L3/H2	pAND076	pAND073	1.00
L2/F37V H2	pAND072	pAND089	0.65
L2/V93A H2	pAND072	pAND090	0.75
L3/F37V H2	pAND076	pAND089	0.80
HuCBE11#4	pAND076	pAND090	1.00

Conditioned medium from transiently transfected cells was used to stain HT-29 cells by incubating for 30 min on ice, washing cells twice with FACS buffer (PBS with 5% FBS and 0.05% sodium azide), staining with PE-conjugated anti-human IgG (H + L), Jackson ImmunoResearch Laboratories, Inc., for 30 min on ice in FACS buffer, washing cells twice with FACS buffer, and resuspending in FACS buffer for analysis. Relative MFI refers to mean MFI normalized to that observed for chCBE11. Data shown represents the average from two independent transfections.

Example 3

IL-8 Agonism on A375 cells

Purified mAbs were assayed for activity. Results of an IL-8 release assay on A375 human melanoma cells are shown in Figure 1A (captured) and Figure 1B (soluble) which measures the amount of IL-8 released upon the binding of anti-LT-β-R antibodies with an LT-β-R expressed on the surface of A375 human melanoma cells.

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A375 cells were plated at 10^5/ml into 96 well plates containing either soluble antibodies or antibodies captured onto goat anti-human

IgG Fc (Jackson ImmunoResearch Laboratories)-coated wells. The culture plates were incubated overnight. Conditioned medium was harvested and analyzed for IL-8 by ELISA.

Example 4

Cytotoxicity on WiDr cells

Results of a cytotoxicity assay using WiDr colon cancer cells with soluble anti-LT-β-R antibodies onto anti-human IgG Fc coated wells which demonstrate that anti-LT-β-R antibodies increases cytotoxicity in cancer cells as shown in Figure 2A (captured) and Figure 2B (soluble). WiDr cells were plated at $6x10^4$/ml in the presence of 80 units/ml huIFN-gamma into 96 well plates containing either soluble antibodies or antibodies captured onto goat anti-human IgG Fc (Jackson ImmunoResearch Laboratories)-coated wells. The culture plates were incubated for 5 days. MTT was added for 4 hrs and the resulting precipitate was dissolved by overnight incubation with 10% SDS in 10 mM HCl, and O.D.s were read on a microplate reader.

Example 5

The antibody consisting of reshaped huCBE11 version 3 light variable chain (VL#3) and reshaped huCBE11 version 4 heavy variable chain, named huCBE11#4 or hCBE11, was made and the cell line producing the antibody was deposited with the A.T.C.C. depository (ATCC patent deposit designation PTA-3357). The full polypeptide sequences of each of the light and heavy chains, including the constant domains are as follows:

Sequence of mature huCBE11 version 3 light chain (SEQ ID NO: 32):

1 DIQMTQSPSS LSASVGDRVT ITCKAGQDIK SYLSWYQQKP GKAPKLLIYY

51 ATRLADGVPS RFSGSGSGTD YTLTISSLQP EDFATYYCLQ HGESPWTFGG

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151 DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG

201 LSSPVTKSFN RGEC]

CDRs are underlined; back mutation F71Y is bolded; constant domain is bracketed.

Sequence of mature huCBE11 version 4 heavy chain (SEQ ID NO: 33):

V37F S49A

1 EVQLVESGGG LVKPGGSLRL SCAASGFTFS DYYMYWFRQA PGKGLEWVAT

51 ISDGGSYTYY PDSVKGRFTI SRDNAKNSLY LQMSSLRAED TAVYYCAREE

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351 VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV

401 LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG]

CDRs are underlined; back mutations V37F and S49A are bolded; constant domain is bracketed.

6 week old nude mice were injected intraperitoneally with 100 ug of anti-LFA3 antibody (1E6), 100 ug anti-LTBR antibody (huCBE11#4) or not injected (control). The animals were then injected subcutaneously with $1x10^6$ WIDR colon adenocarcinoma cells. The huCBE11#4-treated mice were retreated weekly with 100 ug of antibody and the mCBE11 animals were retreated on day 14 only. Tumor size was measured weekly and the volume of the tumor sphere calculated (**Figure 3**). Animals were sacrificed when their tumors reached a volume of 2.0 cm^3 (16 mm diameter) and their death was noted on the survival chart (**Figure 4**).

Example 6

6 week old nude mice were either injected intraperitoneally with 100 ug anti-LTBR antibody (huCBE11#4) or were not injected (control). All animals were then injected subcutaneously with $1x10^6$ WIDR colon adenocarcinama cells. The huCBE11#4-treated mice were retreated weekly with 100 ug of huCBE11#4. Tumor size was measured weekly and the volume of the tumor sphere calculated. Tumor volumes shown represent the average of 10 control animals and 8 huCBE11#4-treated animals (**Figure 5**). Weekly treatment with huCBE11#4 significantly inhibits the growth rate of WIDR tumors implanted subcutaneously in nude mice. Animals treated with antibody through day 21 continue to show reduced tumor growth rates two week following the cessation of treatment.

Example 7

huCBE11#4 Slows Growth of Pregrown WIDR Tumors and Increases Survival in WIDR Tumor-bearing Nude Mice

10^6 WIDR cells were pregrown subcutaneously for 10 days in Nude mice. The mice received subcutaneous injections of either PBS or huCBE11#4 weekly or mCBE11 alternate weeks. Tumor weights were calculated from width and length measurements and animals with tumors over 2000 mg were sacrificed, their tumor weights at time of sacrifice continued into the statistical averaging. Error bars represent standard error. Tumor weights were calculated using the formula: (Width x Width x Length)/2= tumor weight in mg. The results are graphed in **Figure 6** and show that huCBE11#4 is able to slow pre-grown tumors in vivo.

In addition, tumors were grown and treated as described above and percent survival of the animals was measured. The results are graphed in **Figure 7** and show that huCBE11#4 is able to induce prolonged survival in vivo in mice with pregrown tumors.

Example 8

Antibody affinity measurement

HT-29 cells were grown in DMEM supplemented with L-glutamine, non-essential amino acids, sodium pyruvate and 10% fetal bovine serum. Cells were washed once with PBS and removed from the plate by incubating at room temperature for five minutes with PBS plus 20 mM EDTA. Cells were centrifuged at 1000 rpm (110 x g) for five minutes and resuspended to a density of 1 x 10^7 cells/mL in PBS.

HuCBE11#4 anti-LTβR antibody and humanized anti-CD40L as a negative control were diluted in PBS and a 12 point serial 1:4 dilution was made to a final concentration range of 2.37 pM – 10 μM. 100 μL cell suspension and 100 μL antibody dilution were added together to each well of a 96 well V-bottom microtiter plate. The antibody and the cells were incubated at 4°C for 2 hours. The plate was centrifuged at 1000 rpm (110 x g) for 10 minutes at 4°C. The supernatant was discarded and the cell pellet was washed with cold PBS six times.

Goat-anti human IgG-phycoerythrin conjugate (Jackson Immunoresearch) was diluted 1:100 in PBS and 200 μL was added to each well. The cells were incubated with this secondary antibody for one hour at 4°C, centrifuged as described above, and washed once in cold PBS. The cells were then transferred to polystyrene test tubes.

Fluorescence intensity was measured on a FACS Calibur instrument (Beckton Dickinson).

The mean fluorescence intensity values of the staining for anti-CD40L non-specific binding control were plotted against the antibody concentration in Delta Graph. The values were fit to a straight line and the theoretical non-specific binding values for each antibody concentration were subtracted from each data point for the huCBE11#4 dilution series.

These specific fluorescence intensity values were then plotted against huCBE11#4 concentrations on a log scale. The resulting curve is bell shaped and symmetrical, and reflects self-inhibition of the antibody binding at high concentrations. The left half of this curve was fit to a four parameter equation to find the functional affinity of the antibody. The resulting curve fit gives an EC_{50} value of 60 pM for the huCBE11#4 binding to HT-29 cells.

It will be apparent to those skilled in the art that various modifications and variations can be made in the polypeptides, compositions and methods of the invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided that they come within the scope of the appended claims and their equivalents.

What is claimed is:

1. A humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody whose light chain complementary determining regions are defined by amino acid residues 24 to 34, 50 to 56 and 89 to 97 of SEQ ID NO:1, and whose heavy chain complementary determining regions are defined by amino acid residues 31 to 35, 50 to 66 and 99 to 109 of SEQ ID NO: 2 and wherein the antibody comprises at least one of the following residues in its light chain: K3, W41, I46, Q69 and Y71; or at least one of the following residues in its heavy chain: F37, T40, A49, M89 and V93 (Kabat numbering convention).

2. A humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody whose light chain complementary determining regions are defined by amino acid residues 24 to 34, 50 to 56 and 89 to 97 of SEQ ID NO:1, and whose heavy chain complementary determining regions are defined by amino acid residues 31 to 35, 50 to 66 and 99 to 109 of SEQ ID NO:2 and wherein the antibody comprises residue Y71 in its light chain (Kabat numbering convention).

3. A humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody whose light chain complementary determining regions are defined by amino acid residues 24 to 34, 50 to 56 and 89 to 97 of SEQ ID NO:1, and whose heavy chain complementary determining regions are defined by amino acid residues 31 to 35, 50 to 66 and 99 to 109 of SEQ ID NO:2 and wherein the antibody comprises residues F37 and A49 in its heavy chain (Kabat numbering convention).

4. The antibody of claim 1, wherein the antibody comprises a light chain variable domain sequence defined by amino acid residues 1 to 107 of SEQ ID NO:8.

5. The antibody of claim 1, wherein the antibody comprises a heavy chain variable domain sequence defined by amino acid residues 1 to 120 of SEQ ID NO:16.

6. The antibody of claim 4, wherein the antibody further comprises a heavy chain variable domain sequence defined by amino acid residues 1 to 120 of SEQ ID NO:16

7. An antibody comprising the same heavy and light chain polypeptide sequences as an antibody produced by cell line E46.4 (ATCC patent deposit designation PTA-3357) or cell line E77.4 (ATCC patent deposit designation 3765).

8. The antibody according to anyone of claims 1-7 wherein the antibody substantially retains the binding properties of the parent antibody.

9. The antibody according to anyone of claims 1-7 wherein the antibody is further linked to an immunotoxin.

10. The antibody according to anyone of claims 1-7 wherein the antibody is further linked to a chemotherapeutic drug.

11. A composition comprising an antibody of any one of claims 1-7 and a pharmaceutically acceptable carrier.

12. A use of the composition of claim 11 for the preparation of a medicament for treating or reducing the advancement, severity or effects of neoplasia in a human.

13. An isolated nucleic acid comprising a coding sequence for the light chain of an antibody produced by cell line E46.4 (ATCC patent deposit designation PTA-3357) or cell line E77.4 (ATCC patent deposit designation 3765).

14. An isolated nucleic acid comprising a coding sequence for the heavy chain of an antibody produced by cell line E46.4 (ATCC patent deposit designation PTA-3357) or cell line E77.4 (ATCC patent deposit designation 3765).

15. An isolated nucleic acid comprising a coding sequence for residues 1 to 107 of SEQ ID NO:8.

16. An isolated nucleic acid comprising a coding sequence for residues 1 to 120 of SEQ ID NO:16.

17. A cell of cell line E46.4 (ATCC patent deposit designation PTA-3357) or cell line E77.4 (ATCC patent deposit designation 3765).

18. A humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody, or an antigen binding fragment thereof, comprising

a) a light chain comprising complementary determining regions (CDRs) as set forth in SEQ ID NO:1,

b) a heavy chain comprising CDRs as set forth in SEQ ID NO: 2, and

c) a variable framework region from a human acceptor framework comprising a residue in the light chain selected from the group consisting of: K3, W41, I46, Q69 and Y71; or a residue in the heavy chain selected from the group consisting of: F37, T40, A49, M89 and V93 (Kabat numbering convention).

19. A humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody, or an antigen binding fragment thereof, comprising

a) a light chain comprising complementary determining regions (CDRs) as set forth in SEQ ID NO:1,

b) a heavy chain comprising CDRs as set forth in SEQ ID NO:2, and

c) a variable framework region from a human acceptor framework comprising Y71 in the light chain (Kabat numbering convention).

20. A humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody, or an antigen binding fragment thereof, comprising

a) a light chain comprising complementary determining regions (CDRs) as set forth in SEQ ID NO:1,

b) a heavy chain comprising CDRs as set forth in SEQ ID NO:2, and

c) a variable framework region from a human acceptor framework comprising F37 and A49 in the heavy chain (Kabat numbering convention).

21. A humanized antibody light chain comprising variable complementary determining regions (CDRs) as set forth in SEQ ID NO:1, and a variable human acceptor

framework region comprising a residue selected from the group consisting of: K3, W41, I46, Q69 and Y71 (Kabat numbering convention).

22. A humanized antibody heavy chain comprising variable complementary determining regions (CDRs) as set forth in SEQ ID NO:2, and a variable human acceptor framework region comprising a residue selected from the group consisting of: F37, T40, A49, M89 and V93 (Kabat numbering convention).

23. A humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody, or antigen binding fragment thereof, comprising

a) a light chain comprising complementary determining regions (CDRs) as set forth in SEQ ID NO:1,

b) a heavy chain comprising CDRs as set forth in SEQ ID NO:2,

c) a variable framework region from a human acceptor framework comprising Y71 in the light chain (Kabat numbering convention), or a conservative amino acid substitution thereof ; and

d) a variable framework region from a human acceptor framework comprising A49 in the heavy chain (Kabat numbering convention), or a conservative amino acid substitution thereof.

24. The humanized antibody, or antigen binding fragment thereof, of claim 23, wherein the framework region further comprises W41 and I46 in the light chain (Kabat numbering convention), or a conservative amino acid substitution thereof.

25. The humanized antibody, or antigen binding fragment thereof, of claim 23 or 24, wherein the framework region further comprises K3 and Q69 in the light chain (Kabat numbering convention), or a conservative amino acid substitution thereof.

26. The humanized antibody, or antigen binding fragment thereof, of any one of claims 23 to 25, wherein the framework region further comprises F37 in the heavy chain (Kabat numbering convention), or a conservative amino acid substitution thereof.

27. The humanized antibody, or antigen binding fragment thereof, of any one of claims 23 to 26, wherein the framework region further comprises V93 in the heavy chain (Kabat numbering convention), or a conservative amino acid substitution thereof.

28. The humanized antibody, or antigen binding fragment thereof, of any one of claims 23 to 27, wherein the framework region further comprises T40 and M89 in the heavy chain (Kabat numbering convention), or a conservative amino acid substitution thereof.

29. A humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody, or antigen binding fragment thereof, comprising an antibody light chain comprising the amino acid sequence set forth in SEQ ID NO: 32 and an antibody heavy chain set comprising the amino acid sequence set forth in SEQ ID NO: 33.

30. The humanized antibody, or antigen binding fragment thereof, of any one of the preceding claims further linked to a chemotherapeutic drug.

31. The humanized antibody of claim 30, wherein the chemotherapeutic drug is selected from the group consisting of adriamycin, 5FU, vinblastine, actinomycin D, etoposide, cisplatin, methotrexate, and doxorubicin.

32. The humanized antibody of any one of the preceding claims further linked to an immunotoxin.

33. The humanized antibody, or antigen binding fragment thereof, of any one of the preceding claims , wherein the antibody is an IgG1.

34. The humanized antibody, or antigen binding fragment thereof, of any one of the preceding claims, wherein the antigen binding fragment is selected from the group consisting of a Fab fragment, an $F(ab')_2$, and an Fv fragment.

35. A pharmaceutical composition comprising a therapeutically effective amount of the humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody, or antigen binding fragment thereof, of any one of the preceding claims.

36. Use of the humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody, or antigen binding fragment thereof, of any one of the preceding claims, in the preparation of a pharmaceutical composition for treating or reducing the severity or effects of neoplasia in a human.

37. Use of the humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody, or antigen binding fragment thereof, of any one of the preceding claims in the preparation of a pharmaceutical composition for reducing tumor volume in a human.

38. Use of the humanized anti-lymphotoxin-beta receptor (LT-β-R) antibody, or antigen binding fragment thereof, of any one of the preceding claim, in the preparation of a pharmaceutical composition for treating a solid tumor in a human.

39. The use of claim 37 or 38, wherein the tumor is selected from the group consisting of non small cell lung cancer (NSCLC), colorectal cancer (CRC), prostate cancer, gastric cancer, skin cancer, stomach cancer, esophagus cancer, bladder cancer, colon adenocarcinoma, cervical carcinoma, melanoma, breast carcinoma, and primary colon tumors.

40 The use of any one of claims 37 to 39, wherein the composition is designed to be administered in the presence of or conjunction with a chemotherapeutic drug.

41. The use of claim 40, wherein the chemotherapeutic drug is selected from the group consisting of adriamycin, 5FU, vinblastine, actinomycin D, etoposide, cisplatin, methotrexate, and doxorubicin.

42. The use of any one of claims 36 to 41, wherein the composition is designed to be administered in the presence of or conjunction with a cytotoxic factor or an immunotoxin.

43. The use of claim 42, wherein the cytotoxic factor is selected from the group consisting of IFN-γ, TNF-α, TNF-β, IL-1, and IL-2.

44. An isolated nucleic acid encoding an antibody light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, and SEQ ID NO: 8.

45. An isolated nucleic acid encoding an antibody heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 10, SEQ ID NO: 12. SEQ ID NO: 14, and SEQ ID NO: 16.

46. An isolated nucleic acid encoding an antibody light chain comprising an amino acid sequence set forth in SEQ ID NO: 32.

47. An isolated nucleic acid encoding an antibody heavy chain comprising an amino acid sequence set forth in SEQ ID NO: 33.

SEQUENCE LISTING

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GARBER, Ellen
LYNE, Paul
SALDHANA, Jose W.

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Gly Thr Thr Val Thr Val Ser Ser
        115   120

<210> 13
<211> 360
<212> DNA
<213> Homo Sapien

<400> 13
gaggtacaac tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaggctc
 60
tcctgtgcag cctctggatt cactttcagt gactattaca tgtattggtt tcgccaggcc
120
ccgggaaagg ggctggagtg ggtcgcaacc attagtgatg gtggtagtta cacctactat
180
ccagacagtg tgaaggggcg attcaccatc tccagagaca atgccaagaa cagcctctac
240
ctgcagatga gcagcctgag ggctgaggac acagctgtgt attactgtgt aagagaggag
300
aatggtaact tttactactt tgactactgg ggccaaggga ccacggtcac cgtctcctca
360

<210> 14
<211> 120
<212> PRT
<213> Homo Sapien

<400> 14
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1   5   10   15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20   25   30
Tyr Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45
Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val

```
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Glu Glu Asn Gly Asn Phe Tyr Tyr Phe Asp Tyr Trp Gly Gln
            100                 105                 110
Gly Thr Thr Val Thr Val Ser Ser
        115                 120
```

<210> 15
<211> 360
<212> DNA
<213> Homo Sapien

<400> 15
gaggtacaac tggtggagtc tgggggaggc ttagtgaagc ctggagggtc cctgaggctc
60
tcctgtgcag cctctggatt cactttcagt gactattaca tgtattggtt tcgccaggcc
120
ccgggaaagg ggctggagtg ggtcgcaacc attagtgatg gtggtagtta cacctactat
180
ccagacagtg tgaaggggcg attcaccatc tccagagaca atgccaagaa cagcctctac
240
ctgcagatga gcagcctgag ggctgaggac acagctgtgt attactgcgc aagagaggag
300
aatggtaact tttactactt tgactactgg ggccaaggga ccacggtcac cgtctcctca
360

<210> 16
<211> 120
<212> PRT
<213> Homo Sapien

<400> 16

```
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr
            20                  25                  30
Tyr Met Tyr Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45
Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val
    50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80
Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95
Ala Arg Glu Glu Asn Gly Asn Phe Tyr Tyr Phe Asp Tyr Trp Gly Gln
            100                 105                 110
Gly Thr Thr Val Thr Val Ser Ser
        115                 120
```

<210> 17
<211> 36
<212> DNA
<213> Homo Sapien

<400> 17
gcctggaggg tccctgaggc tctcctgtgc agcctc
36

<210> 18
<211> 42
<212> DNA
<213> Homo Sapien

<400> 18
gtttcgccag actccgggaa aggggctgga gtgggtcgca ac
42

<210> 19
<211> 67
<212> DNA
<213> Homo Sapien

<400> 19
cagagacaat gccaagaaca gcctctacct gcagatgagc agcctgaggg ctgaggacac
60
agccatg
67

<210> 20
<211> 38
<212> DNA
<213> Homo Sapien

<400> 20
catgtattgg tttcgccagg ccccgggaaa ggggctgg
38

<210> 21
<211> 36
<212> DNA
<213> Homo Sapien

<400> 21
gggctgagga cacagctgtg tattactgtg taagag
36

<210> 22
<211> 50
<212> DNA

<213> Homo Sapien

<400> 22
gtgactatta catgtattgg gtgcgccagg ccccgggaaa ggggctggag
50

<210> 23
<211> 52
<212> DNA
<213> Homo Sapien

<400> 23
gagggctgag gacacagctg tgtattactg cgcaagagag gagaatggta ac
52

<210> 24
<211> 64
<212> DNA
<213> Homo Sapien

<400> 24
cttgcaagtg atagtgaccc tgtctcccac cgatgcagac aaggatgatg gagactgggt
60
catc
64

<210> 25
<211> 43
<212> DNA
<213> Homo Sapien

<400> 25
cataatagat caggatctta ggcgctttcc atggtttctg ctg
43

<210> 26
<211> 72
<212> DNA
<213> Homo Sapien

<400> 26
gtagacagta ataagttgcg aaatcctcag gctgcaggct gctgatggtt agagtataat
60
cttgcccaga tc
72

<210> 27
<211> 40
<212> DNA
<213> Homo Sapien

<400> 27
gatggagact gggtcatctg gatatcacct ctggcacctg
40

<210> 28
<211> 40
<212> DNA
<213> Homo Sapien

<400> 28
gatggttaga gtataatctg taccagatcc actgccactg
40

<210> 29
<211> 40
<212> DNA
<213> Homo Sapien

<400> 29
gatggagact gggtcatctg gatatcacct ctggcacctg
40

<210> 30
<211> 59
<212> DNA
<213> Homo Sapien

<400> 30
caaccttgtt gcataataga tcagaagctt aggcgctttc cctggtttct gctggtacc
59

<210> 31
<211> 40
<212> DNA
<213> Homo Sapien

<400> 31
gatggttaga gtataatctg taccagatcc actgccactg
40

<210> 32
<211> 663
<212> PRT
<213> Homo Sapien

<400> 32
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Lys Ala Gly Gln Asp Ile Lys Ser Tyr
20 25 30
Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile

```
                35                  40                  45
Tyr Tyr Ala Thr Arg Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60
Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80
Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Trp
                85                  90                  95
Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110
Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160
Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190
Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205
Phe Asn Arg Gly Glu Cys Glu Val Gln Leu Val Glu Ser Gly Gly Gly
    210                 215                 220
Leu Val Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly
225                 230                 235                 240
Phe Thr Phe Ser Asp Tyr Tyr Met Tyr Trp Phe Arg Gln Ala Pro Gly
                245                 250                 255
Lys Gly Leu Glu Trp Val Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr
            260                 265                 270
Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn
        275                 280                 285
Ala Lys Asn Ser Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp
    290                 295                 300
Thr Ala Val Tyr Tyr Cys Ala Arg Glu Glu Asn Gly Asn Phe Tyr Tyr
305                 310                 315                 320
Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser
                325                 330                 335
Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr
            340                 345                 350
Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro
        355                 360                 365
Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
    370                 375                 380
His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser
385                 390                 395                 400
Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
                405                 410                 415
Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val
            420                 425                 430
Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
        435                 440                 445
```

```
Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
    450                 455                 460
Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
465                 470                 475                 480
Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
                485                 490                 495
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
            500                 505                 510
Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
        515                 520                 525
Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala
    530                 535                 540
Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
545                 550                 555                 560
Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
                565                 570                 575
Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser
            580                 585                 590
Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
        595                 600                 605
Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
    610                 615                 620
Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
625                 630                 635                 640
Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
                645                 650                 655
Ser Leu Ser Leu Ser Pro Gly
            660
```

<210> 33
<211> 4852
<212> PRT
<213> Homo Sapien

<400> 33

```
Pro Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr Ala Ser Leu
 1                   5                  10                  15
Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Gly Gln Asp Ile Lys Ser
            20                  25                  30
Tyr Leu Ser Trp Tyr Gln Gln Lys Pro Trp Lys Ser Pro Lys Ile Leu
        35                  40                  45
Ile Tyr Tyr Ala Thr Arg Leu Ala Asp Gly Val Pro Ser Arg Phe Ser
    50                  55                  60
Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser Ser Leu Glu
65                  70                  75                  80
Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro
                85                  90                  95
Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Pro Glu Val Gln
            100                 105                 110
Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Lys
        115                 120                 125
```

```
Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr Tyr Met Tyr
    130                 135                 140
Trp Phe Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val Ala Thr Ile
145                 150                 155                 160
Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg
                165                 170                 175
Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Asn Leu Tyr Leu Gln Met
            180                 185                 190
Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys Val Arg Glu
        195                 200                 205
Glu Asn Gly Asn Phe Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr
    210                 215                 220
Val Thr Val Ser Ser Asp Gly Ala Thr Ala Thr Thr Ala Ala Gly Ala
225                 230                 235                 240
Thr Gly Ala Cys Cys Cys Ala Gly Thr Cys Thr Cys Cys Ala Thr Cys
                245                 250                 255
Ala Thr Cys Cys Thr Thr Gly Thr Cys Thr Gly Cys Ala Thr Cys Gly
            260                 265                 270
Gly Thr Gly Gly Gly Ala Gly Ala Cys Ala Gly Gly Gly Thr Cys Ala
        275                 280                 285
Cys Thr Ala Thr Cys Ala Cys Thr Thr Gly Cys Ala Ala Gly Gly Cys
    290                 295                 300
Gly Gly Gly Thr Cys Ala Gly Gly Ala Cys Ala Thr Thr Ala Ala Ala
305                 310                 315                 320
Ala Gly Cys Thr Ala Thr Thr Thr Ala Ala Gly Cys Thr Gly Gly Thr
                325                 330                 335
Ala Cys Cys Ala Gly Cys Ala Gly Ala Ala Ala Cys Cys Ala Thr Gly
            340                 345                 350
Gly Ala Ala Ala Gly Cys Gly Cys Cys Thr Ala Ala Gly Ala Thr Cys
        355                 360                 365
Cys Thr Gly Ala Thr Cys Thr Ala Thr Thr Ala Thr Gly Cys Ala Ala
    370                 375                 380
Cys Ala Ala Gly Gly Thr Thr Gly Gly Cys Ala Gly Ala Thr Gly Gly
385                 390                 395                 400
Gly Gly Thr Cys Cys Cys Ala Thr Cys Ala Ala Gly Ala Thr Thr Cys
                405                 410                 415
Ala Gly Thr Gly Gly Cys Ala Gly Thr Gly Gly Ala Thr Cys Thr Gly
            420                 425                 430
Gly Gly Cys Ala Ala Gly Ala Thr Thr Ala Thr Ala Cys Thr Cys Thr
        435                 440                 445
Ala Ala Cys Cys Ala Thr Cys Ala Gly Cys Ala Gly Cys Cys Thr Gly
    450                 455                 460
Cys Ala Gly Cys Cys Thr Gly Ala Gly Gly Ala Thr Thr Thr Cys Gly
465                 470                 475                 480
Cys Ala Ala Cys Thr Thr Ala Thr Thr Ala Cys Thr Gly Thr Cys Thr
                485                 490                 495
Ala Cys Ala Gly Cys Ala Thr Gly Gly Thr Gly Ala Gly Ala Gly Cys
            500                 505                 510
Cys Cys Gly Thr Gly Gly Ala Cys Gly Thr Thr Cys Gly Gly Thr Gly
        515                 520                 525
Gly Ala Gly Gly Cys Ala Cys Cys Ala Ala Gly Cys Thr Gly Gly Ala
```

```
    530                 535                 540
Gly Ala Thr Cys Ala Ala Ala Pro Asp Ile Lys Met Thr Gln Ser Pro
545                 550                 555                 560
Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys
                565                 570                 575
Ala Gly Gln Asp Ile Lys Ser Tyr Leu Ser Trp Tyr Gln Gln Lys Pro
            580                 585                 590
Trp Lys Ala Pro Lys Ile Leu Ile Tyr Tyr Ala Thr Arg Leu Ala Asp
        595                 600                 605
Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Thr
    610                 615                 620
Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys
625                 630                 635                 640
Leu Gln His Gly Glu Ser Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu
                645                 650                 655
Glu Ile Lys Asp Gly Ala Thr Ala Thr Cys Cys Ala Gly Ala Thr Gly
            660                 665                 670
Ala Cys Cys Cys Ala Gly Thr Cys Thr Cys Cys Ala Thr Cys Ala Thr
        675                 680                 685
Cys Cys Thr Thr Gly Thr Cys Thr Gly Cys Ala Thr Cys Gly Gly Thr
    690                 695                 700
Gly Gly Gly Ala Gly Ala Cys Ala Gly Gly Gly Thr Cys Ala Cys Thr
705                 710                 715                 720
Ala Thr Cys Ala Cys Thr Thr Gly Cys Ala Ala Gly Gly Cys Gly Gly
                725                 730                 735
Gly Thr Cys Ala Gly Gly Ala Cys Ala Thr Thr Ala Ala Ala Ala Gly
            740                 745                 750
Cys Thr Ala Thr Thr Thr Ala Ala Gly Cys Thr Gly Gly Thr Ala Cys
        755                 760                 765
Cys Ala Gly Cys Ala Gly Ala Ala Ala Cys Cys Ala Thr Gly Gly Ala
    770                 775                 780
Ala Ala Gly Cys Gly Cys Cys Thr Ala Ala Gly Ala Thr Cys Cys Thr
785                 790                 795                 800
Gly Ala Thr Cys Thr Ala Thr Thr Ala Thr Gly Cys Ala Ala Cys Ala
                805                 810                 815
Ala Gly Gly Thr Thr Gly Gly Cys Ala Gly Ala Thr Gly Gly Gly Gly
            820                 825                 830
Thr Cys Cys Cys Ala Thr Cys Ala Ala Gly Ala Thr Thr Cys Ala Gly
        835                 840                 845
Thr Gly Gly Cys Ala Gly Thr Gly Gly Ala Thr Cys Thr Gly Gly Thr
    850                 855                 860
Ala Cys Ala Gly Ala Thr Thr Ala Thr Ala Cys Thr Cys Thr Ala Ala
865                 870                 875                 880
Cys Cys Ala Thr Cys Ala Gly Cys Ala Gly Cys Cys Thr Gly Cys Ala
                885                 890                 895
Gly Cys Cys Thr Gly Ala Gly Gly Ala Thr Thr Thr Cys Gly Cys Ala
            900                 905                 910
Ala Cys Thr Thr Ala Thr Thr Ala Cys Thr Gly Thr Cys Thr Ala Cys
        915                 920                 925
Ala Gly Cys Ala Thr Gly Gly Thr Gly Ala Gly Ala Gly Cys Cys Cys
    930                 935                 940
```

```
Gly Thr Gly Gly Ala Cys Gly Thr Thr Cys Gly Gly Thr Gly Gly Ala
945                 950                 955                 960
Gly Gly Cys Ala Cys Cys Ala Ala Gly Cys Thr Gly Gly Ala Gly Ala
                965                 970                 975
Thr Cys Ala Ala Ala Pro Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
            980                 985                 990
Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Gly
        995                 1000                1005
Gln Asp Ile Lys Ser Tyr Leu Ser Trp Tyr Gln Gln Lys Pro Trp Lys
    1010                1015                1020
Ala Pro Lys Ile Leu Ile Tyr Tyr Ala Thr Arg Leu Ala Asp Gly Val
1025                1030                1035                1040
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr
                1045                1050                1055
Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln
            1060                1065                1070
His Gly Glu Ser Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
        1075                1080                1085
Lys Asp Gly Ala Thr Ala Thr Cys Cys Ala Gly Ala Thr Gly Ala Cys
    1090                1095                1100
Cys Cys Ala Gly Thr Cys Thr Cys Cys Ala Thr Cys Ala Thr Cys Cys
1105                1110                1115                1120
Thr Thr Gly Thr Cys Thr Gly Cys Ala Thr Cys Gly Gly Thr Gly Gly
                1125                1130                1135
Gly Ala Gly Ala Cys Ala Gly Gly Gly Thr Cys Ala Cys Thr Ala Thr
            1140                1145                1150
Cys Ala Cys Thr Thr Gly Cys Ala Ala Gly Gly Cys Gly Gly Gly Thr
        1155                1160                1165
Cys Ala Gly Gly Ala Cys Ala Thr Thr Ala Ala Ala Ala Gly Cys Thr
    1170                1175                1180
Ala Thr Thr Thr Ala Ala Gly Cys Thr Gly Gly Thr Ala Cys Cys Ala
1185                1190                1195                1200
Gly Cys Ala Gly Ala Ala Ala Cys Cys Ala Gly Gly Gly Ala Ala Ala
                1205                1210                1215
Gly Cys Gly Cys Cys Thr Ala Ala Gly Cys Cys Thr Cys Thr Gly Ala
            1220                1225                1230
Thr Cys Thr Ala Thr Thr Ala Thr Gly Cys Ala Ala Cys Ala Ala Gly
        1235                1240                1245
Gly Thr Thr Gly Gly Cys Ala Gly Ala Thr Gly Gly Gly Gly Thr Cys
    1250                1255                1260
Cys Cys Ala Thr Cys Ala Ala Gly Ala Thr Thr Cys Ala Gly Thr Gly
1265                1270                1275                1280
Gly Cys Ala Gly Thr Gly Gly Ala Thr Cys Thr Gly Gly Thr Ala Cys
                1285                1290                1295
Ala Gly Ala Thr Thr Ala Thr Ala Cys Thr Cys Thr Ala Ala Cys Cys
            1300                1305                1310
Ala Thr Cys Ala Gly Cys Ala Gly Cys Cys Thr Gly Cys Ala Gly Cys
        1315                1320                1325
Cys Thr Gly Ala Gly Gly Ala Thr Thr Thr Cys Gly Cys Ala Ala Cys
    1330                1335                1340
Thr Thr Ala Thr Thr Ala Cys Thr Gly Thr Cys Thr Ala Cys Ala Gly
```

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1345                1350                1355                1360
Cys Ala Thr Gly Gly Thr Gly Ala Gly Ala Gly Cys Cys Cys Gly Thr
                1365                1370                1375
Gly Gly Ala Cys Gly Thr Thr Cys Gly Gly Thr Gly Gly Ala Gly Gly
            1380                1385                1390
Cys Ala Cys Cys Ala Ala Gly Cys Thr Gly Gly Ala Gly Ala Thr Cys
        1395                1400                1405
Ala Ala Ala Pro Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser
    1410                1415                1420
Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Gly Gln Asp
1425                1430                1435                1440
Ile Lys Ser Tyr Leu Ser Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro
                1445                1450                1455
Lys Leu Leu Ile Tyr Tyr Ala Thr Arg Leu Ala Asp Gly Val Pro Ser
            1460                1465                1470
Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser
        1475                1480                1485
Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Leu Gln His Gly
    1490                1495                1500
Glu Ser Pro Trp Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Asp
1505                1510                1515                1520
Gly Ala Gly Gly Thr Ala Cys Ala Ala Cys Thr Gly Gly Thr Gly Gly
                1525                1530                1535
Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys Thr Thr
            1540                1545                1550
Ala Gly Thr Gly Ala Ala Gly Cys Cys Thr Gly Gly Ala Gly Gly Gly
        1555                1560                1565
Thr Cys Cys Cys Thr Gly Ala Gly Gly Cys Thr Cys Thr Cys Cys Thr
    1570                1575                1580
Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala Thr Thr
1585                1590                1595                1600
Cys Ala Cys Thr Thr Thr Cys Ala Gly Thr Gly Ala Cys Thr Ala Thr
                1605                1610                1615
Thr Ala Cys Ala Thr Gly Thr Ala Thr Thr Gly Gly Thr Thr Thr Cys
            1620                1625                1630
Gly Cys Cys Ala Gly Ala Cys Thr Cys Cys Gly Gly Gly Ala Ala Ala
        1635                1640                1645
Gly Gly Gly Gly Cys Thr Gly Gly Ala Gly Thr Gly Gly Gly Thr Cys
    1650                1655                1660
Gly Cys Ala Ala Cys Cys Ala Thr Thr Ala Gly Thr Gly Ala Thr Gly
1665                1670                1675                1680
Gly Thr Gly Gly Thr Ala Gly Thr Thr Ala Cys Ala Cys Cys Thr Ala
                1685                1690                1695
Cys Thr Ala Thr Cys Cys Ala Gly Ala Cys Ala Gly Thr Gly Thr Gly
            1700                1705                1710
Ala Ala Gly Gly Gly Gly Cys Gly Ala Thr Thr Cys Ala Cys Cys Ala
        1715                1720                1725
Thr Cys Thr Cys Cys Ala Gly Ala Gly Ala Cys Ala Ala Thr Gly Cys
    1730                1735                1740
Cys Ala Ala Gly Ala Ala Cys Ala Gly Cys Cys Thr Cys Thr Ala Cys
1745                1750                1755                1760
```

Cys Thr Gly Cys Ala Gly Ala Thr Gly Ala Gly Cys Ala Gly Cys Cys
1765 1770 1775
Thr Gly Ala Gly Gly Gly Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys
1780 1785 1790
Ala Gly Cys Cys Ala Thr Gly Thr Ala Thr Thr Ala Cys Thr Gly Thr
1795 1800 1805
Gly Thr Ala Ala Gly Ala Gly Ala Gly Gly Ala Gly Ala Ala Thr Gly
1810 1815 1820
Gly Thr Ala Ala Cys Thr Thr Thr Thr Ala Cys Thr Ala Cys Thr Thr
1825 1830 1835 1840
Thr Gly Ala Cys Thr Ala Cys Thr Gly Gly Gly Gly Cys Cys Ala Ala
1845 1850 1855
Gly Gly Gly Ala Cys Cys Ala Cys Gly Gly Thr Cys Ala Cys Cys Gly
1860 1865 1870
Thr Cys Thr Cys Cys Thr Cys Ala Pro Glu Val Gln Leu Val Glu Ser
1875 1880 1885
Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
1890 1895 1900
Ala Ser Gly Phe Thr Phe Ser Asp Tyr Tyr Met Tyr Trp Phe Arg Gln
1905 1910 1915 1920
Thr Pro Gly Lys Gly Leu Glu Trp Val Ala Thr Ile Ser Asp Gly Gly
1925 1930 1935
Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
1940 1945 1950
Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Ser Ser Leu Arg
1955 1960 1965
Ala Glu Asp Thr Ala Met Tyr Tyr Cys Val Arg Glu Glu Asn Gly Asn
1970 1975 1980
Phe Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser
1985 1990 1995 2000
Ser Asp Gly Ala Gly Gly Thr Ala Cys Ala Ala Cys Thr Gly Gly Thr
2005 2010 2015
Gly Gly Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly Gly Cys
2020 2025 2030
Thr Thr Ala Gly Thr Gly Ala Ala Gly Cys Cys Thr Gly Gly Ala Gly
2035 2040 2045
Gly Gly Thr Cys Cys Cys Thr Gly Ala Gly Gly Cys Thr Cys Thr Cys
2050 2055 2060
Cys Thr Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly Gly Ala
2065 2070 2075 2080
Thr Thr Cys Ala Cys Thr Thr Thr Cys Ala Gly Thr Gly Ala Cys Thr
2085 2090 2095
Ala Thr Thr Ala Cys Ala Thr Gly Thr Ala Thr Thr Gly Gly Thr Thr
2100 2105 2110
Thr Cys Gly Cys Cys Ala Gly Gly Cys Cys Cys Cys Gly Gly Gly Ala
2115 2120 2125
Ala Ala Gly Gly Gly Gly Cys Thr Gly Gly Ala Gly Thr Gly Gly Gly
2130 2135 2140
Thr Cys Gly Cys Ala Ala Cys Cys Ala Thr Thr Ala Gly Thr Gly Ala
2145 2150 2155 2160
Thr Gly Gly Thr Gly Gly Thr Ala Gly Thr Thr Ala Cys Ala Cys Cys

2165 2170 2175
Thr Ala Cys Thr Ala Thr Cys Cys Ala Gly Ala Cys Ala Gly Thr Gly
2180 2185 2190
Thr Gly Ala Ala Gly Gly Gly Gly Cys Gly Ala Thr Thr Cys Ala Cys
2195 2200 2205
Cys Ala Thr Cys Thr Cys Cys Ala Gly Ala Gly Ala Cys Ala Ala Thr
2210 2215 2220
Gly Cys Cys Ala Ala Gly Ala Ala Cys Ala Gly Cys Cys Thr Cys Thr
2225 2230 2235 2240
Ala Cys Cys Thr Gly Cys Ala Gly Ala Thr Gly Ala Gly Cys Ala Gly
2245 2250 2255
Cys Cys Thr Gly Ala Gly Gly Gly Cys Thr Gly Ala Gly Gly Ala Cys
2260 2265 2270
Ala Cys Ala Gly Cys Thr Gly Thr Gly Thr Ala Thr Thr Ala Cys Thr
2275 2280 2285
Gly Thr Gly Thr Ala Ala Gly Ala Gly Ala Gly Gly Ala Gly Ala Ala
2290 2295 2300
Thr Gly Gly Thr Ala Ala Cys Thr Thr Thr Thr Ala Cys Thr Ala Cys
2305 2310 2315 2320
Thr Thr Thr Gly Ala Cys Thr Ala Cys Thr Gly Gly Gly Gly Cys Cys
2325 2330 2335
Ala Ala Gly Gly Gly Ala Cys Cys Ala Cys Gly Gly Thr Cys Ala Cys
2340 2345 2350
Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala Pro Glu Val Gln Leu Val
2355 2360 2365
Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg Leu Ser
2370 2375 2380
Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr Tyr Met Tyr Trp Phe
2385 2390 2395 2400
Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Thr Ile Ser Asp
2405 2410 2415
Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr
2420 2425 2430
Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met Ser Ser
2435 2440 2445
Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Val Arg Glu Glu Asn
2450 2455 2460
Gly Asn Phe Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr
2465 2470 2475 2480
Val Ser Ser Asp Gly Ala Gly Gly Thr Ala Cys Ala Ala Cys Thr Gly
2485 2490 2495
Gly Thr Gly Gly Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly Ala Gly
2500 2505 2510
Gly Cys Thr Thr Ala Gly Thr Gly Ala Ala Gly Cys Cys Thr Gly Gly
2515 2520 2525
Ala Gly Gly Gly Thr Cys Cys Cys Thr Gly Ala Gly Gly Cys Thr Cys
2530 2535 2540
Thr Cys Cys Thr Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Thr Gly
2545 2550 2555 2560
Gly Ala Thr Thr Cys Ala Cys Thr Thr Thr Cys Ala Gly Thr Gly Ala
2565 2570 2575

Cys Thr Ala Thr Thr Ala Cys Ala Thr Gly Thr Ala Thr Thr Gly Gly
2580 2585 2590
Thr Thr Thr Cys Gly Cys Cys Ala Gly Gly Cys Cys Cys Cys Gly Gly
2595 2600 2605
Gly Ala Ala Ala Gly Gly Gly Gly Cys Thr Gly Gly Ala Gly Thr Gly
2610 2615 2620
Gly Gly Thr Cys Gly Cys Ala Ala Cys Cys Ala Thr Thr Ala Gly Thr
2625 2630 2635 2640
Gly Ala Thr Gly Gly Thr Gly Gly Thr Ala Gly Thr Thr Ala Cys Ala
2645 2650 2655
Cys Cys Thr Ala Cys Thr Ala Thr Cys Cys Ala Gly Ala Cys Ala Gly
2660 2665 2670
Thr Gly Thr Gly Ala Ala Gly Gly Gly Gly Cys Gly Ala Thr Thr Cys
2675 2680 2685
Ala Cys Cys Ala Thr Cys Thr Cys Cys Ala Gly Ala Gly Ala Cys Ala
2690 2695 2700
Ala Thr Gly Cys Cys Ala Ala Gly Ala Ala Cys Ala Gly Cys Cys Thr
2705 2710 2715 2720
Cys Thr Ala Cys Cys Thr Gly Cys Ala Gly Ala Thr Gly Ala Gly Cys
2725 2730 2735
Ala Gly Cys Cys Thr Gly Ala Gly Gly Gly Cys Thr Gly Ala Gly Gly
2740 2745 2750
Ala Cys Ala Cys Ala Gly Cys Thr Gly Thr Gly Thr Ala Thr Thr Ala
2755 2760 2765
Cys Thr Gly Thr Gly Thr Ala Ala Gly Ala Gly Ala Gly Gly Ala Gly
2770 2775 2780
Ala Ala Thr Gly Gly Thr Ala Ala Cys Thr Thr Thr Thr Ala Cys Thr
2785 2790 2795 2800
Ala Cys Thr Thr Thr Gly Ala Cys Thr Ala Cys Thr Gly Gly Gly Gly
2805 2810 2815
Cys Cys Ala Ala Gly Gly Gly Ala Cys Cys Ala Cys Gly Gly Thr Cys
2820 2825 2830
Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala Pro Glu Val Gln
2835 2840 2845
Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
2850 2855 2860
Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr Tyr Met Tyr
2865 2870 2875 2880
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Thr Ile
2885 2890 2895
Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys Gly Arg
2900 2905 2910
Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Gln Met
2915 2920 2925
Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Glu
2930 2935 2940
Glu Asn Gly Asn Phe Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Thr
2945 2950 2955 2960
Val Thr Val Ser Ser Asp Gly Ala Gly Gly Thr Ala Cys Ala Ala Cys
2965 2970 2975
Thr Gly Gly Thr Gly Gly Ala Gly Thr Cys Thr Gly Gly Gly Gly Gly

2980 2985 2990
Ala Gly Gly Cys Thr Thr Ala Gly Thr Gly Ala Ala Gly Cys Cys Thr
2995 3000 3005
Gly Gly Ala Gly Gly Gly Thr Cys Cys Cys Thr Gly Ala Gly Gly Cys
3010 3015 3020
Thr Cys Thr Cys Cys Thr Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys
3025 3030 3035 3040
Thr Gly Gly Ala Thr Thr Cys Ala Cys Thr Thr Thr Cys Ala Gly Thr
3045 3050 3055
Gly Ala Cys Thr Ala Thr Thr Ala Cys Ala Thr Gly Thr Ala Thr Thr
3060 3065 3070
Gly Gly Thr Thr Thr Cys Gly Cys Cys Ala Gly Gly Cys Cys Cys Cys
3075 3080 3085
Gly Gly Gly Ala Ala Ala Gly Gly Gly Gly Cys Thr Gly Gly Ala Gly
3090 3095 3100
Thr Gly Gly Gly Thr Cys Gly Cys Ala Ala Cys Cys Ala Thr Thr Ala
3105 3110 3115 3120
Gly Thr Gly Ala Thr Gly Gly Thr Gly Gly Thr Ala Gly Thr Thr Ala
3125 3130 3135
Cys Ala Cys Cys Thr Ala Cys Thr Ala Thr Cys Cys Ala Gly Ala Cys
3140 3145 3150
Ala Gly Thr Gly Thr Gly Ala Ala Gly Gly Gly Gly Cys Gly Ala Thr
3155 3160 3165
Thr Cys Ala Cys Cys Ala Thr Cys Thr Cys Cys Ala Gly Ala Gly Ala
3170 3175 3180
Cys Ala Ala Thr Gly Cys Cys Ala Ala Gly Ala Ala Cys Ala Gly Cys
3185 3190 3195 3200
Cys Thr Cys Thr Ala Cys Cys Thr Gly Cys Ala Gly Ala Thr Gly Ala
3205 3210 3215
Gly Cys Ala Gly Cys Cys Thr Gly Ala Gly Gly Gly Cys Thr Gly Ala
3220 3225 3230
Gly Gly Ala Cys Ala Cys Ala Gly Cys Thr Gly Thr Gly Thr Ala Thr
3235 3240 3245
Thr Ala Cys Thr Gly Cys Gly Cys Ala Ala Gly Ala Gly Ala Gly Gly
3250 3255 3260
Ala Gly Ala Ala Thr Gly Gly Thr Ala Ala Cys Thr Thr Thr Thr Ala
3265 3270 3275 3280
Cys Thr Ala Cys Thr Thr Thr Gly Ala Cys Thr Ala Cys Thr Gly Gly
3285 3290 3295
Gly Gly Cys Cys Ala Ala Gly Gly Gly Ala Cys Cys Ala Cys Gly Gly
3300 3305 3310
Thr Cys Ala Cys Cys Gly Thr Cys Thr Cys Cys Thr Cys Ala Pro Glu
3315 3320 3325
Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser
3330 3335 3340
Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asp Tyr Tyr
3345 3350 3355 3360
Met Tyr Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala
3365 3370 3375
Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro Asp Ser Val Lys
3380 3385 3390

Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu
3395 3400 3405

Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala
3410 3415 3420

Arg Glu Glu Asn Gly Asn Phe Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly
3425 3430 3435 3440

Thr Thr Val Thr Val Ser Ser Asp Gly Cys Cys Thr Gly Gly Ala Gly
3445 3450 3455

Gly Gly Thr Cys Cys Cys Thr Gly Ala Gly Gly Cys Thr Cys Thr Cys
3460 3465 3470

Cys Thr Gly Thr Gly Cys Ala Gly Cys Cys Thr Cys Ser Glu Gln Asp
3475 3480 3485

Gly Thr Thr Thr Cys Gly Cys Cys Ala Gly Ala Cys Thr Cys Cys Gly
3490 3495 3500

Gly Gly Ala Ala Ala Gly Gly Gly Gly Cys Thr Gly Gly Ala Gly Thr
3505 3510 3515 3520

Gly Gly Gly Thr Cys Gly Cys Ala Ala Cys Ser Glu Gln Asp Cys Ala
3525 3530 3535

Gly Ala Gly Ala Cys Ala Ala Thr Gly Cys Cys Ala Ala Gly Ala Ala
3540 3545 3550

Cys Ala Gly Cys Cys Thr Cys Thr Ala Cys Cys Thr Gly Cys Ala Gly
3555 3560 3565

Ala Thr Gly Ala Gly Cys Ala Gly Cys Cys Thr Gly Ala Gly Gly Gly
3570 3575 3580

Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys Ala Gly Cys Cys Ala Thr
3585 3590 3595 3600

Gly Asp Cys Ala Thr Gly Thr Ala Thr Thr Gly Gly Thr Thr Thr Cys
3605 3610 3615

Gly Cys Cys Ala Gly Gly Cys Cys Cys Cys Gly Gly Gly Ala Ala Ala
3620 3625 3630

Gly Gly Gly Gly Cys Thr Gly Gly Asp Gly Gly Gly Cys Thr Gly Ala
3635 3640 3645

Gly Gly Ala Cys Ala Cys Ala Gly Cys Thr Gly Thr Gly Thr Ala Thr
3650 3655 3660

Thr Ala Cys Thr Gly Thr Gly Thr Ala Ala Gly Ala Gly Asp Gly Thr
3665 3670 3675 3680

Gly Ala Cys Thr Ala Thr Thr Ala Cys Ala Thr Gly Thr Ala Thr Thr
3685 3690 3695

Gly Gly Gly Thr Gly Cys Gly Cys Cys Ala Gly Gly Cys Cys Cys Cys
3700 3705 3710

Gly Gly Gly Ala Ala Ala Gly Gly Gly Gly Cys Thr Gly Gly Ala Gly
3715 3720 3725

Asp Gly Ala Gly Gly Gly Cys Thr Gly Ala Gly Gly Ala Cys Ala Cys
3730 3735 3740

Ala Gly Cys Thr Gly Thr Gly Thr Ala Thr Thr Ala Cys Thr Gly Cys
3745 3750 3755 3760

Gly Cys Ala Ala Gly Ala Gly Ala Gly Gly Ala Gly Ala Ala Thr Gly
3765 3770 3775

Gly Thr Ala Ala Cys Asp Cys Thr Thr Gly Cys Ala Ala Gly Thr Gly
3780 3785 3790

Ala Thr Ala Gly Thr Gly Ala Cys Cys Cys Thr Gly Thr Cys Thr Cys

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        3795                3800                3805
Cys Cys Ala Cys Cys Gly Ala Thr Gly Cys Ala Gly Ala Cys Ala Ala
    3810                3815                3820
Gly Gly Ala Thr Gly Ala Thr Gly Gly Ala Gly Ala Cys Thr Gly Gly
3825                3830                3835                3840
Gly Thr Cys Ala Thr Cys Asp Cys Ala Thr Ala Ala Thr Ala Gly Ala
                3845                3850                3855
Thr Cys Ala Gly Gly Ala Thr Cys Thr Thr Ala Gly Gly Cys Gly Cys
            3860                3865                3870
Thr Thr Thr Cys Cys Ala Thr Gly Gly Thr Thr Thr Cys Thr Gly Cys
        3875                3880                3885
Thr Gly Asp Gly Thr Ala Gly Ala Cys Ala Gly Thr Ala Ala Thr Ala
    3890                3895                3900
Ala Gly Thr Thr Gly Cys Gly Ala Ala Ala Thr Cys Cys Thr Cys Ala
3905                3910                3915                3920
Gly Gly Cys Thr Gly Cys Ala Gly Gly Cys Thr Gly Cys Thr Gly Ala
                3925                3930                3935
Thr Gly Gly Thr Thr Ala Gly Ala Gly Thr Ala Thr Ala Ala Thr Cys
            3940                3945                3950
Thr Thr Gly Cys Cys Cys Ala Gly Ala Thr Cys Asp Gly Ala Thr Gly
        3955                3960                3965
Gly Ala Gly Ala Cys Thr Gly Gly Gly Thr Cys Ala Thr Cys Thr Gly
    3970                3975                3980
Gly Ala Thr Ala Thr Cys Ala Cys Cys Thr Cys Thr Gly Gly Cys Ala
3985                3990                3995                4000
Cys Cys Thr Gly Asp Gly Ala Thr Gly Gly Thr Thr Ala Gly Ala Gly
                4005                4010                4015
Thr Ala Thr Ala Ala Thr Cys Thr Gly Thr Ala Cys Cys Ala Gly Ala
            4020                4025                4030
Thr Cys Cys Ala Cys Thr Gly Cys Cys Ala Cys Thr Gly Asp Gly Ala
        4035                4040                4045
Thr Gly Gly Ala Gly Ala Cys Thr Gly Gly Gly Thr Cys Ala Thr Cys
    4050                4055                4060
Thr Gly Gly Ala Thr Ala Thr Cys Ala Cys Cys Thr Cys Thr Gly Gly
4065                4070                4075                4080
Cys Ala Cys Cys Thr Gly Asp Cys Ala Ala Cys Cys Thr Thr Gly Thr
                4085                4090                4095
Thr Gly Cys Ala Thr Ala Ala Thr Ala Gly Ala Thr Cys Ala Gly Ala
            4100                4105                4110
Ala Gly Cys Thr Thr Ala Gly Gly Cys Gly Cys Thr Thr Thr Cys Cys
        4115                4120                4125
Cys Thr Gly Gly Thr Thr Thr Cys Thr Gly Cys Thr Gly Gly Thr Ala
    4130                4135                4140
Cys Cys Asp Gly Ala Thr Gly Gly Thr Thr Ala Gly Ala Gly Thr Ala
4145                4150                4155                4160
Thr Ala Ala Thr Cys Thr Gly Thr Ala Cys Cys Ala Gly Ala Thr Cys
                4165                4170                4175
Cys Ala Cys Thr Gly Cys Cys Ala Cys Thr Gly Pro Asp Ile Gln Met
            4180                4185                4190
Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
        4195                4200                4205
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Ile Thr Cys Lys Ala Gly Gln Asp Ile Lys Ser Tyr Leu Ser Trp Tyr
4210 4215 4220
Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Tyr Ala Thr
4225 4230 4235 4240
Arg Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly
4245 4250 4255
Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala
4260 4265 4270
Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Trp Thr Phe Gly Gly
4275 4280 4285
Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe
4290 4295 4300
Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val
4305 4310 4315 4320
Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp
4325 4330 4335
Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr
4340 4345 4350
Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr
4355 4360 4365
Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val
4370 4375 4380
Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly
4385 4390 4395 4400
Glu Cys Pro Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys
4405 4410 4415
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
4420 4425 4430
Ser Asp Tyr Tyr Met Tyr Trp Phe Arg Gln Ala Pro Gly Lys Gly Leu
4435 4440 4445
Glu Trp Val Ala Thr Ile Ser Asp Gly Gly Ser Tyr Thr Tyr Tyr Pro
4450 4455 4460
Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
4465 4470 4475 4480
Ser Leu Tyr Leu Gln Met Ser Ser Leu Arg Ala Glu Asp Thr Ala Val
4485 4490 4495
Tyr Tyr Cys Ala Arg Glu Glu Asn Gly Asn Phe Tyr Tyr Phe Asp Tyr
4500 4505 4510
Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly
4515 4520 4525
Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
4530 4535 4540
Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
4545 4550 4555 4560
Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
4565 4570 4575
Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
4580 4585 4590
Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
4595 4600 4605
Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys

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    4610                4615                4620
Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
4625                4630                4635                4640
Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
                4645                4650                4655
Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
            4660                4665                4670
Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
        4675                4680                4685
Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
    4690                4695                4700
Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
4705                4710                4715                4720
Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
                4725                4730                4735
Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
            4740                4745                4750
Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
        4755                4760                4765
Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
    4770                4775                4780
Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
4785                4790                4795                4800
Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
                4805                4810                4815
Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
            4820                4825                4830
Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
        4835                4840                4845
Leu Ser Pro Gly
    4850
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