



(12) **CORRECTED EUROPEAN PATENT SPECIFICATION**

(15) Correction information:

**Corrected version no 1 (W1 B1)**

**Corrections, see**

**Description Paragraph(s) 22, 29, 55, 63, 64,  
65, 66, 67, 70, 71, 76**

**Claims EN 1**

(48) Corrigendum issued on:

**21.12.2022 Bulletin 2022/51**

(45) Date of publication and mention  
of the grant of the patent:

**28.09.2022 Bulletin 2022/39**

(21) Application number: **18212095.6**

(22) Date of filing: **13.03.2013**

(51) International Patent Classification (IPC):

<b>C07K 14/495</b> <small>(2006.01)</small>	<b>C07K 14/65</b> <small>(2006.01)</small>
<b>C07K 14/705</b> <small>(2006.01)</small>	<b>C07K 16/28</b> <small>(2006.01)</small>
<b>A61K 39/00</b> <small>(2006.01)</small>	<b>A61K 38/00</b> <small>(2006.01)</small>
<b>A61K 38/10</b> <small>(2006.01)</small>	<b>A61K 39/395</b> <small>(2006.01)</small>
<b>A61K 45/06</b> <small>(2006.01)</small>	<b>A61K 48/00</b> <small>(2006.01)</small>
<b>C07K 16/30</b> <small>(2006.01)</small>	<b>C07K 14/71</b> <small>(2006.01)</small>
<b>C07K 7/08</b> <small>(2006.01)</small>	<b>C07K 16/32</b> <small>(2006.01)</small>
<b>C12N 5/00</b> <small>(2006.01)</small>	<b>C12N 15/62</b> <small>(2006.01)</small>
<b>G01N 33/68</b> <small>(2006.01)</small>	<b>A61K 38/17</b> <small>(2006.01)</small>

(52) Cooperative Patent Classification (CPC):

**C12N 15/62; A61K 38/10; A61K 38/179;  
A61P 35/00; A61P 37/04; C07K 14/495;  
C07K 14/65; C07K 14/70532; C07K 14/70596;  
C07K 16/2818; C07K 16/2863; C07K 16/2866;  
C07K 16/2878; C07K 16/2887; C07K 16/32;**

(Cont.)

(54) **TARGETED/IMMUNOMODULATORY FUSION PROTEINS AND METHODS FOR MAKING SAME**

**GEZIELTE/IMMUNOMODULATORISCHE FUSIONSPROTEINE UND VERFAHREN ZUR  
HERSTELLUNG DAVON**

**PROTÉINES DE FUSION CIBLÉES/IMMUNOMODULATRICES ET LEURS PROCÉDÉS DE  
FABRICATION**

(84) Designated Contracting States:

**AL AT BE BG CH CY CZ DE DK EE ES FI FR GB  
GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO  
PL PT RO RS SE SI SK SM TR**

(30) Priority: **30.04.2012 IN 1689CH2012**  
**30.04.2012 IN 1690CH2012**

(43) Date of publication of application:  
**29.05.2019 Bulletin 2019/22**

(62) Document number(s) of the earlier application(s) in  
accordance with Art. 76 EPC:  
**18153517.0 / 3 333 183**  
**13734158.2 / 2 844 667**

(73) Proprietor: **Biocon Limited**  
**Bangalore 560 100 Karnataka (IN)**

(72) Inventors:

- **GOVINDAPPA, Nagaraj**  
**562130 Bangalore, Karnataka (IN)**

- **SASTRY, Kedarnath**

**560085 Bangalore, Karnataka (IN)**

- **SOARES, Maria Melina**

**560067 Bangalore, Karnataka (IN)**

(74) Representative: **ABG Intellectual Property Law,  
S.L.**

**Avenida de Burgos, 16D**

**Edificio Euromor**

**28036 Madrid (ES)**

(56) References cited:

**WO-A1-2009/027471 WO-A1-2014/164427**

**WO-A2-2011/109789 WO-A2-2012/147048**

**US-A1- 2011 104 734**

- **BIRCH J R ET AL: "Antibody production",  
ADVANCED DRUG DELIVERY REVIEWS,  
ELSEVIER, vol. 58, no. 5-6, 7 August 2006  
(2006-08-07), pages 671-685, XP024892148, ISSN:  
0169-409X, DOI: 10.1016/J.ADDR.2005.12.006  
[retrieved on 2006-08-07]**

Note: Within nine months of the publication of the mention of the grant of the European patent in the European Patent Bulletin, any person may give notice to the European Patent Office of opposition to that patent, in accordance with the Implementing Regulations. Notice of opposition shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

- KALWY S ET AL: "Toward more efficient protein expression", MOLECULAR BIOTECHNOLOGY, HUMANA PRESS, INC, US, vol. 34, no. 2, Sp. Iss. SI, 1 October 2006 (2006-10-01), pages 151-156, XP002592403, ISSN: 1073-6085
- JUN LUO ET AL: "Comparative metabolite analysis to understand lactate metabolism shift in Chinese hamster ovary cell culture process", BIOTECHNOLOGY AND BIOENGINEERING, vol. 109, no. 1, 16 October 2011 (2011-10-16), pages 146-156, XP055135701, ISSN: 0006-3592, DOI: 10.1002/bit.23291
- LIU H ET AL: "Heterogeneity of monoclonal antibodies", JOURNAL OF PHARMACEUTICAL SCIENCES, AMERICAN CHEMICAL SOCIETY AND AMERICAN PHARMACEUTICAL ASSOCIATION, US, vol. 97, no. 7, 1 July 2008 (2008-07-01), pages 2426-2447, XP002530921, ISSN: 0022-3549, DOI: 10.1002/JPS.21180

(52) Cooperative Patent Classification (CPC): (Cont.)  
A61K 38/00; C07K 2317/732; C07K 2319/00;  
C07K 2319/33

**Description**

## BACKGROUND OF THE INVENTION

5 Technical Field

**[0001]** The present invention relates generally to the field of a method of preparing a therapeutically active antibody-peptide fusion protein..

10 Related Art

**[0002]** The immune system provides the human body with a means to recognize and defend itself against microorganisms and substances recognized as foreign or potentially harmful. While passive immunotherapy of cancer with monoclonal antibodies and passive transfer of T cells to attack tumor cells have demonstrated clinical efficacy, the goal of active therapeutic vaccination to induce these immune effectors and establish immunological memory against tumor cells has remained challenging. Several tumor-specific and tumor-associated antigens have been identified, yet these antigens are generally weakly immunogenic and tumors employ diverse mechanisms to create a tolerogenic environment that allows them to evade immunologic attack. Strategies to overcome such immune tolerance and activating robust levels of antibody and/or T cell responses hold the key to effective cancer immunotherapy. More important, the individual proteins and how to create an active chimeric polypeptide with an active tertiary structure needs to be explored.

**[0003]** WO 2011/109789 A2 discloses chimeric molecules comprising a targeting moiety fused with an immunomodulatory moiety, wherein the targeting moiety, either light chain or heavy chain, is fused to the immunomodulatory moiety through linkers. Document WO 2009/027471 A1 discloses methods of increasing the titer of a cellular protein comprising modifying a nucleic acid coding for the protein in CHO cells by deleting the C terminal lysine residue of the heavy chain of a monoclonal antibody resulting in higher titer compared to wild type antibody. In addition, US 2011/104734 A1 relates to the production of a recombinant protein by a cell based on culturing the CHO cells in a sufficient concentration of exogenous as well as the addition of Zn salt, such as Zn lactate, to media. Moreover, methods for the production of antibodies in CHO cells and codon optimisation by increase the CG content is disclosed in Birch J Retal: Advanced drug delivery reviews, elsevier, vol. 58, no. 5-6, 7 august 2006 pages 671-685 and in Kalwy S et al: Molecular Biotechnology, Humana press, Inc, us, vol. 34, no. 2, sp. lss. si, 1 october 2006 pages 151-156,

## SUMMARY OF THE INVENTION

**[0004]** The present disclosure (not covered by the claimed invention) provides for chimeric polypeptides containing at least one targeting moiety to target a cancer cell and at least one immunomodulating moiety that counteracts immune tolerance of cancer cell, wherein the targeting moiety and the immunomodulating moiety are linked by a amino acid spacer of sufficient length of amino acid residues so that both moieties can successfully bond to their individual target. In the alternative, the targeting moiety and the immunomodulating moiety that counteract immune tolerance of cancer cell may be bound directly to each other. The chimeric/fusion polypeptides of the disclosure (not covered by the claimed invention) are useful for binding to a cancer cell receptor and reducing the ability of cancer cells to avoid an immune response.

**[0005]** The present invention is based on preparing chimeric/fusion proteins by expression of polynucleotides encoding the fusion proteins that counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. Such immunosuppressive mechanisms include immunosuppressive cytokines (for example, Transforming growth factor beta (TGF- $\beta$ )) and regulatory T cells and/or immunosuppressive myeloid dendritic cells (DCs). By counteracting tumor-induced immune tolerance, the present disclosure (not covered by the claimed invention) provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment. The present disclosure provides strategies to counteract tumor-induced immune tolerance and enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor against resistant or disseminated cancer cells.

**[0006]** It is also disclosed a molecule (not covered by the claimed invention) including at least one targeting moiety fused with at least one immunomodulatory moiety. The targeting moiety specifically binds a target molecule, and the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- $\beta$ ); (ii) Programmed death- 1 ligand 1 (PD-L1) or Programmed death-1 ligand 2 (PD-L2); (iii) Receptor activator of nuclear factor-KB (RANK) ligand (RANKL); (iv) Transforming growth factor-beta receptor (TGF-pR); (v) Programmed death-1 (PD-1 ); (vi) 4-1BB receptor or (vii) Receptor activator of nuclear factor- $\kappa$ B (RANK).

**[0007]** In a further aspect (not covered by the claimed invention), the targeting moiety includes an antibody, antibody

fragment including the light or heavy chains of the antibody, scFv, or Fc-containing polypeptide that specifically binds a component of a tumor cell, tumor antigen, tumor vasculature, tumor microenvironment, or tumor-infiltrating immune cell. Preferably, the targeting moiety is an antibody or a fragment thereof having binding affinity for a component on a tumor cell. Notably each of the heavy chain and light chain may individually be linked to a separate and distinct immunomodulatory moiety. Further, a heavy or light chain of an antibody targeting moiety may be linked to an immunomodulatory moiety which in turn can be further linked to a second immunomodulatory moiety wherein there is a linker between the two immunomodulatory moieties.

**[0008]** It is also disclosed a chimeric polypeptide (not forming part of the invention), that comprised a tumor targeting moiety and an immunomodulatory moiety comprising a molecule that binds transforming growth factor beta (TGF- $\beta$ ), wherein the tumor targeting moiety is an antibody that binds to EGFR1, where in the antibody can be the full antibody, heavy chain or light chain. The tumor targeting moiety may include monoclonal antibodies that target a cancer cell, including but not limited to cetuximab, trastuzumab, rituximab, ipilimumab, tremelimumab, muromonab-CD3, abciximab, daclizumab, basiliximab, palivizumab, infliximab, gemtuzumab ozogamicin, alemtuzumab, ibritumomab tiuxetan, adalimumab, omalizumab, tositumomab, 1-131 tositumomab, efalizumab, bevacizumab, panitumumab, pertuzumab, natalizumab, etanercept, IGN101 (Aphton), volociximab (Biogen Idec and PDL BioPharm), Anti-CD80 mAb (Biogen Idec), Anti-CD23 mAb (Biogen Idec), CAT-3888 (Cambridge Antibody Technology), CDP-791 (Imclone), eraptuzumab (Immunomedics), MDX-010 (Medarex and BMS), MDX-060 (Medarex), MDX-070 (Medarex), matuzumab (Merck), CP-675,206 (Pfizer), CAL (Roche), SGN-30 (Seattle Genetics), zanolimumab (Serono and Genmab), adecatumumab (Sereno), oregovomab (United Therapeutics), nimotuzumab (YM Bioscience), ABT-874 (Abbott Laboratories), denosumab (Amgen), AM 108 (Amgen), AMG 714 (Amgen), fontolizumab (Biogen Idec and PDL BioPharm), daclizumab (Biogen Idec and PDL BioPharm), golimumab (Centocor and Schering-Plough), CNTO 1275 (Centocor), ocrelizumab (Genetech and Roche), HuMax-CD20 (Genmab), belimumab (HGS and GSK), epratuzumab (Immunomedics), MLN1202 (Millennium Pharmaceuticals), visilizumab (PDL BioPharm), tocilizumab (Roche), ocrerlizumab (Roche), certolizumab pegol (UCB, formerly Celltech), eculizumab (Alexion Pharmaceuticals), pexelizumab (Alexion Pharmaceuticals and Procter & Gamble), abciximab (Centocor), ranibizumab (Genetech), mepolizumab (GSK), TNX-355 (Tanox), or MYO-029 (Wyeth).

**[0009]** In another aspect (not covered by the claimed invention), the tumor targeting moiety is a monoclonal antibody that binds to HER2/Neu, CD20, CTLA4, EGFR1 and wherein the antibody can be the full antibody, heavy chain or light chain.

**[0010]** In yet another aspect (not covered by the claimed invention), the targeting moiety is a molecule that specifically binds epidermal growth factor receptor (EGFR1, Erb-B 1), HER2/neu (Erb-B2), CD20, cytotoxic T-lymphocyte antigen-4 (CTLA-4) which is essential for Treg function (CD 152); H-1 and Interleukin- 6 (IL-6).

**[0011]** In a still further aspect (not covered by the claimed invention), the targeting moiety specifically binds a component of a regulatory T cell (treg), myeloid suppressor cell, or dendritic cell. In another aspect (not covered by the claimed invention), the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2 receptor; IL-2aR); (iii) Transforming growth factor-beta receptor (TGF- $\beta$ R); (vi) Transforming growth factor-beta (TGF- $\beta$ ); (vii) Programmed Death- 1 (PD-1); (viii) Programmed death- 1 ligand (PD-L1 or PD-L2).

**[0012]** In another aspect (not covered by the claimed invention), the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF- $\beta$ ); (ii) Programmed death-1 ligand (PD-L1 or PD-L2); or 4-1BB receptor.

**[0013]** In yet another aspect (not covered by the claimed invention), the immunomodulatory moiety includes a molecule that binds TGF- $\beta$  and inhibits the function thereof. Specifically the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF- $\beta$ RII, TGF- $\beta$ RIIb, or TGF- $\beta$ RIII. In another aspect the immunomodulatory moiety includes an extracellular ligand-binding domain (ECD) of TGF- $\beta$ RII. Still further the immunomodulatory moiety may include H-4-1BB ligand which binds to the 4-1BB receptor to stimulate T-cells to help eradicate tumor.

**[0014]** In a still further aspect (not covered by the claimed invention), the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, or cytotoxic T-lymphocyte antigen-4 (CTLA-4) and wherein the immunomodulatory moiety includes an extracellular ligand-binding domain of TGF- $\beta$ RII.

**[0015]** In yet another aspect (not covered by the claimed invention), the immunomodulatory moiety includes a molecule that specifically binds to and inhibit the activity of Programmed death- 1 ligand 1 (PD-L 1) or Programmed death- 1 ligand 2 (PD-L2). In another aspect (not covered by the claimed invention), the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death- 1 (PD-1).

**[0016]** In a further aspect (not covered by the claimed invention), the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFR1, CD20, cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (1L-2a receptor; IL-2aR), or CD4 and wherein, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death- 1 (PD-1).

**[0017]** In a still further aspect (not covered by the claimed invention), the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, and the immunomodulatory moiety includes a sequence from trans-

forming growth factor- $\beta$  (TGF- $\beta$ ).

**[0018]** In one aspect (not covered by the claimed invention), the present disclosure provides for optimized genes encoding for a fusion polypeptide comprising at least one targeting moiety and at least one immunomodulatory moiety for treating cancer in a human subject wherein the optimized genes have been modified to increase expression in a human subject. Preferably the optimized genes comprise sequences for encoding a targeting moiety or an immunomodulatory moiety selected from SEQ ID NOs: 12 to 28 (aspect not covered by the claimed invention).

**[0019]** In another aspect, the present disclosure provides for a vector comprising optimized genes for treating cancer in a human subject wherein the optimized genes have been modified to increase CG sequences (aspect not covered by the claimed invention). Preferably, the vector includes sequences for encoding at least one targeting moiety and at least one immunomodulatory moiety selected from SEQ ID NOs: 12 to 28.

**[0020]** In an alternative aspect (aspect not covered by the claimed invention), the present disclosure provides an expression vector comprising polynucleotides of optimized genes that encode at least one targeting moiety and at least one immunomodulatory moiety selected from SEQ ID NOs: 12 to 28.

**[0021]** In yet another aspect, the present disclosure provides a recombinant host cell transfected with a polynucleotide that encodes a fusion protein peptide of the present invention (aspect not covered by the claimed invention).

**[0022]** In one aspect, the present invention provides for a method of preparing therapeutically active antibody-peptide fusion proteins, the method comprising;

preparing a codon optimized nucleotide sequence of the antibody-peptide fusion protein, wherein the codon optimized nucleotide sequence is optimized for expression in a Chinese Hamster Ovary (CHO) host cell, wherein the antibody-protein fusion protein comprises a targeting moiety and immunomodulating moiety, wherein the targeting moiety and the immunomodulating moiety are linked by an amino acid spacer selected from SEQ ID NO: 3 or SEQ ID NO: 11, wherein the immunomodulating moiety is TGF- $\beta$ RII comprising an amino acid sequence of SEQ ID NO: 4; wherein the targeting moiety is selected from the group consisting of an Anti-EGFR1 antibody, consisting of heavy chain SEQ ID NO: 5 and light chain SEQ ID NO: 6, an Anti-HER2/Neu antibody consisting of heavy chain SEQ ID NO: 1 and light chain SEQ ID NO: 2; and anti-CTLA4 antibody consisting of heavy chain of SEQ ID NO: 7 and a light chain of SEQ ID NO: 8, wherein SEQ ID NO: 4 is attached via the amino acid spacer to the C-terminus of SEQ ID NO: 1 or SEQ ID NO: 2 of Anti-HER2/Neu; C-terminus of SEQ ID NO: 5 or SEQ ID NO: 6 of Anti-EGFR1; or C-terminus of SEQ ID NO: 7 or SEQ ID NO: 8 of Anti-CTLA-4;

cloning the optimized sequence of said antibody-peptide fusion protein in a Chinese Hamster Ovary (CHO) host cell capable of transient or continued expression;

growing the CHO host cell in a feed batch mode in a fermentation medium under suitable conditions for growing and allowing the CHO host cell to express a cloned protein, wherein the fermentation medium comprises a divalent transitional metallic salt; , wherein the divalent transitional metallic salt includes a zinc ion, wherein the divalent transitional metallic salt is zinc sulphate hepta hydrate salt and purifying the expressed antibody-peptide fusion protein and optionally checking the bi-specific binding capabilities of the antibody-peptide fusion protein to its targets.

**[0023]** The method of the present invention provides nucleotide sequences that encode the therapeutically active antibody-peptide fusion proteins and such expression may be conducted in a transient cell line or a stable cell line. The transient expression is accomplished by transfecting or transforming the host with vectors carrying the fusion proteins into mammalian host cells

**[0024]** Once the fusion peptides are expressed, they are subjected to purification and optionally to in-vitro tests to check its bi-specificity, that being, having the ability to bind to both the target moiety and immunomodulating moiety. Such tests may include in-vitro test such as ELISA or NK/T-cell binding assays to validate bi-functional target binding or immune cell stimulation.

**[0025]** Notably once the specific fusion peptides demonstrate the desired bi-specificity, such fusion peptides are selected for sub-cloning into a stable cell line for larger scale expression and purification. Such stable cell lines is, CHO.

**[0026]** In a further aspect, the culture medium can be improved by additions to such medium. The culture medium include a divalent transitional metallic salt which is added to the cell culture either initially or in fed-batch mode to reduce accumulation of lactate during culturing and/or reduce heterogeneity of the fusion proteins. The transitional metallic salt includes a zinc ion, wherein the divalent transitional metallic salt is zinc sulphate hepta hydrate salt and the addition of the metal ion may be carried out during different phases of the production.

**[0027]** Other features and advantages of the invention will be apparent from the following detailed description, drawings and claims.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**[0028]** Figures 16-21 and 46-65 are not covered by the present invention and are present for illustration purposes only

[0029]

Figure 1 shows the amino acid sequences of with the amino acid sequence of Anti-HER2/neu-TGF $\beta$ RII fusion protein at LC constant region with the amino acid sequence of anti-HER2/neu heavy chain (SEQ ID NO: 1) and anti-HER2/neu light chain (SEQ ID NO: 2) attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters and wherein a linker (SEQ ID NO: 3) is positioned between the anti-HER2/neu light chain and TGF- $\beta$ RII and shown in italics.

Figure 2 shows the amino acid sequences of Anti-EGFR1-TGF $\beta$ RII fusion protein at LC constant region with amino acid sequence of Anti-EGFR1 heavy chain (SEQ ID NO: 5) and the amino acid sequence of Anti-EGFR1 light chain (SEQ ID NO: 6) attached to amino acid residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters and wherein a linker (SEQ ID NO: 3) is positioned between the Anti-EGFR1 light chain and TGF- $\beta$ RII and shown in italics.

Figure 3 shows the amino acid sequences of Anti-CTLA4-TGF $\beta$ RII fusion protein at LC constant region with amino acid sequence of anti-CTLA4 heavy chain (SEQ ID NO: 7) and amino acid sequence of anti-CTLA4 light chain (SEQ ID NO: 8) attached to amino acid residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters and wherein a linker (SEQ ID NO: 3) is positioned between the anti-CTLA4 light chain and TGF- $\beta$ RII and shown in italics.

Figure 4 shows the amino acid sequences of Anti-HER2/neu HC-4-1BB and LC-TGF $\beta$ RII fusion protein with amino acid sequence of Anti-HER2/neu/HC-4-1BB fusion protein wherein the amino acid sequence for Anti-HER2/neu heavy chain (SEQ ID NO: 1) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font and amino acid sequence of anti-HER2/neu light chain (SEQ ID NO: 2) attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters and wherein a linker (SEQ ID NO: 3) is positioned between the anti-HER2/neu light chain and TGF- $\beta$ RII and shown in italics.

Figure 5 shows the amino acid sequence of Anti-EGFR1 HC-4-1BB and LC-TGF $\beta$ RII fusion protein with amino acid sequence of Anti-EGFR1 heavy chain-4-1BB fusion protein wherein the amino acid sequence for Anti-EGFR1 heavy chain (SEQ ID NO: 5) is attached to a linker (SEQ ID NO: 3) is shown in italics and the sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font and amino acid sequence of light chain Anti-EGFR1 (SEQ ID NO: 6) attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 6 shows the amino acid sequence of Anti-CTLA4 HC-4-1BB and LC-TGF $\beta$ RII fusion protein with amino acid sequence of Anti-CTLA4 heavy chain-4-1BB fusion protein wherein the amino acid sequence for Anti-CTLA4 heavy chain (SEQ ID NO: 7) is attached to a linker (SEQ ID NO: 3) is shown in italics and the sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font and amino acid sequence of Anti-CTLA4 light chain (SEQ ID NO: 8) is attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 7 shows the amino acid sequence of Anti-HER2/neu HC-PD1 and LC-TGF $\beta$ RII fusion protein with amino acid sequence of Anti-HER2/neu heavy chain-PDI fusion protein wherein the amino acid sequence for the Anti-HER2/neu heavy chain (SEQ ID NO: 1) is attached to a linker (SEQ ID NO: 3) is shown in italics and the sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font and amino acid sequence of Anti-HER2/neu light chain (SEQ ID NO: 2) is attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 8 shows the amino acid sequence of Anti-EGFR1 HC-PD1 and LC-TGF $\beta$ RII fusion protein with amino acid sequence of Anti-EGFR1 heavy chain-PDI fusion protein wherein the amino acid sequence Anti-EGFR1 heavy chain (SEQ ID NO: 5) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font and amino acid sequence of Anti-EGFR1 light chain (SEQ ID NO: 6) attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 9 shows the amino acid sequence of Anti-CTLA4 HC-PD1 and LC-TGF $\beta$ RII fusion protein with amino acid sequence of Anti-CTLA4 heavy chain-PDI fusion protein wherein the amino acid sequence Anti-CTLA4 heavy chain

(SEQ ID NO: 7) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font and amino acid sequence of Anti-CTLA4 light chain (SEQ ID NO: 8) attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 10 shows the amino acid sequence of Anti-HER2/neu HC-TGF $\beta$ RII-4-1BB fusion protein with amino acid sequence of Anti-HER2/neu heavy chain-TGF $\beta$ RII-4-1BB fusion protein wherein the amino acid sequence for Anti-HER2/neu heavy chain (SEQ ID NO: 1 with an additional Lys on the C-terminal) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Anti-HER2/neu light chain (SEQ ID NO: 2).

Figure 11 shows the amino acid sequence of Anti-EGFR1 HC-TGF $\beta$ RII-4-1BB fusion protein with amino acid sequence of Anti-EGFR1 heavy chain-TGF $\beta$ RII-4-1BB fusion protein wherein the amino acid sequence for Anti-EGFR1 heavy chain (SEQ ID NO: 5 with an additional Lys on the C-terminal) sequence is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font with linker between (SEQ ID NO: 11) and including the amino acid sequence of Anti-EGFR1 light chain (SEQ ID NO: 6).

Figure 12 shows the amino acid sequence of Anti-CTLA4 HC-TGF $\beta$ RII-4-1BB fusion protein with amino acid sequence of Anti-CTLA4 heavy chain-TGF $\beta$ RII-4-1BB fusion protein wherein the amino acid sequence Anti-CTLA4 heavy chain (SEQ ID NO: 7 with an additional Lys on the C-terminal) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font with linker between (SEQ ID NO: 11) and including the amino acid sequence of Anti-CTLA4 light chain (SEQ ID NO: 8).

Figure 13 shows the amino acid sequence of Anti-HER2/neu HC-TGF $\beta$ RII-PD1 fusion protein with amino acid sequence of Anti-HER2/neu heavy chain-TGF $\beta$ RII-PD1 fusion protein wherein the amino acid sequence Anti-HER2/neu heavy chain (SEQ ID NO: 1 with an additional Lys on the C-terminal) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for PD-1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Anti-HER2/neu light chain (SEQ ID NO: 2).

Figure 14 shows the amino acid sequence of Anti-EGFR1 HC-TGF $\beta$ RII-PD1 fusion protein with amino acid sequence of Anti-EGFR1 heavy chain-TGF $\beta$ RII-PD1 fusion protein wherein the amino acid sequence Anti-EGFR1 heavy chain (SEQ ID NO: 5 with an additional Lys on the C-terminal) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for PD-1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Anti-EGFR1 light chain (SEQ ID NO: 6).

Figure 15 shows the of Anti-CTLA4 HC-TGF $\beta$ RII-PD1 fusion protein with amino acid sequence of Anti-CTLA4 heavy chain-TGF $\beta$ RII-PD21 fusion protein wherein the amino acid sequence Anti-CTLA4 heavy chain (SEQ ID NO: 7 with an additional Lys on the C-terminal) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for PD-1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font with linker between (SEQ ID NO: 11) and including the amino acid sequence of Anti-CTLA4 light chain (SEQ ID NO: 8).

Figure 16 shows the nucleotide sequence of Anti-HER2/neu heavy chain constant region with linker (SEQ ID NO: 12) and TGF $\beta$ RII ECD (SEQ ID NO: 13) that have been codon optimized for expression in CHO cell.

Figure 17 shows the nucleotide sequence of Anti-HER2/neu heavy chain variable region (SEQ ID NO: 14), Anti-HER2/neu light chain variable region (SEQ ID NO: 15) and Anti-EGFR1 heavy chain constant region with linker (SEQ ID NO: 16) that have been codon optimized for expression in CHO cell.

Figure 18 shows the nucleotide sequence of Anti-EGFR1 heavy chain variable region (SEQ ID NO: 17), Anti-EGFR1 light chain variable region (SEQ ID NO: 18), Anti-CTLA4 heavy chain variable region (SEQ ID NO: 19) and Anti-

CTLA4 light chain variable region (SEQ ID NO: 20) that have been codon optimized for expression in CHO cell.

Figure 19 shows the nucleotide sequence of Anti CD20 IgG1 molecule (SEQ ID NO: 21), Anti-CD20 heavy chain variable region (SEQ ID NO: 22) and Anti-CD20 light chain variable region (SEQ ID NO: 23) that have been codon optimized for expression in CHO cell.

Figure 20 shows the nucleotide sequence of 4-1BB (SEQ ID NO: 24) and Anti-IL6R heavy chain (SEQ ID NO: 25) that have been codon optimized for expression in CHO cell.

Figure 21 shows the nucleotide sequence of Anti-IL6R light chain variable region (SEQ ID NO: 26), Anti-4-1BB heavy chain (SEQ ID NO: 27) and Anti-4-1BB light chain variable region (SEQ ID NO: 28) that have been codon optimized for expression in CHO cell.

Figure 22 shows the analysis of Protein A purified Anti-HER2/neu-TGFβRII and Anti-EGFR1-TGFβRII at 12 % PAGE

Figure 23 A shows Anti-HER2/neu-TGFβRII samples analyzed by Protein A/SEC Chromatography and B Anti-EGFR1-TGFβRII samples analyzed by Protein A/SEC Chromatography.

Figure 24 A shows that Anti-HER2/neu-TGFβRII and Anti-EGFR1-TGFβRII molecules bind to the TGFβ indicating that the fusion protein is functional and B shows that Anti-HER2-TGFβRII inhibits the proliferation of BT474 cell line similar to the Bmab200 (Herceptin).

Figure 25 shows that Anti-EGFR1-TGFβRII-inhibits the proliferation of A431 cell line similar to the Cetuximab.

Figure 26 shows the ADCC activity of Anti-HER2-TGFβRII on BT474 cells is similar to that of Bmab200 (Herceptin).

Figure 27 shows the ADCC activity of Anti-EGFR1-TGFβRII on A431 cells wherein the ADCC activities are similar to that of Cetuximab.

Figure 28 shows the ADCC activity of ADCC activity of Anti-EGFR1-4-1BB in comparison with Anti-EGFR1-TGFβRII and cetuximab.

Figure 29 A shows that the binding activity of Anti-CTLA4-TGFβRII to TGFβ1 is comparable to Anti-EGFR1-TGFβRII and B shows that the binding activity of Anti-CTLA4-TGFβRII to CTLA4.

Figure 30 A shows the binding activity of Anti-CTLA4-TGFβRII to determine the level of PD1-Fc binding and B shows the binding activity of Anti-EGFR1-4-1BB to determine the binding of 4-1BBL.

Figure 31 A shows the binding activity of Anti-EGFR1-4-1BB to EGFR and B shows the binding activity of PD1-Fc-4-1BB to find out PDL1-Fc.

Figure 32 shows the binding activity of Anti-EGFR1-PD1 to EGFR and PD1.

Figure 33 shows photographs of expressed proteins and reduction alkylation thereof.

Figure 34 A shows the mass spectrum Mass Spectrum of light chain (LC) (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion and B shows Deconvoluted Mass Spectrum of LC (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion.

Figure 35 shows the Mass Spectrum of heavy chain (HC) (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion.

Figure 36 A shows the Mass Spectrum of LC (Reduced) of Anti-EGFR1-TGFβRII ECD and B shows the Deconvoluted Mass Spectrum of LC (Reduced) of Anti-EGFR1-TGFβRII ECD.

Figure 37 shows the Mass Spectrum of HC (Reduced) of Anti-EGFR1-TGFβRII ECD.

Figure 38 A shows the UV Chromatogram of Tryptic Peptides of Anti-HBR2/neu-TGFβRII ECD fusion protein and B shows the Total Ion Chromatogram (TIC) of Tryptic Peptides of Anti-HBR2/neu-TGFβRII ECD fusion protein.



Figures 39, 40 and 41 provide lists of expected/observed tryptic peptide of the light chain, heavy chain and linked motif of the Anti-HER2/neu-TGFβRII ECD fusion protein, respectively.

Figure 42 A shows the UV Chromatogram of Tryptic Peptides of Anti-EGFR1-TGFβRII ECD fusion protein and B shows the Total Ion Chromatogram (TIC) of Tryptic Peptides of Anti-EGFR1-TGFβRII ECD fusion protein.

Figure 43 provides a list of expected/observed tryptic peptide of the light chain of the Anti-EGFR1-TGFβRII ECD fusion protein.

Figure 44 shows the list of expected/observed tryptic peptide of the heavy chain of the Anti-EGFR1-TGFβRII ECD fusion protein.

Figure 45 shows the list of expected/observed tryptic peptide of the heavy chain of the Anti-EGFR1-TGFβRII ECD fusion protein.

Figure 46 shows the amino acid sequences of Cantuzumab-TGFβRII fusion protein at LC constant region with amino acid sequence of Cantuzumab heavy chain (SEQ ID NO: 29) and amino acid sequence of Cantuzumab light chain (SEQ ID NO: 30) attached to amino acid residues for TGF-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters and wherein a linker (SEQ ID NO: 3) is positioned between the Cantuzumab light chain and TGF-βRII and shown in italics.

Figure 47 shows the amino acid sequences of Cixutumumab-TGFβRII fusion protein at LC constant region with amino acid sequence of Cixutumumab heavy chain (SEQ ID NO: 31) and amino acid sequence of Cixutumumab light chain (SEQ ID NO: 32) attached to amino acid residues for TGF-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters and wherein a linker (SEQ ID NO: 3) is positioned between the Cixutumumab light chain and TGF-βRII and shown in italics.

Figure 48 shows the amino acid sequences of Clivatuzumab-TGFβRII fusion protein at LC constant region with amino acid sequence of Clivatuzumab heavy chain (SEQ ID NO: 33) and amino acid sequence of Clivatuzumab light chain (SEQ ID NO: 34) attached to amino acid residues for TGF-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters and wherein a linker (SEQ ID NO: 3) is positioned between the Clivatuzumab light chain and TGF-βRII and shown in italics.

Figure 49 shows the amino acid sequences of Pritumumab-TGFβRII fusion protein at LC constant region with amino acid sequence of Pritumumab heavy chain (SEQ ID NO: 35) and amino acid sequence of Pritumumab light chain (SEQ ID NO: 36) attached to amino acid residues for TGF-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters and wherein a linker (SEQ ID NO: 3) is positioned between the Pritumumab light chain and TGF-βRII and shown in italics.

Figure 50 shows the amino acid sequence of Cantuzumab HC-4-1BB and LC-TGFβRII fusion protein wherein the amino acid sequence for the Cantuzumab heavy chain (SEQ ID NO: 29) is attached to a linker (SEQ ID NO: 3) which is shown in italics and the sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font and amino acid sequence of Cantuzumab light chain (SEQ ID NO: 30) is attached to amino residues for TGF-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 51 shows the amino acid sequence of Cixutumumab HC-4-1BB and LC-TGFβRII fusion protein wherein the amino acid sequence for the Cixutumumab heavy chain (SEQ ID NO: 31) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font and amino acid sequence of Cixutumumab light chain (SEQ ID NO: 32) is attached to amino residues for TGF-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 52 shows the amino acid sequence of Clivatuzumab HC-4-1BB and LC-TGFβRII fusion protein wherein the amino acid sequence for the Clivatuzumab heavy chain (SEQ ID NO: 33) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font and amino acid sequence of Clivatuzumab light chain (SEQ ID NO: 34) is attached to amino residues for TGF-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 53 shows the amino acid sequence of Pritumumab HC-4-1BB and LC-TGFβRII fusion protein wherein the

amino acid sequence for the Pritumumab heavy chain (SEQ ID NO: 35) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font and amino acid sequence of Pritumumab light chain (SEQ ID NO: 36) is attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 54 shows the amino acid sequence of Cantuzumab - HC-PD1 and LC-TGF $\beta$ RII fusion protein wherein the amino acid sequence for the Cantuzumab heavy chain (SEQ ID NO: 29) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font and amino acid sequence of Cantuzumab light chain (SEQ ID NO: 30) is attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 55 shows the amino acid sequence of Cixutumumab - HC-PD1 and LC-TGF $\beta$ RII fusion protein wherein the amino acid sequence for the Cixutumumab heavy chain (SEQ ID NO: 31) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font and amino acid sequence of Cixutumumab light chain (SEQ ID NO: 32) is attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 56 shows the amino acid sequence of Clivatuzumab - HC-PD1 and LC-TGF $\beta$ RII fusion protein wherein the amino acid sequence for the Clivatuzumab heavy chain (SEQ ID NO: 33) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font and amino acid sequence of Clivatuzumab light chain (SEQ ID NO: 34) is attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 57 shows the amino acid sequence of Pritumumab - HC-PD1 and LC-TGF $\beta$ RII fusion protein wherein the amino acid sequence for the Pritumumab heavy chain (SEQ ID NO: 35) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font and amino acid sequence of Pritumumab light chain (SEQ ID NO: 36) is attached to amino residues for TGF- $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

Figure 58 shows the amino acid sequence of Cantuzumab HC-TGF $\beta$ RII-4-1BB fusion protein wherein the amino acid sequence for Cantuzumab heavy chain (SEQ ID NO: 29) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Cantuzumab light chain (SEQ ID NO: 30).

Figure 59 shows the amino acid sequence of Cixutumumab HC-TGF $\beta$ RII-4-1BB fusion protein wherein the amino acid sequence for Cixutumumab heavy chain (SEQ ID NO: 31) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Cixutumumab light chain (SEQ ID NO: 32).

Figure 60 shows the amino acid sequence of Clivatuzumab HC-TGF $\beta$ RII-4-1BB fusion protein wherein the amino acid sequence for Clivatuzumab heavy chain (SEQ ID NO: 33) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Clivatuzumab light chain (SEQ ID NO: 34).

Figure 61 shows the amino acid sequence of Pritumumab HC-TGF $\beta$ RII-4-1BB fusion protein wherein the amino acid sequence for Pritumumab heavy chain (SEQ ID NO: 35) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for 4-1BB (immunomodulatory moiety) (SEQ ID NO: 9) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Pritumumab light chain (SEQ ID NO: 36).

Figure 62 shows the amino acid sequence of Cantuzumab HC-TGF $\beta$ RII-PD1 fusion protein wherein the amino acid sequence for Cantuzumab heavy chain (SEQ ID NO: 29) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGF $\beta$ RII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Cantuzumab light chain (SEQ ID NO: 30).

Figure 63 shows the amino acid sequence of Cixutumumab HC-TGFβRII-PD1 fusion protein wherein the amino acid sequence for Cixutumumab heavy chain (SEQ ID NO: 31) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGFβRII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Cixutumumab light chain (SEQ ID NO: 32).

Figure 64 shows the amino acid sequence of Clivatuzumab HC-TGFβRII-PD1 fusion protein wherein the amino acid sequence for Clivatuzumab heavy chain (SEQ ID NO: 33) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGFβRII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Clivatuzumab light chain (SEQ ID NO: 34).

Figure 65 shows the amino acid sequence of Pritumumab HC-TGFβRII-PD1 fusion protein wherein the amino acid sequence for Pritumumab heavy chain (SEQ ID NO: 35) is attached to a linker (SEQ ID NO: 3) shown in italics and the sequence for TGFβRII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for PD1 (immunomodulatory moiety) (SEQ ID NO: 10) is in written text font with linker between (SEQ ID No: 11) and including the amino acid sequence of Pritumumab light chain (SEQ ID NO: 36).

## DETAILED DESCRIPTION OF THE INVENTION

**[0030]** The practice of the present invention will employ, unless otherwise indicated, conventional techniques of immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook, et al. MOLECULAR CLONING: A LABORATORY MANUAL, 2nd edition (1989); CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (F. M. Ausubel, et al. eds., (1987)); the series METHODS IN ENZYMOLOGY (Academic Press, Inc.); PCR 2: A PRACTICAL APPROACH (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE (R. I. Freshney, ed. (1987)).

### Definitions

**[0031]** Unless otherwise defined, all technical and scientific terms used herein have the meaning commonly understood by one of ordinary skill in the art to which this invention belongs. As used in the description of the invention and the appended claims, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. The following terms have the meanings given:

**[0032]** The term "polynucleotide" as used herein means a sequence of nucleotides connected by phosphodiester linkages. Polynucleotides are presented herein in the direction from the 5' to the 3' direction. A polynucleotide used in the present invention can be a deoxyribonucleic acid (DNA) molecule or ribonucleic acid (RNA) molecule. Where a polynucleotide is a DNA molecule, that molecule can be a gene or a cDNA molecule. Nucleotide bases are indicated herein by a single letter code: adenine (A), guanine (G), thymine (T), cytosine (C), inosine (I) and uracil (U). A polynucleotide used in the present invention can be prepared using standard techniques well known to one of skill in the art.

**[0033]** The term, "optimized" as used herein means that a nucleotide sequence has been altered to encode an amino acid sequence using codons that are preferred in, a Chinese Hamster Ovary cell (CHO). The optimized nucleotide sequence is engineered to retain completely or as much as possible the amino acid sequence originally encoded by the starting nucleotide sequence, which is also known as the "parental" sequence. The optimized sequences herein have been engineered to have codons that are preferred in CHO mammalian cells. The amino acid sequences encoded by optimized nucleotide sequences are also referred to as optimized. The term "expression" as used herein is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

**[0034]** The term "transfection" of a cell as used herein means that genetic material is introduced into a cell for the purpose of genetically modifying the cell. Transfection can be accomplished by a variety of means known in the art, such as transduction or electroporation.

**[0035]** The term "cancer" as used herein is defined as disease characterized by the rapid and uncontrolled growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, ocular cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, lymphoma, leukemia, lung cancer and the like.

**[0036]** The term "transgene" is used in a broad sense to mean any heterologous nucleotide sequence incorporated in a vector for expression in a target cell and associated expression control sequences, such as promoters. It is appreciated by those of skill in the art that expression control sequences will be selected based on ability to promote expression of

the transgene in the target cell. An example of a transgene is a nucleic acid encoding a chimeric fusion protein of the present disclosure.

**[0037]** The term "expression vector" as used herein means a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. Expression vectors can contain a variety of control sequences, which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operatively linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well. The term also includes a recombinant plasmid or virus that comprises a polynucleotide to be delivered into a host cell, either in vitro or in vivo. The host cell is Chinese Hamster Ovary (CHO) cell.

**[0038]** The term "subject," as used herein means a human or vertebrate animal including a dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, rat, and mouse.

**[0039]** The term "therapeutically effective amount" as used herein means the amount of the subject compound that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

**[0040]** The term "pharmaceutically acceptable" as used herein means the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

**[0041]** The term "recombinant" as used herein means a genetic entity distinct from that generally found in nature. As applied to a polynucleotide or gene, this means that the polynucleotide is the product of various combinations of cloning, restriction and/or ligation steps, and other procedures that result in the production of a construct that is distinct from a polynucleotide found in nature.

**[0042]** The term "substantial identity" or "substantial similarity," as used herein when referring to a nucleic acid or fragment thereof, indicates that when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 95 to 99% of the sequence.

**[0043]** The term "peptide," "polypeptide" and "protein" are used interchangeably to denote a sequence polymer of at least two amino acids covalently linked by an amide bond.

**[0044]** The term "homologous" as used herein and relating to peptides refers to amino acid sequence similarity between two peptides. When an amino acid position in both of the peptides is occupied by identical amino acids, they are homologous at that position. Thus by "substantially homologous" means an amino acid sequence that is largely, but not entirely, homologous, and which retains most or all of the activity as the sequence to which it is homologous. As used herein, "substantially homologous" as used herein means that a sequence is at least 50% identical, and preferably at least 75% and more preferably 95% homology to the reference peptide. Additional peptide sequence modification are included, such as minor variations, deletions, substitutions or derivitizations of the amino acid sequence of the sequences disclosed herein, so long as the peptide has substantially the same activity or function as the unmodified peptides. Notably, a modified peptide will retain activity or function associated with the unmodified peptide, the modified peptide will generally have an amino acid sequence "substantially homologous" with the amino acid sequence of the unmodified sequence.

**[0045]** The term "administering" as used herein is defined as the actual physical introduction of the composition into or onto (as appropriate) the host subject. Any and all methods of introducing the composition into the subject are contemplated herein; the method is not dependent on any particular means of introduction and is not to be so construed. Means of introduction are well-known to those skilled in the art, and preferably, the composition is administered subcutaneously or intratumorally. One skilled in the art will recognize that, although more than one route can be used for administration, a particular route can provide a more immediate and more effective reaction than another route. Local or systemic delivery can be accomplished by administration comprising application or instillation of the immunovaccines into body cavities, inhalation or insufflation of an aerosol, or by parenteral introduction, comprising intramuscular, intravenous, intraportal, intrahepatic, peritoneal, subcutaneous, or intradermal administration. In the event that the tumor is in the central nervous system, the composition must be administered intratumorally because there is no priming of the immune system in the central nervous system.

**[0046]** Although chemotherapeutic agents can induce "immunogenic" tumor cell death and facilitate cross-presentation of antigens by dendritic cells, tumors create a tolerogenic environment that allows them to suppress the activation of innate and adaptive immune responses and evade immunologic attack by immune effector cells. The present disclosure provides strategies (not forming part of the claimed invention) to counteract tumor-induced immune tolerance in the tumor microenvironment and can enhance the antitumor efficacy of chemotherapy by activating and leveraging T cell-mediated adaptive antitumor immunity against disseminated cancer cells.

**[0047]** The present disclosure is based on the discovery that targeted immunomodulatory antibodies or fusion proteins of the present invention can counteract or reverse immune tolerance of cancer cells. Cancer cells are able to escape elimination by chemotherapeutic agents or tumor-targeted antibodies via specific immunosuppressive mechanisms in the tumor microenvironment and such ability of cancer cells is recognized as immune tolerance. By counteracting tumor-

induced immune tolerance, the present disclosure (not forming part of the claimed invention) provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment.

**[0048]** The present disclosure provides compositions and methods for producing fusion proteins that counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of durable long-term protection against recurrent or disseminated cancers (not forming part of the claimed invention). These fusion proteins are designed to facilitate effective long term T cell-mediated immune responses against tumor cells by at least one of the following:

- a. promoting death of tumor cells via enhancement of antibody-dependent cellular cytotoxicity (ADCC); and
- b. increasing activation and proliferation of antitumor CD8<sup>+</sup> T cells by negating immune suppression mediated by regulatory T cells and myeloid suppressor cells. These antitumor immune responses may be activated in tandem with the sensitization of tumor cells to immune effector-mediated cytotoxicity, thereby establishing a positive feedback loop that augments tumor cytorreduction and reinforces adaptive antitumor immunity.

**[0049]** In addition, the fusion proteins of the present disclosure (not forming part of the claimed invention) are distinguished from and superior to existing therapeutic, molecules in at least one of the following aspects: (i) To counteract immune tolerance in the tumor microenvironment and promote T cell-mediated adaptive antitumor immunity for maintenance of long-term protection against recurrent or disseminated cancers (for prevention or treatment of diverse cancers); (ii) To produce immune cell compositions for adoptive cellular therapy of diverse cancers; and (iii) To serve as immune adjuvants or vaccines for prophylaxis of diverse cancers or infectious diseases.

**[0050]** The targeted immunostimulatory antibodies and/or fusion proteins of the disclosure (not forming part of the claimed invention) provide the ability to disrupt immunosuppressive networks in the tumor microenvironment. Tumors employ a wide array of regulatory mechanisms to avoid or suppress the immune response. Cancer cells actively promote immune tolerance in the tumor microenvironment via the expression of cytokines and molecules that inhibit the differentiation and maturation of antigen-presenting dendritic cells (DC). The immunosuppressive cytokines and ligands produced by tumor cells include the following: (i) Transforming growth factor-beta (TGF- $\beta$ ); (ii) Programmed death-1 ligand 1 (PD-L1 ; B7-H1); (iii) Vascular endothelial growth factor (VEGF); and (iv) Interleukin-10 (IL-10).

**[0051]** In addition to blocking dendritic cell (DC) maturation, these molecules promote the development of specialized subsets of immunosuppressive CD4<sup>+</sup> T cells (regulatory T cells; Treg cells) and myeloid-derived suppressor cells (MDSC). Tregs are a minority sub-population of CD4<sup>+</sup> T cells that constitutively express CD25 [the interleukin-2 (IL-2) receptor  $\alpha$ -chain] and the forkhead box P3 (FOXP3) transcription factor. Tregs (CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> cells) maintain immune tolerance by restraining the activation, proliferation, and effector functions of a wide range of immune cells, including CD4 and CD8 T cells, natural killer (NK) and NKT cells, B cells and antigen presenting cells (APCs) in vitro and in vivo.

**[0052]** The accumulation of Treg cells in the tumor microenvironment reinforces tumor immune tolerance and facilitates tumor progression and metastases. The increased expression of immunosuppressive cytokines (TGF- $\beta$ ; PD-L1 ) and tumor-infiltrating Tregs is correlated with a reduction of survival of patients with diverse types of cancers. The fusion proteins disclosed herein (not forming part of the claimed invention) inhibit key immunosuppressive molecules expressed by the targeted tumor cell or tumor-infiltrating Treg cells and myeloid suppressor cells (DCs or MDSC). As such, they provide the targeted ability to inhibit the development or function of Tregs within the tumor microenvironment.

**[0053]** As used herein, the term "antibody" includes natural or artificial mono- or polyvalent antibodies including, but not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The antibody may be from any animal origin including birds and mammals. In one aspect, the antibody is, or derived from, a human, murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken. Further, such antibody may be a humanized version of an antibody. The antibody may be monospecific, bispecific, trispecific, or of greater multispecificity. The antibody herein specifically include a "chimeric" antibody in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity.

**[0054]** Various methods have been employed to produce antibodies. Hybridoma technology, which refers to a cloned cell line that produces a single type of antibody, uses the cells of various species, including mice (murine), hamsters, rats, and humans. Another method to prepare an antibody uses genetic engineering including recombinant DNA techniques. For example, antibodies made from these techniques include, among others, chimeric antibodies and humanized antibodies. A chimeric antibody combines DNA encoding regions from more than one type of species. For example, a chimeric antibody may derive the variable region from a mouse and the constant region from a human. A humanized antibody comes predominantly from a human, even though it contains nonhuman portions. Like a chimeric antibody, a

humanized antibody may contain a completely human constant region. But unlike a chimeric antibody, the variable region may be partially derived from a human. The nonhuman, synthetic portions of a humanized antibody often come from CDRs in murine antibodies. In any event, these regions are crucial to allow the antibody to recognize and bind to a specific antigen.

**[0055]** According to the invention, the method comprises preparing a codon optimized nucleotide sequence of the antibody-peptide fusion protein, wherein the codon optimized nucleotide sequence is optimized for expression in a Chinese Hamster Ovary (CHO) host cell, wherein the antibody-protein fusion protein comprises a targeting moiety and immunomodulating moiety, wherein the targeting moiety and the immunomodulating moiety are linked by an amino acid spacer selected from SEQ ID NO: 3 or SEQ ID NO: 11, wherein the immunomodulating moiety is TGF- $\beta$ RII comprising an amino acid sequence of SEQ ID NO: 4; wherein the targeting moiety is selected from the group consisting of an Anti-EGFR1 antibody, consisting of heavy chain SEQ ID NO: 5 and light chain SEQ ID NO: 6, an Anti-HER2/Neu antibody consisting of heavy chain SEQ ID NO: 1 and light chain SEQ ID NO: 2; and anti-CTLA4 antibody consisting of heavy chain of SEQ ID NO: 7 and a light chain of SEQ ID NO: 8, wherein SEQ ID NO: 4 is attached via the amino acid spacer to the C-terminus of SEQ ID NO: 1 or SEQ ID NO: 2 of Anti-HER2/Neu; C-terminus of SEQ ID NO: 5 or SEQ ID NO: 6 of Anti-EGFR1; or C-terminus of SEQ ID NO: 7 or SEQ ID NO: 8 of Anti-CTLA-4.

**[0056]** An antibody fragment can include a portion of an intact, antibody, e.g. including the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub>, and Fv fragments; Fc fragments or Fc-fusion products; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragment(s). An intact antibody is one which includes an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variant thereof for any other modified Fc (e.g. glycosylation or other engineered Fc).

**[0057]** . One preparing a codon optimized nucleotide sequence of the antibody-peptide fusion protein as previously described,, they are cloned into any suitable vector for expression. Numerous cloning vectors are known to those of skill in the art, and the selection of an appropriate cloning vector is a matter of choice. The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired polypeptide is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. Heterologous leader sequences can be added to the coding sequence that causes the secretion of the expressed polypeptide from the host organism. Other regulatory sequences may also be desirable which allow for regulation of expression of the protein sequences relative to the growth of the host cell. Such regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

**[0058]** The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

**[0059]** The expression vector may then used to transform an appropriate host cell, which is a Chinese Hamster Ovary (CHO) host cell capable of transient or continued expression.

**[0060]** The method of the invention further comprises growing the CHO host cell in a feed batch mode in a fermentation medium under suitable conditions for growing and allowing the CHO host cell to express a cloned protein, wherein the fermentation medium comprises a divalent transitional metallic salt; , wherein the divalent transitional metallic salt includes a zinc ion, wherein the divalent transitional metallic salt is zinc sulphate hepta hydrate salt.. The protein can then be isolated from the host cells and is purified. If the expression system secretes the protein into growth media, the protein can be purified directly from the media. If the protein is not secreted, it is isolated from cell lysates. The method comprises purifying the expressed antibody-peptide fusion protein and optionally checking the bi-specific binding capabilities of the antibody-peptide fusion protein to its targets. The selection of the appropriate growth conditions and recovery methods are within the skill of the art. Once purified, the amino acid sequences of the proteins can be determined, i.e., by repetitive cycles of Edman degradation, followed by amino acid analysis by HPLC. Other methods of amino acid sequencing are also known in the art.

**[0061]** Once produced, the inhibitory activity of a candidate polypeptide can be tested by assessing the ability of the candidate to inhibit the lipopolysaccharide-induced nuclear translocation of NF- $\kappa$ B by, for example, using murine endothelial cells.

## Experimental

**[0062]** Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have

been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

#### Example 1

**[0063]** The Fusion proteins comprising of IgG heavy chain linked to immunomodulator (either suppressor or activator) ligands were expressed by codon optimized genes for the expression of CHO cells. The codon optimized nucleotide sequences defined by SEQ ID NOs: 12 to 28 were expressed in (CHO) cells and the expressed chimeric/fusion proteins are shown in Table 1

Fusion protein Details	
Anti-HER2/neu heavy chain + TGFβ-RII ECD and Anti-HER2/neu light chain	
Anti-EGFR1 heavy chain + TGFβ-RII ECD and Anti- EGFR1 light chain	
Anti-CTLA4 heavy chain + TGFβ-RII ECD and Anti-CTLA4 light chain	
Anti-CTLA4 heavy chain + PD1 ectodomain and Anti-CTLA4 light chain	
Anti-HER2/neu heavy chain + 4-1BBL and Anti-HER2/neu light chain	
Anti-EGFR1 heavy chain + 4-1BBL and Anti- EGFR1 light chain	
Anti-CTLA4 heavy chain + 4-1BBL and Anti-CTLA4 light chain	
PD1 ectodomain-Fc-4-1BBL	
TGFβRII ECD-Fc-4-1BBL	
Anti-EGFR1 heavy chain + PD1 ectodomain and Anti- EGFR1 light chain	
Anti-CD20 heavy chain + 4-1BBL and Anti- CD20 light chain	
Anti-HER2/neu heavy chain + PD1 ectodomain and Anti-HER2/neu light chain	
Anti-IL6Rheavy chain + PD1 ectodomain and Anti-IL6R light chain	
Anti-IL6Rheavy chain + TGFβ-RII ECD and Anti-IL6R light chain	
Anti-4-1BB heavy chain + PD1 ectodomain and Anti-4-1BB light chain	

**[0064]** The expressed protein were characterized by using SDS PAGE and the expressed fusion proteins Anti-HER2/neu-TGFβRII and Anti-EGFR1- TGFβRII were purified from culture supernatants using ProteinA column and the results are shown in Figure 22. Notably, Anti-EGFR1-TGFβRII light chain mass is higher and it may be because of the presence of two glycosylation sites on the variable regions light and heavy chain. Both the & Anti-EGFR1-TGFβRII heavy chains mass are higher because of the TGFβRII. Also heavy chain has four N-glycosylation sites while Anti-EGFR1-TGFβRII has five N-glycosylation sites.

#### Example 2

**[0065]** Protein A/SEC chromatography. The and Anti-EGFR1-TGFβRII samples were analyzed by ProteinA/SEC chromatography and the results are shown in Figure 23. Figure 23 A shows a sharp peak of elution of Bmab200(Herceptin) vs a broader elution peak is believed to be a measure of heterogeneity due to presence of glycosylation as there are three additional N-glycosylation sites that are present in the TGFβRII region. Notably storage at -80C did not causing aggregation. The shift in the position or appearance of the peak early in SEC column indicates that the increase in the molecular weight is because of the fusion partner. This once again confirms that the full length molecule is being expressed. Figure 23 B shows a sharp peak of elution of Bmab200(Herceptin) vs a broader elution peak which is believed to be a measure of heterogeneity due to presence of glycosylation sites as there are three additional N-glycosylation sites are present in the TGFβRII region. Again, storage at -80C did not causing aggregation. The shift in the position or appearance of the peak early in SEC column indicates that the increase in the molecular weight is because of the fusion partner. This once again confirms that the full length molecule is being expressed.

## Example 3

**[0066]** Functional assays for the Fusion proteins. ELISA experiment was carried out to check the binding ability Anti-HER2/neu-TGF $\beta$ RII of and Anti-EGFR1-TGF $\beta$ RII to TGF $\beta$ . Figure 24 A shows that Anti-HER2/neu-TGF $\beta$ RII and Anti-EGFR1-TGF $\beta$ RII molecules bind to the TGF $\beta$  indicating that the fusion protein is functional. Figure 24 B shows that Anti-HER2-TGF $\beta$ RII inhibits the proliferation of BT474 cell line similar to the Bmab200 (Herceptin). Figure 25 shows that Anti-EGFR1-TGF $\beta$ RII-inhibits the proliferation of A431 cell line similar to the Cetuximab.

## Example 4

**[0067]** Antibody dependent cellular cytotoxicity ADCC activity for Anti-HER2/neu-TGF $\beta$ RII fusion protein was conducted to determine that the protein binds to the target receptors on the cells. The results are shown in Figure 26 wherein the activity is determined in BT474 cells and it is evident that ADCC activity (%lysis of cells) of Anti-HER2-TGF $\beta$ RII on BT474 cells is similar to that of Bmab200(Herceptin). Figure 27 shows ADCC activity of Anti-EGFR1-TGF $\beta$ RII on A431 cells wherein the ADCC activities are similar to that of Cetuximab. Figure 28 shows the ADCC activity of ADCC activity of Anti-EGFR1-4-1BB in comparison with Anti-EGFR1-TGF $\beta$ RII and cetuximab.

## Example 5

**[0068]** Binding Activity of the expressed proteins. The aim of this assay is to test the functionality of the fusion proteins to bind to the target receptors on the cells in a dose dependent manner. Figure 29 A shows that the binding activity of Anti-CTLA4-TGF $\beta$ RII to TGF $\beta$ 1 is comparable to Anti-EGFR1-TGF $\beta$ RII and B shows that the binding activity of Anti-CTLA4-TGF $\beta$ RII to CTLA4. Figure 30 A shows the binding activity of Anti-CTLA4-TGF $\beta$ RII to determine the level of PD1-Fc binding and B shows the binding activity of Anti-EGFR1-4-1BB to determine the binding of 4-1BBL. Figure 31 A shows the binding activity of Anti-EGFR1-4-1BB to EGFR and B shows the binding activity of PD1-Fc-4-1BB to find out PDL1-Fc. Figure 32 shows the binding activity of Anti-EGFR1-PD1 to EGFR and PD1.

## Example 6

**[0069]** Confirmation of primary structure of molecule. As shown in Figure 33, the expressed proteins are evaluated to determine the molecular weight and the presence of glycosylation. The samples were analyzed by reducing and non-reducing SDS PAGE. The heavy and light chains of the antibody are separated by reduction alkylation so that the reduced structures can be evaluated. Tryptic digestion of the fusion proteins provides for the identification of the primary sequence. MS/MS analysis of the proteins is performed.

**[0070]** Mass Spectrometry Analysis of Anti-HER2/neu-TGF $\beta$ RII and Anti-EGFR1-TGF $\beta$ RII. The fusion protein shown in Figure 1 was expressed and tested. Figure 34 A shows the mass spectrum Mass Spectrum of light chain (LC )(Reduced) of Anti-HER2/neu-TGF $\beta$ RII ECD fusion and B shows Deconvoluted Mass Spectrum of Anti-HER2/neu-TGF $\beta$ RII LC (Reduced) of ECD fusion. Figure 35 shows the Mass Spectrum of heavy chain (HC) (Reduced) of Anti-HER2/neu-TGF $\beta$ RII ECD fusion.

**[0071]** The fusion protein shown in Figure 2 was expressed and tested. Figure 36 A shows the Mass Spectrum of LC (Reduced) of Anti-EGFR1-TGF $\beta$ RII ECD and B shows the Deconvoluted Mass Spectrum of LC (Reduced) of Anti-EGFR1-TGF $\beta$ RII ECD. Figure 37 shows the Mass Spectrum of HC (Reduced) of Anti-EGFR1-TGF $\beta$ RII ECD.

## Example 7

**[0072]** The fusion proteins having amino acid sequences as described in Figures 1 and 2 were inspected using UV chromatography and providing chromatograms resulting from the chromatographic separation of the tryptic digest of the fusion proteins and tested with UV 218-222 nm wavelength. Total Ion Current (TIC) corresponding to UV trace was also evaluated. Figure 38 A shows the UV Chromatogram of Tryptic Peptides of ECD fusion protein and B shows the Total Ion Chromatogram (TIC) of Tryptic Peptides of Anti-HER2/neu-TGF $\beta$ RII ECD fusion protein. Figures 39, 40 and 41 provide lists of expected/observed tryptic peptide of the light chain, heavy chain and linked motif of the Anti-HER2/neu-TGF $\beta$ RII ECD fusion protein, respectively. Notably, all the expected peptides of the molecules were identified including the light and heavy chain peptides and the peptides of the linked motif (TGF  $\beta$ RII).

**[0073]** Figure 42 A shows the UV Chromatogram of Tryptic Peptides of Anti-EGFR1-TGF $\beta$ RII ECD fusion protein and B shows the Total Ion Chromatogram (TIC) of Tryptic Peptides of Anti-EGFR1-TGF $\beta$ RII ECD fusion protein. Figures 43, 44, and 45 provide lists of expected/observed tryptic peptide of the light chain, heavy chain and linked motif of the Anti-EGFR1-TGF $\beta$ RII ECD fusion protein, respectively. Again all the expected peptides of the molecules were identified including the light and heavy chain peptides and the peptides of the linked motif (TGF  $\beta$ RII).



## Example 8

**[0074]** The host cell line used for the expression of recombinant fusion protein expression is CHO cells or the derivative of the CHO cells. The CHO cells referred here is either freedom CHO-S cells; CHO-S Cells are CHO-derived cells adapted to high density, serum-free suspension culture in chemically-defined medium that are capable of producing high levels of secreted, recombinant protein or CHO K1 cells; having the same as ATCC No. CCL-61. It is basically an adherent cell line. The vectors used for stable cell line:

**[0075]** The Freedom pCHO 1.0 vector, designed by ProBioGen AG, to express one or two genes of interest downstream of the vector's two different hybrid CMV promoters. This vector contains the dihydrofolate reductase (DHFR) selection marker and a puromycin resistance gene, allowing selection using MTX and Puromycin simultaneously.

**[0076]** The light chain or the light chain fusion protein coding nucleic acid sequences are cloned into the restriction enzyme sites AvrII and BstZ17 under the control of EF2/CMV promoter. The heavy chain or the heavy chain fusion protein coding nucleic acid sequences are cloned, in restriction enzyme sites EcoRV and Pad under the control of CMV/EF1 promoter.

**[0077]** The construct(s) are transfected into Freedom CHO-S cells/CHOK1 cells. The high producer single, clonal cell strain is selected for producing the recombinant fusion protein. Prepare the MCB and characterize for cell viability, productivity, stability and other parameters. The cells are used for culturing followed by purification.

## Example 9

**[0078]** The cell culture is performed in feed-batch mode. In the cell culture, the mammalian host cells used is Chinese Hamster Ovary (CHO) cells and culture medium are supplied initially. The CHO cells are genetically engineered to produce the Antibody-peptide fusion protein. The zinc sulphate hepta hydrate salt is added in the medium at a concentration of 0.4 mM. In contrast, there is no addition of any zinc salt in the control medium. The production fermentation run starts with an initial cell count of  $0.3\text{--}0.45 \times 10^6$  cells/ml at  $37 \pm 1^\circ\text{C}$ , the first 3-4 days are dedicated to grow the cells in batch phase. Next step involves lowering the temperature to  $31 \pm 1^\circ\text{C}$  and continuing the run till 7th day. Lactate reduces by almost 10-40% throughout the run. The produced fusion protein is then collected from the media using the technique of affinity chromatography.

## Example 10

**[0079]** The cell culture is performed in a feed-batch mode is employed. In the cell cultures the mammalian host cells and culture medium which is Hyclone CDM4Mab are supplied initially. The salts (zinc) is also added in the medium (0.3mM). The production fermentation run starts with an initial cell count of  $0.3\text{--}0.45 \times 10^6$  cells/ml at  $37 \pm 1^\circ\text{C}$ , the first 3-4 days are dedicated to growing the cells in batch phase. Next step involves lowering the temperature to  $31 \pm 1^\circ\text{C}$  and continuing the run till 7th day.

## Example 11

**[0080]** Purification of antibody-peptide fusion immunostimulatory molecules using protein A column. Supernatant culture secreted from recombinant CHO cell line containing the fusion monoclonal antibodies is tested for titer and endotoxins under sterile conditions. The supernatant is subjected to affinity chromatography using Mab Select Xtra Protein A affinity resin, washed and equilibrated with binding buffer. The pH of the supernatant is adjusted using 0.5M phosphate to the same pH as the column; the supernatant is allowed to bind to the column/ pass through the column at the flow rate of 0.5 ml/minute to achieve the maximum binding. All the Antibody-proteins fusion molecules bind through the Fc region while impurities are eliminated as flow through.. The column is washed with equilibration buffer and the bound fusion molecules are eluted using 0.1 M glycine at pH 3.0. The pH of the eluted proteins is adjusted to neutral pH or the stable formulation pH and the purified protein are stored at  $-20^\circ\text{C}$  or at  $2\text{--}8^\circ\text{C}$ .

## Example 12

Differentiating Trastuzumab from Trastuzumab-TGF  $\beta$ RII receptor fusion molecule

**[0081]** A breast cancer tumor overexpressing the ErbB2 receptor will either by constitutive activation or heterodimerization with other members of the ErbB family of receptors lead to tumor progression. This will involve the binding of growth factors associated with the ErbB signaling pathway. In addition to this, the tumor creates a milieu wherein the immune system is suppressed by activating TGF  $\beta$  and specific cytokines involved in the subdued immune response. A novel molecule is generated wherein Trastuzumab (anti ErbB2) is fused with the TGF  $\beta$ RII receptor as a fusion protein.

While it is hypothesized that Trastuzumab will act as a targeted molecule homing into the ErbB2 overexpressing breast cancer cells, the TGF $\beta$ RII receptor will sequester TGF $\beta$  leading to immune activation. The experiment will utilize the growth of Herceptin resistant ErbB2 expressing cell lines (selected by growing BT474 cells in the presence of Herceptin) in the presence of TGF $\beta$ , cytotoxic CD8 positive cells and NK cells. While Trastuzumab will be ineffective in inducing cytotoxicity Trastuzumab TGF $\beta$ RII receptor fusion molecule will sequester the TGF $\beta$  thereby preventing the inhibition of cytotoxic CD8 and NK cells. This will lead to enhanced cytotoxicity observed in Trastuzumab -TGF $\beta$ RII receptor fusion treated cells over cells treated with Trastuzumab alone. The readout for the experiment will use Alamar Blue a resazurin dye which will get activated directly proportional to live cells present. Another method could be to measure cytotoxicity by using cytotox glo which measures protease release which directly corresponds to proportional dead cells. Yet another method could be the use of the flow cytometer directly measuring apoptotic and necrotic cell population by using Annexin V and propidium iodide. Results from these multiple experiments will elucidate understanding of the activity of the conjugate molecule as compared to Trastuzumab alone.

## SEQUENCE LISTING

[0082]

&lt;110&gt; Biocon Limited

&lt;120&gt; TARGETED/IMMUNOMODULATORY FUSION PROTEINS AND METHODS FOR MAKING SAME

&lt;130&gt; P11512EPPC02

&lt;150&gt; 1690/CHE/2012

&lt;151&gt; 2012-04-30

&lt;150&gt; 1689/CHE.2012

&lt;151&gt; 2012-04-30

&lt;160&gt; 39

&lt;170&gt; PatentIn version 3.5

&lt;210&gt; 1

&lt;211&gt; 449

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Synthetic construct

&lt;400&gt; 1

EP 3 489 254 B9

	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	
	1				5					10					15		
5	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asn	Ile	Lys	Asp	Thr	
				20					25					30			
10	Tyr	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
			35					40					45				
15	Ala	Arg	Ile	Tyr	Pro	Thr	Asn	Gly	Tyr	Thr	Arg	Tyr	Ala	Asp	Ser	Val	
	50						55					60					
20	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Ala	Asp	Thr	Ser	Lys	Asn	Thr	Ala	Tyr	
	65					70					75					80	
25	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	
				85						90				95			
30	Ser	Arg	Trp	Gly	Gly	Asp	Gly	Phe	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln	
				100					105					110			
35	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	
			115					120					125				
40	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	
	130						135					140					

EP 3 489 254 B9

	Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser	
	145	150 155 160
5	Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val	
		165 170 175
10	Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro	
		180 185 190
15	Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys	
		195 200 205
20	Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp	
		210 215 220
25	Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly	
		225 230 235 240
30	Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile	
		245 250 255
35	Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu	
		260 265 270
40	Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His	
		275 280 285
45	Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg	
		290 295 300
50	Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys	
		305 310 315 320
55	Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu	
		325 330 335
60	Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr	
		340 345 350
65	Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu	
		355 360 365
70	Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp	
		370 375 380
75	Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val	

# EP 3 489 254 B9

	385		390		395		400									
5	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp
					405					410					415	
10	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His
				420					425					430		
15	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro
			435					440					445			
20																
25																
30																
35																
40																
45																
50																
55																

Gly

<210> 2

<211> 214

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 2

EP 3 489 254 B9

	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	
	1				5					10					15		
5	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Asp	Val	Asn	Thr	Ala	
				20					25					30			
10	Val	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile	
			35					40					45				
15	Tyr	Ser	Ala	Ser	Phe	Leu	Tyr	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly	
		50					55					60					
20	Ser	Arg	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	
	65					70					75					80	
25	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	His	Tyr	Thr	Thr	Pro	Pro	
					85					90					95		
30	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	
				100					105					110			
35	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	
			115					120					125				
40	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	
		130					135					140					
45	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	
	145					150					155					160	
50	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	
					165					170					175		
55	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	
				180					185					190			
60	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	
			195					200					205				
65	Phe	Asn	Arg	Gly	Glu	Cys											
		210															

<210> 3

<211> 15

<212> PRT

<213> Artificial Sequence

EP 3 489 254 B9

<220>

<223> Synthetic construct

<400> 3

5

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

10

<210> 4

<211> 137

<212> PRT

<213> Artificial Sequence

15

<220>

<223> Synthetic construct

<400> 4

20

Thr Ile Pro Pro His Val Gln Lys Ser Val Asn Asn Asp Met Ile Val  
1 5 10 15

25

Thr Asp Asn Asn Gly Ala Val Lys Phe Pro Gln Leu Cys Lys Phe Cys  
20 25 30

30

Asp Val Arg Phe Ser Thr Cys Asp Asn Gln Lys Ser Cys Met Ser Asn  
35 40 45

Cys Ser Ile Thr Ser Ile Cys Glu Lys Pro Gln Glu Val Cys Val Ala  
50 55 60

35

Val Trp Arg Lys Asn Asp Glu Asn Ile Thr Leu Glu Thr Val Cys His

65 70 75 80

40

Asp Pro Lys Leu Pro Tyr His Asp Phe Ile Leu Glu Asp Ala Ala Ser  
85 90 95

45

Pro Lys Cys Ile Met Lys Glu Lys Lys Lys Pro Gly Glu Thr Phe Phe  
100 105 110

50

Met Cys Ser Cys Ser Ser Asp Glu Cys Asn Asp Asn Ile Ile Phe Ser  
115 120 125

Glu Glu Tyr Asn Thr Ser Asn Pro Asp  
130 135

55

<210> 5

<211> 448

<212> PRT

EP 3 489 254 B9

<213> Artificial Sequence

<220>

<223> Synthetic Construct

5

<400> 5

10

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

15

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

20

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

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

25

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

30

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

Arg Ala Leu Thr Tyr Tyr Asp Tyr Glu Phe Ala Tyr Trp Gly Gln Gly  
100 105 110

35

Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val Phe  
115 120 125

40

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu  
130 135 140

45

50

55



EP 3 489 254 B9

	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp
	145					150					155					160
5	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu
					165					170					175	
10	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser
				180					185					190		
15	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro
			195					200					205			
20	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys
	210						215					220				
25	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro
	225					230					235					240
30	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser
					245					250					255	
35	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp
				260					265					270		
40	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn
			275					280					285			
45	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val
	290						295					300				
50	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu
	305					310					315					320
55	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys
					325					330					335	
60	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr
				340					345					350		
65	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr
			355					360					365			
70	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu
	370						375					380				
75	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu

# EP 3 489 254 B9

	385		390		395		400									
5	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys
					405					410					415	
10	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu
				420					425					430		
15	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly
			435					440					445			
	<210> 6															
	<211> 214															
	<212> PRT															
	<213> Artificial Sequence															
20	<220>															
	<223> Synthetic construct															
	<400> 6															
25																
30																
35																
40																
45																
50																
55																

EP 3 489 254 B9

	Asp	Ile	Leu	Leu	Thr	Gln	Ser	Pro	Val	Ile	Leu	Ser	Val	Ser	Pro	Gly	
	1				5					10					15		
5	Glu	Arg	Val	Ser	Phe	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Gly	Thr	Asn	
				20					25					30			
10	Ile	His	Trp	Tyr	Gln	Gln	Arg	Thr	Asn	Gly	Ser	Pro	Arg	Leu	Leu	Ile	
			35					40					45				
15	Lys	Tyr	Ala	Ser	Glu	Ser	Ile	Ser	Gly	Ile	Pro	Ser	Arg	Phe	Ser	Gly	
		50					55					60					
20	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Ser	Ile	Asn	Ser	Val	Glu	Ser	
	65					70					75					80	
25	Glu	Asp	Ile	Ala	Asp	Tyr	Tyr	Cys	Gln	Gln	Asn	Asn	Asn	Trp	Pro	Thr	
					85					90					95		
30	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Arg	Thr	Val	Ala	Ala	
				100					105					110			
35	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	
			115					120					125				
40	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	
		130					135					140					
45	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	
	145					150					155					160	
50	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	
					165				170						175		
55	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	
				180					185					190			
60	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	
			195					200					205				
65	Phe	Asn	Arg	Gly	Glu	Cys											
		210															

<210> 7

<211> 447

<212> PRT

<213> Artificial Sequence

<220>

EP 3 489 254 B9

<223> Synthetic construct

<400> 7

5	Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg	1	5	10	15
10	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr	20	25	30	
15	Thr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val	35	40	45	
20	Thr Phe Ile Ser Tyr Asp Gly Asn Asn Lys Tyr Tyr Ala Asp Ser Val	50	55	60	
25	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr	65	70	75	80
30	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Ile Tyr Tyr Cys	85	90	95	
35	Ala Arg Thr Gly Trp Leu Gly Pro Phe Asp Tyr Trp Gly Gln Gly Thr	100	105	110	
40	Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro	115	120	125	
45	Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly	130	135	140	

EP 3 489 254 B9

	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	145	150	155				160
5	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln		165	170				175
10	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser		180	185				190
	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser		195	200				205
15	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr		210	215				220
20	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser		225	230				235
25	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg		245	250				255
	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro		260	265				270
30	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala		275	280				285
35	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val		290	295				300
40	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr		305	310				315
	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr		325	330				335
45	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu		340	345				350
50	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys		355	360				365
	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser		370	375				380
55	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp		385	390				395
																							400

EP 3 489 254 B9

	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	
					405					410					415		
5	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	
					420					425					430		
10	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly		
					435					440					445		
	<210> 8																
	<211> 215																
	<212> PRT																
15	<213> Artificial Sequence																
	<220>																
	<223> Synthetic construct																
20	<400> 8																
	Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	Leu	Ser	Pro	Gly	
	1				5					10					15		
25	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Arg	Ala	Ser	Gln	Ser	Val	Gly	Ser	Ser	
					20					25					30		
30	Tyr	Leu	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	Leu	Leu	
					35				40					45			
35	Ile	Tyr	Gly	Ala	Phe	Ser	Arg	Ala	Thr	Gly	Ile	Pro	Asp	Arg	Phe	Ser	
					50			55					60				
	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Arg	Leu	Glu	
	65					70					75					80	
40	Pro	Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Tyr	Gly	Ser	Ser	Pro	
					85					90					95		
45	Trp	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	
					100				105					110			
50	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	
					115			120					125				
	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg	Glu	
					130			135				140					
55	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	
					145			150			155					160	

EP 3 489 254 B9

	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	
					165					170					175		
5	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	
				180					185					190			
10	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	
			195					200					205				
15	Ser	Phe	Asn	Arg	Gly	Glu	Cys										
		210					215										
	<210> 9																
	<211> 205																
	<212> PRT																
	<213> Artificial Sequence																
20	<220>																
	<223> Synthetic construct																
	<400> 9																
25																	
	Ala	Cys	Pro	Trp	Ala	Val	Ser	Gly	Ala	Arg	Ala	Ser	Pro	Gly	Ser	Ala	
	1				5					10					15		
30	Ala	Ser	Pro	Arg	Leu	Arg	Glu	Gly	Pro	Glu	Leu	Ser	Pro	Asp	Asp	Pro	
				20					25					30			
35	Ala	Gly	Leu	Leu	Asp	Leu	Arg	Gln	Gly	Met	Phe	Ala	Gln	Leu	Val	Ala	
			35					40					45				
40	Gln	Asn	Val	Leu	Leu	Ile	Asp	Gly	Pro	Leu	Ser	Trp	Tyr	Ser	Asp	Pro	
		50					55					60					
45	Gly	Leu	Ala	Gly	Val	Ser	Leu	Thr	Gly	Gly	Leu	Ser	Tyr	Lys	Glu	Asp	
	65					70					75				80		
50	Thr	Lys	Glu	Leu	Val	Val	Ala	Lys	Ala	Gly	Val	Tyr	Tyr	Val	Phe	Phe	
					85					90					95		
55	Gln	Leu	Glu	Leu	Arg	Arg	Val	Val	Ala	Gly	Glu	Gly	Ser	Gly	Ser	Val	
				100					105					110			
	Ser	Leu	Ala	Leu	His	Leu	Gln	Pro	Leu	Arg	Ser	Ala	Ala	Gly	Ala	Ala	
			115					120					125				
	Ala	Leu	Ala	Leu	Thr	Val	Asp	Leu	Pro	Pro	Ala	Ser	Ser	Glu	Ala	Arg	
		130					135					140					

# EP 3 489 254 B9

Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser Ala Gly  
145 150 155 160

5 Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg His Ala  
165 170 175

10 Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg Val Thr  
180 185 190

15 Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu  
195 200 205

<210> 10

<211> 150

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 10



EP 3 489 254 B9

	Pro	Gly	Trp	Phe	Leu	Asp	Ser	Pro	Asp	Arg	Pro	Trp	Asn	Pro	Pro	Thr
	1				5					10					15	
5	Phe	Ser	Pro	Ala	Leu	Leu	Val	Val	Thr	Glu	Gly	Asp	Asn	Ala	Thr	Phe
				20					25					30		
	Thr	Cys	Ser	Phe	Ser	Asn	Thr	Ser	Glu	Ser	Phe	Val	Leu	Asn	Trp	Tyr
10			35					40					45			
	Arg	Met	Ser	Pro	Ser	Asn	Gln	Thr	Asp	Lys	Leu	Ala	Ala	Phe	Pro	Glu
		50					55					60				
15	Asp	Arg	Ser	Gln	Pro	Gly	Gln	Asp	Cys	Arg	Phe	Arg	Val	Thr	Gln	Leu
	65					70					75					80
	Pro	Asn	Gly	Arg	Asp	Phe	His	Met	Ser	Val	Val	Arg	Ala	Arg	Arg	Asn
20					85					90					95	
	Asp	Ser	Gly	Thr	Tyr	Leu	Cys	Gly	Ala	Ile	Ser	Leu	Ala	Pro	Lys	Ala
25				100					105					110		
	Gln	Ile	Lys	Glu	Ser	Leu	Arg	Ala	Glu	Leu	Arg	Val	Thr	Glu	Arg	Arg
			115					120					125			
30	Ala	Glu	Val	Pro	Thr	Ala	His	Pro	Ser	Pro	Ser	Pro	Arg	Pro	Ala	Gly
		130					135					140				
35	Gln	Phe	Gln	Thr	Leu	Val										

145

150

40 <210> 11  
 <211> 7  
 <212> PRT  
 <213> Artificial Sequence

45 <220>  
 <223> Synthetic construct

<400> 11

50 Glu Pro Lys Ser Cys Asp Lys  
 1 5

55 <210> 12  
 <211> 1032  
 <212> DNA  
 <213> Artificial Sequence

# EP 3 489 254 B9

<220>

<223> Synthetic construct

<400> 12

5  
gctagcacca agggcccctc cgtgttccct ctggccccct ccagcaagtc cacctctggc 60  
ggcaccgccg ctctgggctg cctgggtcaag gactacttcc ccgagcccgt gaccgtgtcc 120  
10 tggaaactctg gcgctctgac ctccggcgtg cacaccttcc ctgccgtgct gcagtcctcc 180  
ggcctgtact ccctgtcctc cgtcgtgacc gtgccctcca gctctctggg caccagacc 240  
tacatctgca acgtgaacca caagccctcc aacaccaagg tggacaagaa ggtggaaccc 300  
15 aagtcctgcg acaagaccca cacctgtccc ccctgccctg cccctgagct cctgggaggg 360  
cctagcgtgt tcctgttccc cccaaagccc aaggacaccc tgatgatctc ccggaccccc 420  
20 gaagtgacct gcgtgggtgg ggacgtgtcc cacgaggacc ctgaagtgaa gttcaattgg 480  
tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ccagagagga acagtacaac 540  
tccacctacc ggggtggtgtc cgtgctgacc gtgctgcacc aggactggct gaacggcaaa 600  
25 gagtacaagt gcaaggtgtc caacaaggcc ctgcctgccc ccacgaaaa gaccatctcc 660  
aaggccaagg gccagccccg cgagcctcag gtgtacaccc tgccccctag ccgggaagag 720  
atgaccaaga accaggtgtc cctgacctgt ctgggtcaagg gcttctaccc ctccgatatc 780  
30 gccgtggaat gggagtccaa cggccagccc gagaacaact acaagaccac cccccctgtg 840  
ctggactccg acggctcatt cttcctgtac tccaagctga ccgtggacaa gtcccgggtg 900  
cagcagggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc 960  
35 cagaagtccc tgtccctgag ccagggcaaa ggcgaggagg gatctggcgg cggaggatct 1020  
ggtggcggat cc 1032

<210> 13

<211> 425

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 13

# EP 3 489 254 B9

ggatccacca tccccccaca cgtgcagaaa tccgtgaaca acgacatgat cgtgaccgac 60  
 aacaacggcg ctgtgaagtt cccccagctg tgcaagttct gcgacgtgcg gttctctacc 120  
 5 tgcgacaacc agaaatcctg catgtccaac tgctccatca cctccatctg cgagaagccc 180  
 caggaagtgt gcgtcgccgt ctggcggaag aacgacgaga acatcaccct ggaaaccgtg 240  
 tgccacgacc ccaagctgcc ctaccacgac ttcatcctgg aagatgccgc ctcccccaag 300  
 10 tgcacatga aggaaaagaa gaagcccggc gagactttct tcatgtgcag ctgctcctcc 360  
 gacgagtgc aacgacaacat catcttctcc gaagagtaca acacctcaa ccccgactga 420  
 15 agctt 425

<210> 14  
 <211> 430  
 <212> DNA  
 20 <213> Artificial Sequence  
 <220>  
 <223> Synthetic construct  
 25 <400> 14

gcggccgcca tgaacttcgg cctgcggctg atcttcctgg tgctgaccct gaagggcgtg 60  
 30 cagtgcgagg tgcagctggt ggaatccggc ggaggcctgg tccagcctgg cggatctctg 120  
 agactgtcct gcgccgcctc cggcttcaac atcaaggaca cctacatcca ctgggtccga 180  
 caggcccctg gcaagggcct ggaatgggtg gcccgatct accccaccaa cggctacacc 240  
 35 agatacgccg actccgtgaa gggccgggtc accatctccg ccgacacctc caagaacacc 300  
 gcctacctgc agatgaactc cctgcggggc gaggacaccg ccgtgtacta ctgctccaga 360  
 tggggaggcg acggcttcta cgccatggac tactggggcc agggcaccct ggtcaccgtg 420  
 40 ctccgctagc 430

<210> 15  
 <211> 442  
 45 <212> DNA  
 <213> Artificial Sequence  
 <220>  
 <223> Synthetic construct  
 50 <400> 15

gcggccgcca tggaatccca gaccaggtg ctgatctccc tgctgttctg ggtgtccggc 60  
 55

EP 3 489 254 B9

	acctgtggcg	acatccagat	gacccagtc	ccctccagcc	tgtccgcctc	tgtgggagac	120
	agagtgacca	tcacctgtcg	ggcctcccag	gacgtgaaca	ccgccgtggc	ctggtatcag	180
5	cagaagcccg	gcaaggcccc	caagctgctg	atctactccg	cctccttcct	gtactccggc	240
	gtgccctccc	ggttctccgg	ctctagatcc	ggcaccgact	ttaccctgac	catctccagc	300
	ctgcagcccg	aggacttcgc	cacctactac	tgccagcagc	actacaccac	cccccccacc	360
10	tttggccagg	gcaccaaggt	ggaaatcaag	cggaccgtgg	ccgctccctc	cgtgttcac	420
	cccaccctcc	gacgagcagc	tg				442
15	<210> 16						
	<211> 1032						
	<212> DNA						
	<213> Artificial Sequence						
20	<220>						
	<223> Synthetic construct						
	<400> 16						
25	gctagcacca	agggccctc	cgtgtttccc	ctggccccct	ccagcaagtc	cacctctggc	60
	ggcaccgccg	ctctgggctg	cctggtcaag	gactacttcc	ccgagcccgt	gaccgtgtcc	120
	tggaaactctg	gcgctctgac	ctccggcgtg	cacaccttcc	ctgccgtgct	gcagtccctc	180
30	ggcctgtact	ccctgtcctc	cgtcgtgacc	gtgccctcca	gctctctggg	caccagacc	240
	tacatctgca	acgtgaacca	caagccctcc	aacaccaagg	tggacaagcg	ggtggaaccc	300
35	aagtccctgcg	acaagaccca	cacctgtccc	ccctgccctg	cccctgaact	gctgggaggc	360
	ccttccgtgt	tcctgttccc	cccaaagccc	aaggacaccc	tgatgatctc	ccggaccccc	420
	gaagtgacct	gcgtgggtgt	ggacgtgtcc	cacgaggacc	ctgaagtga	gttcaattgg	480
40	tacgtggacg	gcgtggaagt	gcacaacgcc	aagaccaagc	ccagagagga	acagtacaac	540
	tccacctacc	gggtggtgtc	cgtgctgacc	gtgctgcacc	aggactggct	gaacggcaaa	600
	gagtacaagt	gcaaggtgtc	caacaaggcc	ctgcctgccc	ccatcgaaaa	gaccatctcc	660
45	aaggccaagg	gccagccccg	cgagcctcag	gtgtacaccc	tgcctcccag	ccgggacgag	720
	ctgaccaaga	accaggtgtc	cctgacctgt	ctggtcaagg	gcttctaccc	ctccgatatc	780
50	gccgtggaat	gggagtccaa	cggccagccc	gagaacaact	acaagaccac	ccccctgtg	840
	ctggactccg	acggctcatt	cttcctgtac	tccaagctga	ccgtggacaa	gtcccgggtg	900
	cagcagggca	acgtgttctc	ctgctccgtg	atgcacgagg	ccctgcacaa	ccactacacc	960
55	cagaagtccc	tgtctctgag	ccccggcaaa	ggcggcggag	gatctggcgg	tggcggatca	1020
	ggcggaggat	cc					1032

# EP 3 489 254 B9

<210> 17  
<211> 427  
<212> DNA  
<213> Artificial Sequence

5

<220>  
<223> Synthetic construct

10

gcggccgcca tgaacttcgg cctgcggctg atcttcctgg tgctgaccct gaagggcgtg	60
cagtgccagg tgcagctgaa gcagtccgga cctggcctgg tgcagccttc ccagtccctg	120
tccatcacct gtaccgtgtc cggcttctcc ctgaccaact acggcgtgca ctgggtccga	180
cagtccccag gcaagggcct ggaatggctg ggagtgattt ggagcggcgg caacaccgac	240
tacaacaccc ctttcacctc ccggctgtcc atcaacaagg acaactccaa gtcccagggtg	300
ttcttcaaga tgaactccct gcagtccaac gacaccgcca tctactactg cgccagagcc	360
ctgacctact atgactacga gttcgccctac tggggacagg gcaccctggt caccgtgtct	420
cgctagc	427

25

<210> 18  
<211> 442  
<212> DNA  
<213> Artificial Sequence

30

<220>  
<223> Synthetic construct

35

<400> 18

gcggccgcca tggaatccca gaccaggtg ctgatctccc tgctgttctg ggtgtccggc	60
acctgtggcg acatcctgct gaccaggtcc cccgtgatcc tgtccgtgtc tcctggcgag	120
cgggtgtcct tctcctgccg ggcctcccag tccatcggca ccaacatcca ctggtatcag	180
cagcggacca acggctcccc tcggctgctg attaatgacg cctccgagtc tatctccggc	240
atccccctccc ggttctccgg ctctggctcc ggcaccgact tcaccctgtc catcaactcc	300
gtggaatccg aggatatcgc cgactactac tgccagcaga acaacaactg gccaccacc	360
ttcggcgtg gcaccaagct ggaactgaag cggaccgtgg ccgctccctc cgtgttcac	420
cccaccctcc gacgagcagc tg	442

50

<210> 19  
<211> 424  
<212> DNA  
<213> Artificial Sequence

55

<220>

# EP 3 489 254 B9

<223> Synthetic construct

<400> 19

5	gcggccgcca tgaacttcgg cctgcggctg atcttcctgg tgctgaccct gaagggcgtg	60
	cagtgccagg tgcagctggt ggaatccggc ggaggcgtgg tgcagcctgg cagatccctg	120
10	agactgtcct gcgccgcctc cggcttcacc ttctccagct acaccatgca ctgggtccga	180
	caggccccctg gcaagggcct ggaatgggtc accttcacat gctacgacgg caacaacaag	240
	tactacgccg actccgtgaa gggccgggtc accatctccc gggacaactc caagaacacc	300
15	ctgtacctgc agatgaactc cctgcggggc gaggacaccg ccatctacta ctgcgcccgg	360
	accggctggc tgggcccttt tgattactgg ggccagggca ccctgggtcac cgtgtcctcc	420
20	tagc	424

<210> 20

<211> 445

<212> DNA

25 <213> Artificial Sequence

<220>

<223> Synthetic construct

30 <400> 20

	gcggccgcca tggaatccca gaccaggtg ctgatctccc tgctgttctg ggtgtccggc	60
35	acctgtggcg agatcgtgct gaccaggtcc cccggcacc cgtctctgag ccctggcgag	120
	agagccaccc tgtcctgcag agcctcccag tccgtgggct cctcctacct ggcttggtat	180
	cagcagaagc ccggccaggc ccctcggtg ctgatctacg gcgctttctc tcgggccacc	240
40	ggcatccctg accggttctc tggctccggc tccggcaccg acttcaccct gaccatctcc	300
	cggctggaac ccgaggactt cgccgtgtac tactgccagc agtacggctc ctccccctgg	360
	acctttggcc agggcaccaa ggtggaaatc aagcggaccg tggccgctcc ctccgtgttc	420
45	cttcccaccc tccgacgagc agctg	445

<210> 21

<211> 1035

50 <212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic construct

55 <400> 21

# EP 3 489 254 B9

	gctagcaciaa agggccctag tgtgtttcct ctggctccct cttccaaatc cacttctggt	60
	ggcactgctg ctctgggatg cctgggtgaag gattactttc ctgaacctgt gactgtctca	120
5	tggaactctg gtgctctgac ttctgggtgtc cacactttcc ctgctgtgct gcagtctagt	180
	ggactgtact ctctgtcatc tgtgggtcact gtgccctctt catctctggg aaccagacc	240
10	tacatttgta atgtgaacca caaacatcc aacactaaag tggacaaaaa agccgaaccc	300
	aaatcctgtg acaaaaccca cacctgcca ccttgtcctg cccctgaact gctgggagga	360
	ccttctgtgt ttctgttccc accaaaacca aaagataccc tgatgatctc tagaacccct	420
15	gaggtgacat gtgtgggtggt ggatgtgtct catgaggacc ctgaggtcaa atttaattgg	480
	tacgtcgatg gagtggaagt ccacaatgcc aaaaccaagc ctagagagga acagtacaat	540
20	tcaacctaca gagtcgtcag tgtgctgact gtgctgcac aggattggct gaatggcaag	600
	gaatacaagt gtaaagtctc aaacaaggcc ctgcctgctc caattgagaa aacaatctca	660
	aaggccaagg gacagcctag ggaaccccag gtctacaccc tgccaccttc acgcgacgaa	720
25	ctgacaaaa accaggtgtc cctgacatgc ctggtcaaag gcttctaccc ttctgacatt	780
	gctgtggagt gggagtcaaa tggacagcct gagaacaact acaaaacaac cccccctgtg	840
30	ctggattctg atggctcttt ctttctgtac tccaaactga ctgtggacaa gtctagatgg	900
	cagcagggga atgtcttttc ttgctctgtc atgcatgagg ctctgcataa ccactacact	960
	cagaaatccc tgtctctgtc tcccgggaaa ggcggcggag gatctggcgg aggcgggttct	1020
35	ggtgggtggcg gatcc	1035

<210> 22

<211> 435

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic construct

<400> 22

# EP 3 489 254 B9

gcgggccgcca tgaatttttg actgaggctg attttcctgg tgctgaccct gaaaggcgctc 60  
cagtgtcagg tgcagctgca gcagcctggg gccgagctcg tgaaacctgg cgcctccgtg 120  
5 aagatgtcct gcaaggcctc cggctacacc ttcaccagct acaacatgca ctgggtcaag 180  
cagacccccg gcagaggcct ggaatggatc ggcgctatct accccggcaa cggcgacacc 240  
tcctacaacc agaagttcaa gggcaaggcc accctgaccg ccgacaagtc ctcttccacc 300  
10 gcctacatgc agctgtcctc cctgacctcc gaggactccg ccgtgtacta ctgcgcccgg 360  
tctacctact acggcggcga ctggtacttc aacgtgtggg gcgctggcac caccgtgacc 420  
15 gtgtctgctg ctage 435

<210> 23  
<211> 405  
<212> **DNA**  
20 <213> Artificial Sequence  
<220>  
<223> Synthetic construct  
25 <400> 23

gcgggccgcca tgaatttttg actgaggctg attttcctgg tgctgaccct gaaaggcgctc 60  
30 cagtgtcaga tcgtgctgtc ccagtcacct gccatcctgt ctgctagccc tggcgagaaa 120  
gtgacaatga cctgccgggc ctctctctcc gtgtcctaca tccactggtt ccagcagaag 180  
35 cccggctcca gcccgaagcc ttggatctac gccacctcca acctggcctc tggcgtgcca 240  
gtgcgggtttt ccggctctgg ctctggcacc tctactccc tgaccatctc tcgggtggaa 300  
gccgaggatg ccgccaccta ctactgccag cagtggacca gcaaccccc caccatttggc 360  
40 ggaggcacca agctggaaat caagcggacc gtggcggcgc cctct 405

<210> 24  
<211> 631  
45 <212> **DNA**  
<213> Artificial Sequence  
<220>  
<223> Synthetic construct  
50 <400> 24

55



# EP 3 489 254 B9

	ggatccgcct gtccttgggc cgtgtccggc gctagagcct ctccctggctc tgccgcctcc	60
	cccagactga gagagggccc tgagctgtcc cctgacgata ctgccggcct gctggacctg	120
5	agacagggca tgtttgccca gctggtggcc cagaacgtgc tgctgatcga cggccccctg	180
	tcctggtact ctgatcctgg cctggccggc gtgtccctga ccggcggact gtcctacaaa	240
	gaggacacca aagaactggt ggtggccaag gctggcgtgt actacgtgtt ctttcagctg	300
10	gaactgcggc ggggtggtggc cggcgagggc tctggatctg tgtccctggc cctgcatctg	360
	cagcccctga gatctgccgc tggcgccgct gctctggccc tgacagtgga tctgcctcct	420
15	gcctcctccg aggcccgaa ctccgcattc gggtttcagg gccggctgct gcacctgtct	480
	gctggccaga gactgggagt gcatctgcac accgaggcca gagccagaca cgcctggcag	540
	ctgaccaggg gcgctaccgt gctgggcctg ttcagagtga cccccgagat cccagccggc	600
20	ctgcccagcc ctagatccga gtgataagct t	631

<210> 25

<211> 1458

<212> **DNA**

25 <213> Artificial Sequence

<220>

<223> Synthetic construct

30 <400> 25

	gcggccgcca tgaattttgg actgaggctg attttcctgg tgctgaccct gaaaggcgtc	60
35	cagtgtcagg tgcagctgca ggaatctggc cctggactcg tgccgccttc ccaaaccctg	120
	tctctgacct gtaccgtgtc cggctactcc atcacctccg accacgcctg gtcttggtg	180
	cgacagcctc ctggcagagg cctggaatgg atcggtaca tctcctactc cggcatcacc	240
40	acctacaacc ccagcctgaa gtccagagtg accatgctgc gggacacctc caagaaccag	300
	ttctccctgc ggctgtcctc cgtgaccgct gctgataccg ccgtgtacta ctgcgccaga	360
45	tctctggcca ggaccaccgc catggattac tggggccagg gctccctcgt gaccgtgtcc	420

50

55

EP 3 489 254 B9

	tctgctagca ccaagggccc ctccgtgttc cctctggccc cttcctctaa atctacctct	480
	ggcggcaccg ccgctctggg ctgcctcgtg aaggactact tccccgagcc cgtgacagtg	540
5	tcttgaact ctggcgccct gacctccggc gtgcacacct ttccagctgt gctgcagtcc	600
	tccggcctgt actccctgtc cagcgtcgtg actgtgccct cctcatctct gggcaccag	660
	acctacatct gcaacgtgaa ccacaagccc tccaacacca aggtggacaa gaaggtggaa	720
10	cccaagtcct gcgacaagac ccacacctgt ccccttgtc ctgcccctga actgctgggc	780
	ggaccctctg tgttcctgtt cccaccaaaa ccgaaagaca ccctgatgat ctcccgacc	840
	cccgaagtga cctgcgtggt ggtggatgtg tcccacgagg accctgaagt gaagttcaat	900
15	tggtagctgg acggcgtgga agtgcacaac gccaaagaca agcctagaga ggaacagtac	960
	aactccacct accgggtggt gtccgtgctg accgtgctgc accaggattg gctgaacggc	1020
20	aaagagtaca agtgcaaggt gtccaacaag gccctgcctg ccccatcga aaagaccatc	1080
	tccaaggcca agggccagcc acgggaaccc caggtgtaca cactgcccc tagccgcgac	1140
	gagctgacca agaatcaggt gtccctgaca tgctcgtga aaggcttcta cccctccgat	1200
25	atcgccgtgg aatgggagtc caacggccag cctgagaaca actacaagac cccccccct	1260
	gtgctggact ccgacggctc attcttctct tactcaaagc tgacagtgga caagtcccgg	1320
	tggcagcagg gcaacgtgtt ctctgtctcc gtgatgcacg aggccctgca caaccactac	1380
30	accagaagt ccctgtccct gagccccggg aaaggcggcg gaggatctgg cggaggcgg	1440
	tctggtggtg gcggatcc	1458
35	<210> 26 <211> 405 <212> DNA <213> Artificial Sequence	
40	<220> <223> Synthetic construct	
	<400> 26	
45	gcggccgcca tgaattttgg actgaggctg attttctctg tgctgaccct gaaaggcgtc	60
	cagtgtgaca tccagatgac ccagtcccc tccagcctgt ctgcctctgt gggcgacaga	120
	gtgaccatca cctgtcgggc ctcccaggac atctctctct acctgaactg gtatcagcag	180
50	aagcccggca agggccccaa gctgctgata tactacacct cccggctgca ctccggcgtg	240
	ccctctagat tttccggctc tggctccggc accgacttta ccttcaccat cagctccctg	300
55	cagcccagg atatacgccac ctactactgc cagcaaggca acaccctgcc ctacaccttt	360
	ggccagggca ccaaggtgga aatcaagcgg accgtggcgg cgccc	405

# EP 3 489 254 B9

<210> 27  
 <211> 1455  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Synthetic construct

<400> 27

```

gcgggccgcca tgaat ttttgg actgaggctg attttccctgg tgctgaccct gaaaggcgtc      60
cagtgtcagg tgcagctgca gcagtgggga gctggactgc tgaagccctc cgagacactg      120
tctctgacct gcgctgtgta cggcggctcc ttctccggct actactggtc ctggattcgg      180
cagtcccctg agaagggcct ggaatggatc ggcgagatca accacggcgg ctacgtgacc      240
tacaaccca gcctggaatc cagagtgacc atctccgtgg acacctcaa gaaccagttc      300
tccctgaagc tgtcctccgt gaccgccgct gataccgccg tgtactactg cgccagagac      360
tacggccctg gcaactacga ctggtacttc gacctgtggg gcagaggcac cctcgtgacc      420
gtgtcctctg ctagcaccaa gggcccctcc gtgtttcctc tggccccttg ctcacgctcc      480
acctccgaat ctaccgccgc tctgggctgc ctcgtgaagg actacttccc cgagcccgtg      540
actgtgtctt ggaactctgg cgccctgacc tccggcgtgc acacctttcc agctgtgctg      600
cagtcctccg gcctgtactc cctgtccagc gtcgtgacag tgccctccag ctctctgggc      660
accaagacct acacctgtaa cgtggaccac aagccctcca acaccaaggt ggacaagcgg      720
gtggaatcta aatacggccc tccctgccct ccttgcccag cccctgaatt tctgggcgga      780
ccttccgtgt tctgtttccc cccaaaaccc aaggacaccc tgatgatctc ccggaccccc      840
gaagtgacct gcgtgggtgt ggatgtgtcc caggaagatc ccgagggtgca gttcaattgg      900
tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ctagagagga acagttcaac      960
tccacctacc ggggtggtgtc cgtgctgacc gtgctgcacc aggattggct gaacggcaaa     1020
gagtacaagt gcaaggtgtc caacaagggc ctgcccagct ccatcgaaaa gaccatcagc     1080
aaggccaagg gccagccccg ggaaccccag gtgtacacac tgccctccaag ccaggaagag     1140
atgaccaaga atcaggtgtc cctgacctgt ctcgtgaaag gcttctaccc ctccgatatc     1200
gccgtggaat gggagtccaa cggccagcct gagaacaact acaagaccac cccccctgtg     1260
ctggactccg acggcagctt cttcctgtac tctcgccctga ccgtggacaa gtcccgggtg     1320
caggaaggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc     1380
cagaagtccc tgtccctgtc tctggggaaa ggcggcggag gatctggcgg aggcggttct     1440
ggtggtggcg gatcc                                           1455
  
```

# EP 3 489 254 B9

<210> 28  
 <211> 411  
 <212> DNA  
 <213> Artificial Sequence

5

<220>  
 <223> Synthetic construct

10

g	c	g	g	c	c	c	a		t	g	a	a	t	t	t	t	g		a	c	t	g	a	g	g	c	t	g		a	t	t	t	c	c	t	g		t	g	c	t	g	a	c	c	c	t		g	a	a	a	g	g	c	g	t	c		60					
c	a	g	t	g	t	g	a		t	c	g	t	g	c	t	g	a	c		c	c	a	g	t	c	t	c	c	t		g	c	c	a	c	c	c	t	g	t		c	t	c	t	g	a	g	c	c	c		t	g	g	c	g	a	g	a		120				
g	c	t	a	c	c	c	t	g	t		c	c	t	g	c	c	g	t	g	c		c	t	c	c	c	a	a	t	c	c		g	t	g	t	c	c	t	c	t	t		a	c	c	t	g	g	c	c	t	g		g	t	a	t	c	a	g	c	a	a		180
a	a	g	c	c	c	g	g	c		a	g	g	c	t	c	c	c	g		g	c	t	g	c	t	g	a	t	c		t	a	c	g	a	t	g	c	c	t		c	c	a	a	t	a	g	a	g	c		c	a	c	c	g	g	c	a	t	c		240		
c	c	t	g	c	c	a	g	a	t		t	c	t	c	c	g	g	c	t	c		t	g	g	c	t	c	t	g	g	c		a	c	c	g	a	c	t	t	t	a		c	c	c	t	g	a	c	c	a	t		c	t	c	c	t	c	t	c	t	g		300
g	a	a	c	c	c	g	a	g		a	c	t	t	c	g	c	c	g	t		g	t	a	c	t	a	c	t	g	c		c	a	g	c	a	g	c	g	g	t		c	c	a	a	c	t	g	g	c	c		t	c	c	c	g	c	c	c	t	g		360	
a	c	a	t	t	t	g	g	c	g		g	a	g	g	c	a	c	a	a		g	g	t	g	g	a	a	a	t	c		a	a	g	c	g	g	a	c	c	g		t	g	g	c	g	g	c	g	c	c		c		411										

25

<210> 29  
 <211> 449  
 <212> PRT  
 <213> Artificial Sequence

30

<220>  
 <223> Synthetic construct

<400> 29

35

40

45

50

55

EP 3 489 254 B9

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

EP 3 489 254 B9

	130		135		140												
5	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	
	145					150					155					160	
	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	
10					165					170					175		
	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	
				180					185					190			
15	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	
			195					200					205				
	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	
20		210					215					220					
	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	
	225					230					235					240	
25	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	
					245					250					255		
30	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	
				260					265					270			
	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	
35			275					280					285				
	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	
	290						295					300					
40	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	
	305					310					315					320	
	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	
45					325					330					335		
	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	
50				340					345					350			
	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	
				355				360					365				
55	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	
	370						375					380					

# EP 3 489 254 B9

	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu
	385					390					395					400
5	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys
					405					410					415	
10	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu
				420					425					430		
15	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly
			435					440					445			
	Lys															
20	<210> 30															
	<211> 219															
	<212> PRT															
	<213> Artificial Sequence															
25	<220>															
	<223> Synthetic construct															
	<400> 30															
30																
35																
40																
45																
50																
55																

EP 3 489 254 B9

	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Leu	Ser	Val	Pro	Val	Thr	Pro	Gly	
	1				5					10					15		
5	Glu	Pro	Val	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser	Leu	Leu	His	Ser	
				20					25					30			
10	Asn	Gly	Asn	Thr	Tyr	Leu	Tyr	Trp	Phe	Leu	Gln	Arg	Pro	Gly	Gln	Ser	
			35					40					45				
15	Pro	Gln	Leu	Leu	Ile	Tyr	Arg	Met	Ser	Asn	Leu	Val	Ser	Gly	Val	Pro	
		50					55					60					
20	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ala	Phe	Thr	Leu	Arg	Ile	
	65					70					75				80		
25	Ser	Arg	Val	Glu	Ala	Glu	Asp	Val	Gly	Val	Tyr	Tyr	Cys	Leu	Gln	His	
					85					90					95		
30	Leu	Glu	Tyr	Pro	Phe	Thr	Phe	Gly	Pro	Gly	Thr	Lys	Leu	Glu	Leu	Lys	
				100					105					110			
35	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	
			115					120					125				
40	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	
45																	
50	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	
	145					150					155					160	
55	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	
					165					170					175		
60	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	
				180					185					190			
65	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	
			195					200					205				
70	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys						
		210					215										
75	<210>	31															
	<211>	460															
	<212>	PRT															
	<213>	Artificial Sequence															



# EP 3 489 254 B9

<220>

<223> Synthetic construct

<400> 31

5

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

10

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

15

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

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

20

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

25

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

30

Ala Arg Ala Pro Leu Arg Phe Leu Glu Trp Ser Thr Gln Asp His Tyr  
100 105 110

Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val  
115 120 125

35

40

45

50

55

EP 3 489 254 B9

	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	
	130						135					140					
5	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	
	145					150					155					160	
10	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	
					165					170					175		
15	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	
				180					185					190			
20	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	
			195					200					205				
25	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	
		210					215					220					
30	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	
	225					230				235						240	
35	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	
				245					250						255		
40	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	
				260					265					270			
45	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	
		275						280					285				
50	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	
	290						295					300					
55	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	
	305					310					315					320	
60	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	
				325						330					335		
65	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	
				340					345					350			
70	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	
			355					360					365				
75	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	

# EP 3 489 254 B9

370

375

380

5

Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro  
385 390 395 400

10

Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser  
405 410 415

15

Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln  
420 425 430

25

Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His  
435 440 445

30

Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
450 455 460

<210> 32

<211> 213

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic construct

35

40

45

50

55

EP 3 489 254 B9

	Ser	Ser	Glu	Leu	Thr	Gln	Asp	Pro	Ala	Val	Ser	Val	Ala	Leu	Gly	Gln	
	1				5					10					15		
5	Thr	Val	Arg	Ile	Thr	Cys	Gln	Gly	Asp	Ser	Leu	Arg	Ser	Tyr	Tyr	Ala	
				20					25					30			
10	Thr	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Ile	Leu	Val	Ile	Tyr	
			35					40					45				
15	Gly	Glu	Asn	Lys	Arg	Pro	Ser	Gly	Ile	Pro	Asp	Arg	Phe	Ser	Gly	Ser	
		50					55					60					
20	Ser	Ser	Gly	Asn	Thr	Ala	Ser	Leu	Thr	Ile	Thr	Gly	Ala	Gln	Ala	Glu	
	65					70					75					80	
25	Asp	Glu	Ala	Asp	Tyr	Tyr	Cys	Lys	Ser	Arg	Asp	Gly	Ser	Gly	Gln	His	
					85					90					95		
30	Leu	Val	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Thr	Val	Leu	Gly	Gln	Pro	Lys	
				100					105					110			
35	Ala	Ala	Pro	Ser	Val	Thr	Leu	Phe	Pro	Pro	Ser	Ser	Glu	Glu	Leu	Gln	
			115					120					125				
40	Ala	Asn	Lys	Ala	Thr	Leu	Val	Cys	Leu	Ile	Ser	Asp	Phe	Tyr	Pro	Gly	
		130					135					140					
45	Ala	Val	Thr	Val	Ala	Trp	Lys	Ala	Asp	Ser	Ser	Pro	Val	Lys	Ala	Gly	
	145					150					155				160		
50	Val	Glu	Thr	Thr	Thr	Pro	Ser	Lys	Gln	Ser	Asn	Asn	Lys	Tyr	Ala	Ala	
					165					170					175		
55	Ser	Ser	Tyr	Leu	Ser	Leu	Thr	Pro	Glu	Gln	Trp	Lys	Ser	His	Arg	Ser	
				180					185					190			
60	Tyr	Ser	Cys	Gln	Val	Thr	His	Glu	Gly	Ser	Thr	Val	Glu	Lys	Thr	Val	
			195					200					205				
65	Ala	Pro	Ala	Glu	Cys												
				210													

<210> 33

<211> 449

<212> PRT

<213> Artificial Sequence

<220>

# EP 3 489 254 B9

<223> Synthetic construct

<400> 33

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

EP 3 489 254 B9

	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	
			115					120					125				
5	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	
			130				135					140					
10	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	
	145					150					155					160	
15	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	Asn	Thr	Phe	Pro	Ala	Val	Leu	
					165					170					175		
20	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	
				180					185					190			
25	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	
			195					200					205				
30	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	
		210					215					220					
35	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	
	225					230					235					240	
40	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Asn	Ile	Ser	
				245						250					255		
45	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	
				260					265					270			
50	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	
			275				280						285				
55	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	
		290					295					300					
60	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	
	305					310					315					320	
65	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	
				325						330					335		
70	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	
				340					345					350			
75	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	
			355					360					365				

# EP 3 489 254 B9

	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	
	370						375					380					
5	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	
	385					390					395					400	
10	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	
					405					410					415		
15	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Asn	His	Glu	
				420					425					430			
20	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	
		435						440					445				
25	Lys																
	<210>	34															
	<211>	215															
	<212>	PRT															
	<213>	Artificial Sequence															
	<220>																
	<223>	Synthetic construct															
30	<400>	34															
35	Asp	Ile	Gln	Leu	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	
	1				5					10					15		
40	Asp	Arg	Val	Thr	Met	Thr	Cys	Ser	Ala	Ser	Ser	Ser	Val	Ser	Ser	Ser	
			20						25					30			
45	Tyr	Leu	Tyr	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Trp	
		35						40					45				
50	Ile	Tyr	Ser	Thr	Ser	Asn	Leu	Ala	Ser	Gly	Val	Pro	Ala	Arg	Phe	Ser	
	50					55						60					
55	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	
	65				70						75					80	
60	Pro	Glu	Asp	Ser	Ala	Ser	Tyr	Phe	Cys	His	Gln	Trp	Asn	Arg	Tyr	Pro	
				85					90					95			
65	Tyr	Thr	Phe	Gly	Gly	Gly	Thr	Arg	Leu	Glu	Ile	Lys	Arg	Thr	Val	Ala	
			100						105					110			

EP 3 489 254 B9

	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	
			115					120					125				
5	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Glu	Ala	Lys	
		130					135					140					
10	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	
	145					150					155					160	
15	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	
					165					170					175		
20	Thr	Leu	Thr	Leu	Ser	Pro	Arg	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	
				180					185					190			
25	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	
			195					200					205				
30	Ser	Phe	Asn	Arg	Gly	Glu	Cys										
		210					215										
	<210> 35																
	<211> 450																
	<212> PRT																
	<213> Artificial Sequence																
	<220>																
	<223> Synthetic construct																
35	<400> 35																
40	Glu	Val	Gln	Leu	Leu	Glu	Ser	Gly	Gly	Asp	Leu	Val	Gln	Pro	Gly	Gly	
	1				5					10					15		
45	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Tyr	
				20					25					30			
50	Ala	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
			35					40					45				
55	Ser	Ala	Ile	Thr	Pro	Ser	Gly	Gly	Ser	Thr	Asn	Tyr	Ala	Asp	Ser	Val	
		50					55					60					
60	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Gln	Asn	Thr	Leu	Tyr	
	65					70					75					80	
65	Leu	Gln	Met	Asn	Ser	Leu	Arg	Val	Glu	Asp	Thr	Ala	Val	Tyr	Ile	Cys	
					85					90					95		



EP 3 489 254 B9

	Gly	Arg	Val	Pro	Tyr	Arg	Ser	Thr	Trp	Tyr	Pro	Leu	Tyr	Trp	Gly	Gln	
				100					105					110			
5	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	
			115					120					125				
10	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	
		130					135					140					
15	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	
	145					150					155					160	
20	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	
					165					170					175		
25	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	
				180					185					190			
30	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	
			195					200					205				
35	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	
		210					215					220					
40	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	
	225					230					235					240	
45	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	
				245						250					255		
50	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	
				260					265					270			
55	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	
		275						280					285				
60	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	
		290					295					300					
65	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	
	305					310					315					320	
70	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	
				325						330					335		
75	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	
				340					345					350			

EP 3 489 254 B9

	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	
			355					360					365				
5	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	
		370					375					380					
10	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	
	385					390					395					400	
15	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	
					405					410					415		
20	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	
				420					425					430			
25	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	
			435					440					445				
30	Gly	Lys															
		450															
	<210>	36															
	<211>	214															
	<212>	PRT															
	<213>	Artificial Sequence															
	<220>																
	<223>	Synthetic construct															
35	<400>	36															
40	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	
	1				5					10					15		
45	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Asp	Ile	Ser	Asn	Tyr	
				20					25					30			
50	Leu	Ala	Trp	Phe	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Ser	Leu	Ile	
			35					40					45				
55	Tyr	Ala	Ala	Ser	Ser	Leu	His	Ser	Lys	Val	Pro	Thr	Gln	Phe	Ser	Gly	
	50						55					60					
60	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro	
	65					70					75					80	
65	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Leu	Gln	Tyr	Ser	Thr	Tyr	Pro	Ile	
					85					90					95		

EP 3 489 254 B9

	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	
				100					105					110			
5	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	
			115					120					125				
10	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	
15	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	
					165					170					175		
20	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	
				180					185					190			
25	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											
		210															
30																	
	<210> 37																
	<211> 450																
	<212> PRT																
	<213> Homo sapiens																
35																	
	<400> 37																
40	Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Val	Gln	Pro	Gly	Gly	
	1				5					10					15		
	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Asn	Ile	Lys	Asp	Thr	
				20					25					30			
45	Tyr	Ile	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
			35					40					45				
50	Ala	Arg	Ile	Tyr	Pro	Thr	Asn	Gly	Tyr	Thr	Arg	Tyr	Ala	Asp	Ser	Val	
		50					55					60					
	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Ala	Asp	Thr	Ser	Lys	Asn	Thr	Ala	Tyr	
55	65					70					75					80	
	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	
					85					90					95		

EP 3 489 254 B9

	Ser	Arg	Trp	Gly	Gly	Asp	Gly	Phe	Tyr	Ala	Met	Asp	Tyr	Trp	Gly	Gln	
				100					105					110			
5	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	
			115					120					125				
	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	
10		130					135					140					
	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	
	145					150					155					160	
15	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	
					165					170					175		
	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	
20				180					185					190			
	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	
25			195					200					205				
	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu	Pro	Lys	Ser	Cys	Asp	
		210					215					220					
30	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	
	225					230					235					240	
	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	
35				245						250					255		
	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	
				260					265					270			
40	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	
		275						280					285				
	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	
45		290					295					300					
	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	
50	305					310					315					320	
	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	
					325					330					335		
55	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	
				340					345					350			

EP 3 489 254 B9

	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	
			355					360					365				
5	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	
		370					375					380					
10	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	
	385					390					395					400	
15	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	
					405					410					415		
20	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	
				420					425					430			
25	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	
			435					440					445				
30	Gly	Lys															
		450															
	<210> 38																
	<211> 449																
	<212> PRT																
	<213> Homo sapiens																
	<400> 38																
35	Gln	Val	Gln	Leu	Lys	Gln	Ser	Gly	Pro	Gly	Leu	Val	Gln	Pro	Ser	Gln	
	1				5					10					15		
40	Ser	Leu	Ser	Ile	Thr	Cys	Thr	Val	Ser	Gly	Phe	Ser	Leu	Thr	Asn	Tyr	
				20					25					30			
45	Gly	Val	His	Trp	Val	Arg	Gln	Ser	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Leu	
			35					40					45				
50	Gly	Val	Ile	Trp	Ser	Gly	Gly	Asn	Thr	Asp	Tyr	Asn	Thr	Pro	Phe	Thr	
		50					55					60					
55	Ser	Arg	Leu	Ser	Ile	Asn	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Phe	Phe	
	65					70					75					80	
	Lys	Met	Asn	Ser	Leu	Gln	Ser	Asn	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	Ala	
					85					90					95		
	Arg	Ala	Leu	Thr	Tyr	Tyr	Asp	Tyr	Glu	Phe	Ala	Tyr	Trp	Gly	Gln	Gly	
				100					105					110			

EP 3 489 254 B9

	Thr	Leu	Val	Thr	Val	Ser	Ala	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	
			115					120					125				
5	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	
		130					135					140					
10	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	
	145					150					155					160	
15	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	
					165					170					175		
20	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	
				180					185					190			
25	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	
			195					200					205				
30	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	
		210					215					220					
35	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	
	225					230					235					240	
40	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	
				245						250					255		
45	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	
				260					265					270			
50	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	
			275					280					285				
55	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	
		290					295					300					
60	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	
	305					310					315					320	
65	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	
				325						330					335		
70	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	
				340					345					350			
75	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	
			355					360					365				

EP 3 489 254 B9

	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	
	370						375					380					
5	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	
	385					390					395					400	
10	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	
					405					410					415		
15	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	
				420					425					430			
20	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	
		435						440					445				
25	Lys																
	<210>	39															
	<211>	448															
	<212>	PRT															
	<213>	Homo sapiens															
	<400>	39															
30	Gln	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Arg	
	1			5						10					15		
35	Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Ser	Tyr	
				20					25					30			
40	Thr	Met	His	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val	
			35					40					45				
45	Thr	Phe	Ile	Ser	Tyr	Asp	Gly	Asn	Asn	Lys	Tyr	Tyr	Ala	Asp	Ser	Val	
		50					55					60					
50	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Tyr	
	65					70					75					80	
55	Leu	Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Ile	Tyr	Tyr	Cys	
				85						90					95		
60	Ala	Arg	Thr	Gly	Trp	Leu	Gly	Pro	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	Thr	
				100					105					110			
65	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	
				115				120					125				

EP 3 489 254 B9

	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	
	130						135					140					
5	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	
	145					150					155					160	
	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	
10					165					170					175		
	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	
				180					185					190			
15	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	
			195					200					205				
	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	
20		210					215					220					
	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	
25	225					230					235					240	
	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	
				245						250					255		
30	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	
				260					265					270			
	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	
35			275					280					285				
	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	
40		290					295					300					
	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	
	305					310					315					320	
45	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	
					325					330					335		
	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	
50				340					345					350			
	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	
			355					360					365				
55	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	



370

375

380

5 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
385 390 395 400

10 Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
405 410 415

15 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
420 425 430

20 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
435 440 445

## 20 Claims

1. A method of preparing a therapeutically active antibody-peptide fusion protein, the method comprising,

25 preparing a codon optimized nucleotide sequence of the antibody-peptide fusion protein, wherein the codon optimized nucleotide sequence is optimized for expression in a Chinese Hamster Ovary (CHO) host cell, wherein the antibody-protein fusion protein comprises a targeting moiety and immunomodulating moiety, wherein the targeting moiety and the immunomodulating moiety are linked by an amino acid spacer selected from SEQ ID NO: 3 or SEQ ID NO: 11, wherein the immunomodulating moiety is TGF- $\beta$ RII comprising the amino acid sequence of SEQ ID NO: 4; wherein the targeting moiety is selected from the group consisting of an Anti-EGFR1 antibody, consisting of heavy chain SEQ ID NO: 5 and light chain SEQ ID NO: 6, an Anti-HER2/Neu antibody consisting of heavy chain SEQ ID NO: 1 and light chain SEQ ID NO: 2; and anti-CTLA4 antibody consisting of heavy chain of SEQ ID NO: 7 and a light chain of SEQ ID NO: 8, wherein SEQ ID NO: 4 is attached via the amino acid spacer to the C-terminus of SEQ ID NO: 1 or SEQ ID NO: 2 of Anti-HER2/Neu; C-terminus of SEQ ID NO: 5 or SEQ ID NO: 6 of Anti-EGFR1; or C-terminus of SEQ ID NO: 7 or SEQ ID NO: 8 of Anti-CTLA-4;  
30 cloning the optimized sequence of said antibody-peptide fusion protein in a Chinese Hamster Ovary (CHO) host cell capable of transient or continued expression;  
35 growing the CHO host cell in a feed batch mode in a fermentation medium under suitable conditions for growing and allowing the CHO host cell to express a cloned protein, wherein the fermentation medium comprises a divalent transitional metallic salt; , wherein the divalent transitional metallic salt includes a zinc ion, wherein the divalent transitional metallic salt is zinc sulphate hepta hydrate salt and purifying the expressed antibody-peptide fusion protein and optionally checking the bi-specific binding capabilities of the antibody-peptide fusion protein to its targets.

- 45 2. The method of claim 1, wherein the divalent transitional metallic salt is introduced into the cell culture either initially or in fed-batch mode.

3. The method of claim 2, wherein the zinc sulphate hepta hydrate salt is at an initial concentration of 0.4 mM added to the fermentation medium, the production fermentation starts with initial cell count of  $0.3-0.45 \times 10^6$  cells/ml at  $37 \pm 1^\circ\text{C}$  during the first 3-4 days followed by culture at  $31 \pm 1^\circ\text{C}$  until the 7th day and wherein the accumulation of lactate is reduced by almost 10-40% during the cell culture.

4. The method of claim 1, wherein the expressed antibody-peptide fusion protein is subjected to affinity chromatography using a Mab Select Xtra protein A column having a specific pH.

- 55 5. The method of claim 1, wherein the supernatant binding and passing through the Mab Select Xtra protein A column is pH adjusted to the specific pH of the protein A column.

6. The method of claim 1, wherein the antibody-proteins fusion protein binds through the Fc region of the antibody to

the column while impurities are eliminated as flow through.

7. The method of claim 1, wherein the antibody-protein fusion protein bound to the column is eluted using glycine at pH 3.0 and adjusted to neutral pH for storage.
8. The method of claim 1, wherein the purified protein is stored at -20°C or at 2-8°C.
9. The method of claim 1, wherein optimized nucleotide sequence comprises an increase of CG nucleotides relative to a non-optimized nucleotide sequence.

## Patentansprüche

1. Verfahren zur Herstellung eines therapeutisch aktiven Antikörper-Peptid-Fusionsproteins, wobei das Verfahren umfasst:

Herstellen einer Codon-optimierten Nukleotidsequenz des Antikörper-Peptid-Fusionsproteins, wobei die Codon-optimierte Nukleotidsequenz für die Expression in einer Chinese Hamster Ovary (CHO)-Wirtszelle optimiert ist, wobei das Antikörper-Protein-Fusionsprotein eine Targeting-Einheit und eine immunmodulierende Einheit umfasst, wobei die Targeting-Einheit und die immunmodulierende Einheit durch einen Aminosäure-Spacer verknüpft sind, der ausgewählt ist unter SEQ ID NO: 3 oder SEQ ID NO: 11, wobei die immunmodulierende Einheit TGF-βRII ist, umfassend die Aminosäuresequenz von SEQ ID NO: 4; wobei die Targeting-Einheit ausgewählt ist aus der Gruppe, bestehend aus einem Anti-EGFR1-Antikörper, bestehend aus der schweren Kette mit der SEQ ID NO: 5 und der leichten Kette mit der SEQ ID NO: 6, einem Anti-HER2/Neu-Antikörper, bestehend aus der schweren Kette mit der SEQ ID NO: 1 und der leichten Kette mit der SEQ ID NO: 2; und Anti-CTLA4-Antikörper, bestehend aus der schweren Kette mit SEQ ID NO: 7 und einer leichten Kette mit der SEQ ID NO: 8, wobei SEQ ID NO: 4 über den Aminosäure-Spacer an den C-Terminus von SEQ ID NO: 1 oder SEQ ID NO: 2 von Anti-HER2/Neu gebunden ist; C-Terminus von SEQ ID NO: 5 oder SEQ ID NO: 6 von Anti-EGFR1; oder C-Terminus von SEQ ID NO: 7 oder SEQ ID NO: 8 von Anti-CTLA-4;

Klonieren der optimierten Sequenz des Antikörper-Peptid-Fusionsproteins in eine Chinese Hamster Ovary (CHO)-Wirtszelle, die zur vorübergehenden oder fortgesetzten Expression fähig ist;

Züchten der CHO-Wirtszelle in einem Feed-Batch-Modus in einem Fermentationsmedium unter geeigneten Zuchtbedingungen für das Wachstum und die Expression eines klonierten Proteins durch die (CHO)-Wirtszelle, wobei das Fermentationsmedium ein zweiwertiges Übergangsmetallsalz umfasst; wobei das zweiwertige Übergangsmetallsalz ein Zinkion umfasst, wobei das zweiwertige Übergangsmetallsalz Zinksulfat-Heptahydratsalz ist und Reinigen des exprimierten Antikörper-Peptid-Fusionsproteins und gegebenenfalls Überprüfen der bispezifischen Bindungsfähigkeiten des Antikörper-Peptid-Fusionsproteins an seine Ziele.

2. Verfahren nach Anspruch 1, wobei das zweiwertige Übergangsmetallsalz entweder zu Beginn oder im Fed-Batch-Modus in die Zellkultur eingeführt wird.
3. Verfahren nach Anspruch 2, wobei das Zinksulfat-Heptahydratsalz in einer anfänglichen Konzentration von 0,4 mM dem Fermentationsmedium zugesetzt wird, die Produktionsfermentation mit einer anfänglichen Zellzahl von  $0,3-0,45 \times 10^6$  Zellen/ml bei  $37 \pm 1^\circ\text{C}$  während der ersten 3-4 Tage beginnt, gefolgt von Kultivieren bei  $31 \pm 1^\circ\text{C}$  bis zum 7. Tag, und wobei die Laktatakkumulation während der Zellkultur um fast 10-40 % verringert wird.
4. Verfahren nach Anspruch 1, wobei das exprimierte Antikörper-Peptid-Fusionsprotein einer Affinitätschromatographie unter Verwendung einer Mab Select Xtra Protein A Säule mit einem spezifischen pH-Wert unterzogen wird.
5. Verfahren nach Anspruch 1, wobei der pH-Wert bei Binden und Passieren des Überstands durch die Mab Select Xtra Protein A Säule auf den spezifischen pH-Wert der Protein-A-Säule eingestellt wird.
6. Verfahren nach Anspruch 1, wobei das Antikörper-Protein-Fusionsprotein durch die Fc-Region des Antikörpers an die Säule bindet, während Verunreinigungen als Durchfluss entfernt werden.
7. Verfahren nach Anspruch 1, wobei das an die Säule gebundene Antikörper-Protein-Fusionsprotein unter Verwendung von Glycin bei pH 3,0 eluiert und zur Lagerung auf einen neutralen pH-Wert eingestellt wird.

8. Verfahren nach Anspruch 1, wobei das gereinigte Protein bei -20 °C oder bei 2-8°C gelagert wird.
9. Verfahren nach Anspruch 1, wobei die optimierte Nukleotidsequenz eine Zunahme von CG-Nukleotiden in Vergleich zu einer nicht optimierten Nukleotidsequenz umfasst.

5

## Revendications

10

1. Procédé de préparation d'une protéine de fusion anticorps-peptide thérapeutiquement active, le procédé comprenant,

15

la préparation d'une séquence nucléotidique à codon optimisé de la protéine de fusion anticorps-peptide, dans laquelle la séquence nucléotidique à codon optimisé est optimisée pour l'expression dans une cellule hôte d'ovaire de hamster chinois (CHO), dans laquelle la protéine de fusion anticorps-peptide comprend une fraction de ciblage et une fraction d'immunomodulation, dans laquelle la fraction de ciblage et la fraction d'immunomodulation sont liées par un espaceur d'acides aminés choisi parmi SEQ ID NO : 3 ou SEQ ID NO : 11, dans laquelle la fraction d'immunomodulation est TGF-βRII comprenant la séquence d'acides aminés de SEQ ID NO : 4 ; dans lequel la fraction de ciblage est choisie dans le groupe constitué d'un anticorps Anti-EGFR1, constitué d'une chaîne lourde SEQ ID NO : 5 et d'une chaîne légère SEQ ID NO : 6, d'un anticorps Anti-HER2/Neu constitué d'une chaîne lourde SEQ ID NO : 1 et d'une chaîne légère SEQ ID NO : 2 ; et d'un anticorps anti-CTLA4 constitué d'une chaîne lourde de SEQ ID NO : 7 et d'une chaîne légère de SEQ ID NO : 8, -dans laquelle SEQ ID NO : 4 est fixée via l'espaceur d'acides aminés à l'extrémité C-terminale de SEQ ID NO : 1 ou SEQ ID NO : 2 de Anti-HER2/Neu ; à l'extrémité C-terminale de SEQ ID NO : 5 ou SEQ ID NO : 6 de Anti-EGFR1 ; ou à l'extrémité C-terminale de SEQ ID NO : 7 ou SEQ ID NO : 8 de Anti-CTLA-4 ;

25

le clonage de la séquence optimisée de ladite protéine de fusion anticorps-peptide dans une cellule hôte d'ovaire de hamster chinois (CHO) capable d'expression transitoire ou continue ;

30

la culture de la cellule hôte CHO dans un milieu de fermentation à alimentation discontinue dans des conditions appropriées pour cultiver et permettre à la cellule hôte CHO d'exprimer une protéine clonée, dans laquelle le milieu de fermentation comprend un sel de métal de transition divalent ; dans laquelle le sel de métal de transition divalent comprend un ion zinc, dans laquelle le sel de métal de transition divalent est un sel de sulfate de zinc heptahydraté, et

la purification de la protéine de fusion anticorps-peptide exprimée et optionnellement la vérification des capacités de liaison bispécifique de la protéine de fusion anticorps-peptide à ses cibles.

35

2. Procédé selon la revendication 1, dans lequel le sel de métal de transition divalent est introduit dans la culture cellulaire soit initialement, soit de manière discontinue.

40

3. Procédé selon la revendication 2, dans lequel le sel de sulfate de zinc heptahydraté est à une concentration initiale de 0,4 mM ajouté au milieu de fermentation, la fermentation de production commence avec un comptage cellulaire initial de  $0,3-0,45 \times 10^6$  cellules/ml à  $37 \pm 1^\circ\text{C}$  pendant les premiers 3-4 jours suivie d'une culture à  $31 \pm 1^\circ\text{C}$  jusqu'au 7<sup>ème</sup> jour et dans lequel l'accumulation de lactate est réduite de presque 10-40 % pendant la culture cellulaire.

45

4. Procédé selon la revendication 1, dans lequel la protéine de fusion anticorps-peptide exprimée est soumise à une chromatographie d'affinité en utilisant une colonne de protéine A Mab Select Xtra ayant un pH spécifique.

50

5. Procédé selon la revendication 1, dans lequel le pH du surnageant se liant et passant à travers la colonne de protéine A Mab Select Xtra est ajusté au pH spécifique de la colonne de protéine A.

6. Procédé selon la revendication 1, dans lequel la protéine de fusion anticorps-protéine se lie par la région FC de l'anticorps à la colonne alors que les impuretés sont éliminées en suivant l'écoulement à travers la colonne.

55

7. Procédé selon la revendication 1, dans lequel la protéine de fusion anticorps-protéine liée à la colonne est éluée en utilisant de la glycine à pH 3,0 et est ajustée à un pH neutre pour le stockage.

8. Procédé selon la revendication 1, dans lequel la protéine purifiée est stockée à - 20°C ou à 2-8°C.

9. Procédé selon la revendication 1, dans lequel la séquence nucléotidique optimisée comprend une augmentation de nucléotides CG par rapport à une séquence nucléotidique non-optimisée.

**Anti-HER2/neu-TGF $\beta$ RII fusion protein at LC constant region**

Amino acid sequence of Anti-HER2/neu heavy chain:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGK  
GLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSL  
RAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPS  
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV  
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV  
DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR  
TPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN  
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG  
QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN  
GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS  
VMHEALHNHYTQKSLSLSPG

Amino acid sequence of Anti-HER2/neu light chain fusion protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKA  
PKLLIYSASFLYSGVPSRFSRSGTDFTLTISLQPEDFATYYC  
QQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASV  
VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS  
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGG  
GSGGGGSGGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCK  
**FCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENI**  
**TLETVCHDPKLPYHDFILEDAA**SPKCIMKEKKKKPGETFFMCSC  
SSDECNDNIIFSEYNTSNPD

Figure 1

**Anti-EGFR1-TGF $\beta$ RII fusion protein at LC constant region**

Amino acid sequence of Anti-EGFR1 heavy chain:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPG  
 KGLEWLGVIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSL  
 QSNDTAIYYCARALTYDYEFAYWGQGTLVTVSAASTKGPSVF  
 PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT  
 FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK  
 RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTP  
 EVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST  
 YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP  
 REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ  
 PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVM  
 HEALHNHYTQKSLSLSPG

Amino acid sequence of Anti-EGFR1 light chain fusion protein:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSP  
 RLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQ  
 NNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVC  
 LLNMFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS  
 STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGGS  
 GGGGSGGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFC  
**DVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL**  
**ETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSS**  
**DECNDNIIFSEEYNTSNPD**

Figure 2

## **Anti-CTLA4-TGF $\beta$ RII fusion protein at LC constant region**

Amino acid sequence of anti-CTLA4 heavy chain:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAP  
GKGLEWVTFISYDGNNKYYADSVKGRFTISRDN SKNTLYLQMN  
SLRAEDTAIYYCARTGWLGPFDYWGGTGLVTVSSASTKGPSVF  
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT  
FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK  
RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTP  
EVT CVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST  
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP  
REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ  
PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM  
HEALHNHYTQKSLSLSPG

Amino acid sequence of anti-CTLA4 light chain fusion protein:

EIVLTQSPGTLSSLSPGERATLSCRASQSVGSSYLAWYQQKPGQ  
APRLLIYGAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYY  
CQQYGSSPWTFGQGKVEIKRTVAAPSVFIFPPSDEQLKSGTA  
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST  
YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECG  
GGGSGGGGSGGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQ  
**LCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKN**  
**DENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFM**  
**CSCSSDECNDNIIFSEEYNTSNPD**

Figure 3

**Anti-HER2/neu HC-4-1BB and LC-TGF $\beta$ RII fusion protein:**

Amino acid sequence of heavy chain-4-1BB fusion protein:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVA  
RIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRW  
GGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC  
LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG  
TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP  
PKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE  
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP  
REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSL  
SLSPGGGGGGSGGGGGSGGGGGSACPWAVSGARASPGSAASPRLREGPE  
LSPDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTG  
GLSYKEDTKELVAKAGVYVFFQLELRRVAGEGSGSVSLALHLQPLR  
SAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHT  
EARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE

Amino acid sequence of light chain-TGF $\beta$ RII fusion protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYS  
ASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQ  
GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKV  
DNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEKHKVYACEVTHQ  
GLSSPVTKSFNRGECGGGGSGGGGGSGGGGGSTIPPHVQKS VNNDMIVTD  
NNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW  
RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSC  
SSDECNDNIIFSEEYNTSNPD

Figure 4

**Anti-EGFR1 HC-4-1BB and LC-TGF $\beta$ RII fusion protein:**

Amino acid sequence of heavy chain-4-1BB fusion protein:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWL  
 GVIWSSGGNTDYNTPTFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARA  
 LTTYDYEFAYWGQGTLLTVSAASTKGPSVFPLAPSSKSTSGGTAALGCL  
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGT  
 QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP  
 KPKDTLMISRTPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR  
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS  
 LSPGGGGGGSGGGGGSGGGGGSACPWAVSGARASPGSAASPRLREGPEL  
 SPDDPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGG  
 LSYKEDTKELVAKAGVYYVFFQLELRVWAGEGSGSVSLALHLQPLRS  
 AAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTE  
 ARARHAWQLTQGATVLGLFRVTPEIPAGLPSRSE

Amino acid sequence of light chain-TGF $\beta$ RII fusion protein:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYA  
 SESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAG  
 TKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD  
 NALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQG  
 LSSPVTKSFNRGECGGGGGSGGGGGSGGGGGSTIPPHVQKSVNNDMIVTDN  
 NGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAVWR  
 KNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCS  
 SDECNDNIIFSEEYNTSNPD

Figure 5



**Anti-CTLA4 HC-4-1BB and LC-TGF $\beta$ RII fusion protein:**

Amino acid sequence of heavy chain-4-1BB fusion protein:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLEW  
 VTFISYDGNNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAIYYCA  
 RTGWLGPFDYWGGGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL  
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT  
 QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP  
 KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR  
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLS  
 LSPGGGGGGSGGGGGSGGGGGSACPWAVSGARASPGSAASPRLREGPEL  
 SPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGG  
 LSYKEDTKELVAKAGVYYVFFQLELRRVWAGEGSGSVSLALHLQPLRS  
 AAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTE  
 ARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE

Amino acid sequence of light chain-TGF $\beta$ RII fusion protein:

EIVLTQSPGTL SLSPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLLIY  
 GAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTF  
 GGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW  
 KVDNALQSGNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVT  
 HQGLSSPVTKSFNRGECGGGGGSGGGGSGGGGGSTIPPHVQKSVNNDMIV  
 TDNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEV CVAV  
 WRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMC  
 SCSSDECNDNIIFSEEYNTSNPD

Figure 6

**Anti-HER2/neu HC-PD1 and LC-TGF $\beta$ RII fusion protein:**

Amino acid sequence of heavy chain-PD1 fusion protein:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVA  
 RIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRW  
 GGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC  
 LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG  
 TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP  
 PKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE  
 EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP  
 REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSL  
 SLSPGGGGGGSGGGGGSGGGGGSPGWFLDSPDRPWNPPPTFSPALLVTE  
 GDNATFTCSFSNTSESVLNWYRMSPSNQTDKLAAFPEDRSQPGQD  
 CRFRVTQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESL  
 RAELRVTERRAEVPTAHPSPPRPAGQFQTLV

Amino acid sequence of light chain-TGF $\beta$ RII fusion protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYS  
 ASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQ  
 GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV  
 DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQ  
 GLSSPVTKSFNRGECGGGGSGGGGGSGGGGGSTIPPHVQKSVNNDMIVTD  
 NNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVW  
 RKNDENITLETVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSC  
 SSDECNDNIIFSEEYNTSNPD

Figure 7

**Anti-EGFR1 HC-PD1 and LC-TGF $\beta$ RII fusion protein:**

Amino acid sequence of heavy chain-PD1 fusion protein:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWL  
 GVIWSSGGNTDYNTPTFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARA  
 LTTYDYEFAYWGQGTLLTVSAASTKGPSVFPLAPSSKSTSGGTAALGCL  
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT  
 QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP  
 KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR  
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLS  
 LSPGGGGGGSGGGGGSGGGGGSPGWFLDSPDRPWNPPPTFSPALLVTEG  
 DNATFTCSFSNTSESFVLNWMSPSNQTDKLAAFPEDRSQPGQDC  
 RFRVTQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLR  
 AELRVTERRAEVPTAHPSPPSRPAGQFQTLV

Amino acid sequence of light chain-TGF $\beta$ RII fusion protein:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYA  
 SESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAG  
 TKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD  
 NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG  
 LSSPVTKSFNRGECGGGGSGGGGGSGGGGGSTIPPHVQKSVNNDMIVTDN  
**NGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAVWR**  
**KNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCS**  
**SDECNDNIIFSEYNTSNPD**

Figure 8

**Anti-CTLA4 HC-PD1 and LC-TGF $\beta$ RII fusion protein:**

Amino acid sequence of heavy chain-PD1 fusion protein:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLEW  
 VTFISYDGNNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAIYYCA  
 RTGWLGPFDYWGGGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL  
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT  
 QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP  
 KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR  
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLS  
 LSPGGGGGGSGGGGSGGGGSGPGWFLDSPDRPWNPPPTFSPALLVTEG  
 DNATFTCSFSNTSESFVLN WYRMSPSNQTDKLAAFPEDRSQPGQDC  
 RFRVTQLPNGRDFHMSVVRARRND SGTYLCGAISLAPKAQIKESLR  
 AELRV TERRAEVPTAHPSPSPRPAGQFQTLV

Amino acid sequence of light chain-TGF $\beta$ RII fusionprotein:

EIVLTQSPGTLSSLSPGERATLSCRASQSVGSSYLA WYQQKPGQAPRLLIY  
 GAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTF  
 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW  
 KVDNALQSGNSQESVTEQDSKDSTYSLSSSTLTLSKADYEKHKVYACEVT  
 HQGLSSPVTKSFNRGECGGGGSGGGGSGGGGSTIPPHVQKSVNNDMIV  
 TDNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAV  
 WRKNDENITLETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMC  
 SCSSDECNDNIIFSEEYNTSNPD

Figure 9

**Anti-HER2/neu HC-TGF $\beta$ RII-4-1BB fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-4-1BB fusion protein:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVA  
 RIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRW  
 GGDGFYAMDYWGQGT~~LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGC~~  
 LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG  
 TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP  
 PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE  
 EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP  
 REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSL  
 SLSPGKGGGGSGGGGGSGGGGSTIPPHVQKS~~VNNDMIVTDNNGAVKFP~~  
**QLCKFC**DVRFSTCDN**QKSCMSNCSITSICEK**PQEV**CVAVWRK**NDENITL  
**ETVCHDPKLPYHDFILED**AAS**PKCIMKEKKKPGETFFMCSCSSDECNDN**  
**IIFSEEYNTSNPDEPKSCDKACPWAVSGARASPGSAASPRLREGPELSP**  
*DDPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLS*  
*YKEDTKELVAKAGVYVFFQLELRRVAGEGSGSVSLALHLQPLRSA*  
*AGAAALALTVDLPPASSEARNSAFGFQGRLHLSAGQRLGVHLHTEA*  
*RARHAWQLTQGATVLGLFRVTPEIPAGLPSRSE*

Amino acid sequence of light chain:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYS  
 ASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQ  
 GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNFPYPREAKVQWKV  
 DNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQ  
 GLSSPVTKSFNRGEC

Figure 10

**Anti-EGFR1 HC-TGF $\beta$ RII-4-1BB fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-4-1BB fusion protein:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWL  
 GVIWSSGGNTDYNTPTFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARA  
 LTTYDYEFAYWGQGT~~LV~~TVSAASTKGPSVFPLAPSSKSTSGGTAALGCL  
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGT  
 QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP  
 KPKDTLMISRTP~~EV~~TCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR  
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNV~~F~~SCSVMHEALHNHYTQKSLS  
 LSPGKGGGGSGGGGGSGGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQL  
**CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV**CVAVWRKNDENITLET  
**VCHDPKLPYHDFILED**AASPKCIMKEKKK**PGETFFMCSCSSDECNDNIIF**  
**SE**EYNTSNPDEPKSCDKACPWAVSGARASPGSAASPRLREGPELSPD  
 DPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSY  
 KEDTKELVAKAGVYYVFFQLELRVWAGEGSGSVSLALHLQPLRSAA  
 GAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEAR  
 ARHAWQLTQGATVLGLFRVTPEIPAGLPSRSE

Amino acid sequence of light chain:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYA  
 SESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAG  
 TKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD  
 NALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQG  
 LSSPVTKSFNRGEC

Figure 11

**Anti-CTLA4 HC-TGF $\beta$ RII-4-1BB fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-4-1BB fusion protein:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLEW  
 VTFISYDGNNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAIYYCA  
 RTGWLGPFDYWGGGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL  
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT  
 QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP  
 KPKDTLMISRTPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR  
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLS  
 LSPGKGGGGSGGGGSGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQL  
**CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV**CVAVWRKNDENITLET  
**VCHDPKLPYHDFILED**AASPKCIMKEKKKKPGETFFMCSCSSDECNDNIIF  
**SE**EYNTSNPDEPKSCDKACPWAVSGARASPGSAASPRLREGPELSPD  
 DPAGLLDLRQGMFAQLVAQNVLIDGPLSWYSDPGLAGVSLTGGLSY  
 KEDTKELVAKAGVYYVFFQLELRRVAGEGSGSVSLALHLQPLRSAA  
 GAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEAR  
 ARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE

Amino acid sequence of light chain:

EIVLTQSPGTL SLSPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLLIY  
 GAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTF  
 QGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW  
 KVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVT  
 HQGLSSPVTKSFNRGEC

Figure 12

**Anti-HER2/neu HC-TGF $\beta$ RII-PD1 fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-PD1 fusion protein:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVA  
 RIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRW  
 GGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGC  
 LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG  
 TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP  
 PKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE  
 EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP  
 REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK  
 TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSL  
 SLSPGKGGGGSGGGGGSGGGGGSTIPPHVQKS VNNDMIVTDNNGAVKFP  
**QLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL**  
**ETVCHDPKLPYHDFILED AASPKCIMKEKKKPGETFFMCSCSSDECNDN**  
**IIFSEEYNTSNPDEPKSCDKPGWFLDSPDRPWNPPPTFSPALLVVTED**  
*NATFTCSFSNTSESVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRF*  
*RVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAEL*  
*RVTERRAEVPTAHPSPPRPAGQFQTLV*

Amino acid sequence of light chain:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYS  
 ASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQ  
 GTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKV  
 DNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEKHKVYACEVTHQ  
 GLSSPVTKSFNRGEC

Figure 13



**Anti-EGFR1 HC-TGF $\beta$ RII-PD1 fusion protein:**

Amino acid sequence of heavy chain-TGF $\beta$ RII-PD1 fusion protein:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLEWL  
 GVIWSSGGNTDYNTPTFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARA  
 LTTYDYEFAYWGQGTLLTVSAASTKGPSVFPLAPSSKSTSGGTAALGCL  
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGT  
 QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP  
 KPKDTLMISRTPPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR  
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLS  
 LSPGKGGGGSGGGGSGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQL  
**CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLET**  
**VCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECNDNIIF**  
**SEYNTSNPDEPKSCDKPGWFLDSPDRPWNPPPTFSPALLVVTEDNA**  
*TFTCSFSNTSESFVLNWMSPSNQTDKLAAPFEDRSQPGQDCRFRV*  
*TQLPNGRDFHMSVVRARRNDSTYLCGAISLAPKAQIKESLRAELRV*  
*TERRAEVPTAHPSPPRPAGQFQTLV*

Amino acid sequence of light chain:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYA  
 SESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAG  
 TKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD  
 NALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQG  
 LSSPVTKSFNRGEC

Figure 14

**Anti-CTLA4 HC-TGF $\beta$ RII-PD1 fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-PD1 fusion protein:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLEW  
 VTFISYDGNNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAIYYCA  
 RTGWLGPFDYWGGGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL  
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGT  
 QTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP  
 KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE  
 QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR  
 EPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT  
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLS  
 LSPGKGGGGSGGGGSGGGGSTIPPHV**QKSVNNDMIVTDNNGAVKFPQL**  
**CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV**CVAVWRKNDENITLET  
**VCHDPKLPYHDFILED**AASPKCIMKEKKK**PGETFFMCSCSSDECNDNIIF**  
**SEYNTSNPDEPKSCDKPGWFLDSPDRPWN**PPTFSPALLVTEGDNA  
 TFTCSFSNTSESFVLNWYRMSPSNQTDKLA**AFPEDRSQPGQDCRFRV**  
 TQLPNGRDFHMSVVRARRND**SGTYLCGAISLAPKAQIKESLRAELRV**  
 TERRAEVPTAH**PSPSRPAGQFQTLV**

Amino acid sequence of light chain:

EIVLTQSPGTL~~SL~~SPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLLIY  
 GAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTF  
 GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW  
 KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVT  
 HQGLSSPVTKSFNRGEC

Figure 15

**Nucleotide sequence of Anti-HER2/neu heavy chain constant region with linker:**

```

1 gctagcacca agggccctc cgtgttcct ctggccctc ccagcaagtc cacctctggc
61 ggacccgctg ctctgggctg cctggtaag gactacttc ccgagcccg gaccgtgtcc
121 tggaactctg gcgctctgac ctccggcgtg cacaccttc ctgccgtgct gcagtcctcc
181 ggctgtact cctgtcctc cgtcgtgacc gtgccctcca gctctctgg caccagacc
241 tacatctgca acgtgaacca caagccctcc aacaccaagg tggacaagaa ggtggaaccc
301 aagtctgctg acaagaccca cacctgtccccctgcctg cccctgagct cctgggaggc
361 cctagcgtgt tctgttccc ccaaagccc aaggacacc tgatgatct ccggacccc
421 gaagtgcct gcgtggtgtt ggacgtgtcc cagcaggacc ctgaagtga gttcaattgg
481 tacgtggacg gcgtggaagt gcacaacgc aagaccaagc ccagagagga acagtacaac
541 tccacctacc ggggtgtgtc cgtgtgacc gtgtgcacc aggactggct gaacggcaaa
601 gagtacaagt gcaagggtgc caacaaggcc ctgcctgcc ccatgaaaa gaccatctcc
661 aaggccaagg gccagccccg cgagcctcag gtgtacacc tgcctcctag ccggaagag
721 atgaccaaga accaggtgtc cctgacctgt ctggtcaagg gcttctacc ctccgatctc
781 gccgtggaat gggagtccaa cggccagccc gagaacaact acaagaccac cccccctgtg
841 ctggactccg acggctcatt ctctctgtac tccaagctga ccgtggacaa gtcccgtgtg
901 cagcagggca acgtgttctc ctgtccgtg atgcacgagg cctgcacaa ccactacacc
961 cagaagtccc tgcctctgag ccaggcaaa ggccgaggcg gatctggcgg cggaggatct
....1021 ggtggcg gatcc

```

**Nucleotide sequence of TGF $\beta$ RII ECD:**

```

1 ggatccacca tccccca cgtgcagaaa tccgtgaaca acgacatgat cgtgaccgac
61 aacaacggcg ctgtgaagtt ccccagctg tgcaagttct gcgacgtgcg gttctctacc
121 tgcgacaacc agaaatcctg catgtccaac tgctccatca cctccatctg cgagaagccc
181 caggaagtgt gcgtgccgt ctggcgaag aacgacgaga acatcacctt ggaaaccgtg
241 tgccacgacc ccaagctgcc ctaccacgac ttcacctctg aagatgccgc ctcccccaag
301 tgcacatga aggaaaagaa gaagcccggc gagactttct tcatgtgcag ctgtctctcc
361 gacgagtgca acgacaacat catcttctcc gaagagtaca acacctcaa ccccgactga
421 agctt

```

**Figure 16**

**Nucleotide sequence of Anti-HER2/neu heavy chain variable region**

```

1  gcggccgcca tgaacttcgg cctgcggctg atcttccctg tgetgaccct gaagggcgtg
61  cagtgcgagg tgcagctggt ggaatccggc ggaggcctgg tccagcctgg cggatctctg
121 agactgtcct gcgcgcctc cggttcaac atcaaggaca cctacatcca ctgggtccga
181 caggccctg gcaagggcct ggaatgggtg gcccggatct accccaccaa cggctacacc
241 agatacgccg actccgtgaa gggccgggtt accatctccg ccgacacctc caagaacacc
301 gcctacctgc agatgaactc cctgcggggc gaggacaccg ccgtgtacta ctgctccaga
361 tggggaggcg acggcttcta cgccatggac tactgggggc agggcaccct ggtcaccgtg
421  ctccgcta gc

```

**Nucleotide sequence of Anti-HER2/neu light chain variable region**

```

1  gcggccgcca tggaatccca gaccaggtg ctgatctccc tgetgttctg ggtgtccggc
61  acctgtggcg acatccagat gaccagtc ccctccagcc tgtccgcctc tgtgggcgac
121 agagtgaaca tcacctgtcg ggcctcccag gacgtgaaca ccgccgtggc ctggtatcag
181 cagaagcccg gcaagggccc caagctgtcg atctactccg cctccttccg gtactccggc
241 gtgcctccc ggttctccgg ctctagatcc ggcaccgact ttacctgac catctccagc
301 ctgcagcccg aggacttcgc cacctactac tgccagcagc actacaccac cccccccacc
361 tttggccagg gcaccaaggt ggaaatcaag cggaccgtgg ccgctccctc cgtgttcato
421  cccaccct ccgacgagca gctg

```

**Nucleotide sequence of Anti-EGFR1 heavy chain constant region with linker:**

```

1  gctagcacca agggcccctc cgtgtttccc ctggccccct ccagcaagtc cacctctggc
61  ggcaccgccc ctctgggctg cctgggtcaag gactacttcc ccgagcccgt gaccgtgtcc
121 tggaactctg gcgctctgac ctccggcgtg cacaccttcc ctgccgtgct gcagtcctcc
181 ggcctgtact cctgtcctc cgtcgtgacc gtgccctcca gctctctggg caccagacc
241 tacatctgca acgtgaacca caagccctcc aacaccaagg tggacaagcg ggtggaaccc
301 aagtcctgcg acaagaccca cacctgtccc ccctgccctg cccctgaact gctgggaggc
361 ccttccgtgt tctgttccc cccaaagccc aaggacaccc tgatgatctc ccggaccccc
421 gaagtgacct gcgtgggtgt ggacgtgtcc caccaggacc ctgaagtga gttcaattgg
481 tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ccagagagga acagtacaac
541 tccacctacc ggggtgggtg cgtgctgacc gtgctgcacc aggactggct gaacggcaaa
601 gagtacaagt gcaaggtgtc caacaaggcc ctgcctgccc ccatcgaaaa gaccatctcc
661 aaggccaagg gccagccccg cgagcctcag gtgtacaccc tgcctcccag ccgggacgag
721 ctgaccaaga accaggtgtc cctgacctgt ctgggtcaagg gcttctaccc ctccgatatc
781 gccgtggaat gggagtccaa cggccagccc gagaacaact acaagaccac cccccctgtg
841 ctggactccg acggctcatt ctctctgtac tccaagctga ccgtggacaa gtcccgggtg
901 cagcagggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc
961 cagaagtccc tgtctctgag ccccgcaaaa ggcgccggag gatctggcgg tggcggatca
1021 ggccggag gatcc

```

Figure 17

**Nucleotide sequence of Anti-EGFR1 heavy chain variable region**

```

1 gcgggccgcca tgaacttcgg cctgcggctg atcttcctgg tgctgacct gaagggcgctg
61 cagtgccagg tgcagctgaa gcagtccgga cctggcctgg tgcagccttc ccagtcctctg
121 tccatcacct gtaccgtgtc cggcttctcc ctgaccaact acggcggtgca ctgggtccga
181 cagtccccag gcaagggcct ggaatggctg ggagtgattt ggagcggcgg caacaccgac
241 tacaacaccc ccttcacctc ccggtgtgtc atcaacaagg acaactccaa gtcccagggtg
301 ttcttcaaga tgaactccct gcagtccaac gacaccgcca tctactactg cgccagagcc
361 ctgacctact atgactacga gttcgcctac tggggacagg gcacctgggt caccgtgtct
421 cgctagc

```

**Nucleotide sequence of Anti-EGFR1light chain variable region**

```

1 gcgggccgcca tggaatccca gaccaggtg ctgatctccc tgctgttctg ggtgtccggc
61 acctgtggcg acatcctgct gaccagtc ccgctgatcc tgtccgtgtc tccctggcgag
121 cgggtgtcct tctcctgccg ggcctcccag tccatcggca ccaacatcca ctggtatcag
181 cagcggacca acggctcccc tcggctgtctg attaatagc cctccgagtc tatctccggc
241 atccccctcc ggttctccgg ctctggctcc ggcaccgact tcacctgtc catcaactcc
301 gtggaatccg aggatatcgc cgactactac tgccagcaga acaacaactg gccaccacc
361 ttcggcgctg gcaccaagct ggaactgaag cggaccgtgg ccgctccctc cgtgttcatc
421 cccaccct ccgacgagca gctg

```

**Nucleotide sequence of Anti-CTLA4 heavy chain variable region**

```

1 gcgggccgcca tgaacttcgg cctgcggctg atcttcctgg tgctgacct gaagggcgctg
61 cagtgccagg tgcagctggt ggaatccggc ggaggcgctgg tgcagcctgg cagatccctg
121 agactgtcct gcgccgcctc cggcttcacc ttctccagct acaccatgca ctgggtccga
181 caggccccctg gcaagggcct ggaatgggtc accttcatca gctacgacgg caacaacaag
241 tactacgccg actccgtgaa gggccgggtc accatctccc gggacaactc caagaacacc
301 ctgtacctgc agatgaactc cctgcggggc gaggacaccg ccactacta ctgcgcccg
361 accggctggc tgggcccttt tgattactgg ggccagggca cctgggtcac cgtgtcctcc
421 tagc

```

**Nucleotide sequence of Anti-CTLA4light chain variable region**

```

1 gcgggccgcca tggaatccca gaccaggtg ctgatctccc tgctgttctg ggtgtccggc
61 acctgtggcg agatcgtgct gaccagtc ccggcacc tgtctctgag ccctggcgag
121 agagccaccc tgtcctgcag agcctcccag tccgtgggt cctcctacct ggcttggtat
181 cagcagaagc ccggccaggc cctcggctg ctgatctacg gcgtttctc tcgggccacc
241 ggcacccctg accggttctc tggctccggc tccggcaccg acttcacct gaccatctcc
301 cggctggaac ccgaggactt cggcgtgtac tactgccagc agtacggctc ctccccctgg
361 acctttggcc agggcaccaa ggtggaaatc aagcggaccg tggccgctcc ctccgtgttc
421 cttcccac cctccgacga gcagctg

```

Figure 18

**Nucleotide sequence of Anti CD20 IgG1 molecule:**

```

1  gctagcacaa agggccctag tgtgttttct ctggctccct cttccaaatc cacttctggt
61  ggcactgctg ctctgggatg cctgggtgaag gattactttc ctgaacctgt gactgtctca
121  tggaactctg gtgctctgac ttctgggtgtc cacactttcc ctgctgtgct gcagtctagt
181  ggactgtact ctctgtcatc tgtgggtcact gtgccctctt catctctggg aaccagacc
241  tacatttgta atgtgaacca caaacctacc aacactaaag tggacaaaaa agccgaacct
301  aaatcctgtg acaaaaccca cacctgcccc ccttgtcctg cccctgaact gctgggagga
361  ccttctgtgt ttctgttccc accaaaacca aaagataccc tgatgatctc tagaacccct
421  gaggtgacat gtgtgggtgt ggatgtgtct catgaggacc ctgaggtcaa atttaattgg
481  tacgtcgatg gagtgggaagt ccacaatgcc aaaaccaagc ctagagagga acagtacaat
541  tcaacctaca gagtgcgtcag tgtgctgact gtgctgcac aggattggct gaatggcaag
601  gaatacaagt gtaaagtctc aaacaaggcc ctgcctgtct caattgagaa aacaatctca
661  aaggccaagg gacagcctag ggaaccccag gtctacaccc tgccaccttc acgcgacgaa
721  ctgacaaaaa accaggtgtc cctgacatgc ctgggtcaaag gcttctaccc ttctgacatt
781  gctgtggagt gggagtcaaa tggacagcct gagaacaact acaaaacaac cccccctgtg
841  ctggattctg atggctcttt ctttctgtac tccaaactga ctgtggacaa gtctagatgg
901  cagcagggga atgtcttttc ttgctctgtc atgcatgagg ctctgcataa ccactacact
961  cagaaatccc tgtctctgtc tcccgggaaa ggcggcggag gatctggcgg aggcggttct
1021 ggtggtggcg gatcc

```

**Nucleotide sequence of Anti-CD20 heavy chain variable region**

```

1  gcggccgcca tgaatttttg actgaggctg attttctctg tgctgacct gaaaggcgctc
61  cagtgtcagg tgcagctgca gcagcctggg gccgagctcg tgaaacctgg cgctccgctg
121  aagatgtcct gcaaggcctc cggctacacc ttcaccagct acaacatgca ctgggtcaag
181  cagacccccg gcagaggcct ggaatggatc ggcgctatct accccggcaa cggcgacacc
241  tcctacaacc agaagttcaa gggcaaggcc accctgaccg ccgacaagtc ctcttcacc
301  gcctacatgc agctgtcctc cctgacctcc gaggactccg ccgtgtacta ctgcgcccg
361  tctacctact acggcgcgca ctggtacttc aacgtgtggg gcgctggcac caccgtgacc
421  gtgtctgctg ctage

```

**Nucleotide sequence of Anti-CD20 light chain variable region**

```

1  gcggccgcca tgaatttttg actgaggctg attttctctg tgctgacct gaaaggcgctc
61  cagtgtcaga tcgtgctgtc ccagtcacct gccatcctgt ctgctagccc tggcgagaaa
121  gtgacaatga cctgccgggc ctctctctcc gtgtcctaca tccactggtt ccagcagaag
181  cccggctcca gccccagcc ttggatctac gccacctcca acctggcctc tggcgtgcca
241  gtgcgggtttt ccggctctgg ctctggcacc tcctactccc tgaccatctc tcgggtggaa
301  gccgaggatg ccgccaccta ctactgccag cagtggacca gcaaccccc cactttggc
361  ggaggcacca agctggaaat caagcggacc gtggcggcgc cctct

```

Figure 19

**Nucleotide sequence of 4-1BB.**

```

1  ggatccgcct gtccttgggc cgtgtccggc gctagagcct ctccctggctc tgccgcctcc
61  cccagactga gagagggccc tgagctgtcc cctgacgac ctgccggcct gctggacctg
121 agacagggca tgtttgccc gctgggtggc cagaacgtgc tgctgacga cggccccctg
181 tccctggact ctgatacctg cctggccggc gtgtccctga ccggcggact gtcctacaaa
241 gaggacacca aagaactggg ggtggccaag gctggcgtgt actacgtgtt ctttcagctg
301 gaactgcggc ggggtggggc cggcgagggc tctggatctg tgtccctggc cctgcactctg
361 cagccccctg gatctgccgc tggcgccgct gctctggccc tgacagtgga tctgcctcct
421 gcctcctccg agggccggaa ctccgcattc gggtttcagg gccggctgct gcacctgtct
481 gctggccaga gactgggagt gcactctgcac accgaggcca gagccagaca cgcctggcag
541 ctgacccagg gcgctaccgt gctgggcctg ttcagagtga cccccgagat cccagccggc
601 ctgcccagcc ctagatccga gtgataagct t

```

**Nucleotide sequence of Anti-IL6R heavy chain:**

```

1  gcggccgcca tgaattttgg actgaggctg attttccctgg tgctgaccct gaaaggcgtc
61  cagtgtcagg tgcagctgca ggaatctggc cctggactcg tgcggccttc ccaaaccctg
121 tctctgacct gtaccgtgtc cggctactcc atcacctccg accacgcctg gtcttgggtg
181 cgacagcctc ctggcagagg cctggaatgg atcggctaca tctcctactc cggcatcacc
241 acctacaacc ccagcctgaa gtccagagtg accatgctgc gggacacctc caagaaccag
301 ttctccctgc ggctgtcctc cgtgaccgct gctgataccg ccgtgtacta ctgcgccaga
361 tctctggcca ggaccaccgc catggattac tggggccagg gctccctcgt gaccgtgtcc
421 tctgctagca ccaagggccc ctccgtgttc cctctggccc ctccctctaa atctacctct
481 ggcggcaccg ccgctctggg ctgcctcgtg aaggactact tccccgagcc cgtgacagtg
541 tcttggaaact ctggcgccct gacctccggc gtgcacacct ttccagctgt gctgcagtcc
601 tccggcctgt actccctgtc cagcgtcgtg actgtgccct cctcatctct gggcaccag
661 acctacatct gcaacgtgaa ccacaagccc tccaacacca aggtggacaa gaaggtggaa
721 cccaagtcct gcgacaagac ccacacctgt ccccttgtc ctgccctga actgctgggc
781 ggacctctct tgttctctgt cccacaaaaa ccgaaagaca ccctgatgat ctcccgacc
841 cccgaagtga cctgcgtggg ggtggatgtg tcccacgagg accctgaagt gaagttcaat
901 tggtagctgg acggcgtgga agtgcacaac gccaaagaca agcctagaga ggaacagtac
961 aactccacct accgggtggg gtccgtgctg accgtgctgc accaggattg gctgaacggc
1021 aaagagtaca agtgcaagg gtccaacaag gccctgcctg ccccatcga aaagaccatc
1081 tccaaggcca agggccagcc acgggaaccc cagggtgtaca cactgcccc tagccgcgac
1141 gagctgacca agaatacagg gtccctgaca tgctcgtga aaggcttcta cccctccgat
1201 atcgccgtgg aatgggagtc caacggccag cctgagaaca actacaagac cccccccct
1261 gtgctggact ccgacggctc attcttctct tactcaaagc tgacagtgga caagtcccgg
1321 tggcagcagg gcaacgtgtt ctccctgctc gtgatgcacg aggcctgca caaccactac
1381 acccagaagt ccctgtccct gagccccggg aaaggcggcg gaggatctgg cggaggcggg
1441 tctggtgggt gcggatcc

```

**Figure 20**

**Nucleotide sequence of Anti-IL6R light chain variable region:**

```

1  gcggccgcca tgaatttttg actgaggctg attttccttg tgctgaccct gaaaggcgctc
61  cagtgtgaca tccagatgac ccagtcctcc tccagcctgt ctgcctctgt gggcgacaga
121  gtgaccatca cctgtcgggc ctcccaggac atctcctcct acctgaactg gtatcagcag
181  aagccccgca agggccccaa gctgctgata tactacacct cccggctgca ctccggcgctg
241  ccctctagat ttcccggtct tggtccgggc accgacttta ccttcaccat cagctccctg
301  cagcccgagg atatcgccac ctactactgc cagcaaggca acaccctgcc ctacaccttt
361  ggccagggca ccaaggtgga aatcaagcgg accgtggcgg cgccc

```

**Nucleotide sequence of Anti-4-1BB heavy chain**

```

1  gcggccgcca tgaatttttg actgaggctg attttccttg tgctgaccct gaaaggcgctc
61  cagtgtcagg tgcagctgca gcagtgggga gctggactgc tgaagccctc cgagacactg
121  tctctgacct gcgtgtgtga cggcggtctc ttctccggct actactggtc ctggattcgg
181  cagtcctctg agaaggcctt ggaatggatc ggcgagatca accacggcgg ctacgtgacc
241  tacaacccca gctggaatc cagagtgacc atctccgtgg acacctccaa gaaccagttc
301  tccctgaagc tgctcctcgt gaccgcccgt gataccgccg tgtactactg cgccagagac
361  tacggccctg gcaactacga ctggtacttc gacctgtggg gcagaggcac cctcgtgacc
421  gtgtcctctg ctagcaccaa gggccctctc gtgtttctct tggccctctg ctcacgtctc
481  acctccgaat ctaccgccgc tctgggctgc ctctgaagg actacttccc cgagcccgctg
541  actgtgtctt ggaactctgg cgcctgacc tccggcgtgc acacctttcc agctgtgctg
601  cagtctccg gcctgtactc cctgtccagc gtcgtgacag tgccctccag ctctctgggc
661  accaagacct acacctgtaa cgtggaccac aagccctcca acaccaaggt ggacaagcgg
721  gtggaatcta aatacggccc tccctgcctt ccttgcccag cccctgaatt tctgggcgga
781  ccttcctgtg tctgttctcc cccaaaacct aaggacacct tgatgatctc ccggaccccc
841  gaagtgacct gcgtggtggt ggatgtgtcc caggaagatc ccgaggtgca gttcaattgg
901  tacgtggaag gcgtggaagt gcacaacgcc aagaccaagc ctagagagga acagttcaac
961  tccacctacc ggggtggtgt cgtgtgacc gtgctgcacc aggatgggtt gaacggcaaa
1021  gagtacaagt gcaaggtgtc caacaagggc ctgccagctt ccatcgaaaa gaccatcagc
1081  aaggccaagg gccagccccg ggaaccccag gtgtacacac tgccctcaag ccaggaagag
1141  atgaccaaga atcaggtgtc cctgacctgt ctctgaaag gcttctacct ctccgatatc
1201  gccgtggaat gggagtccaa cggccagcct gagaacaact acaagaccac cccccctgtg
1261  ctggactccg acggcagctt ctctctgtac tctgcctga ccgtggacaa gtcccgggtg
1321  caggaaggca acgtgttctc ctgtccgtg atgcacgagg ccctgcacaa ccactacacc
1381  cagaagtccc tgctccctgt tctggggaaa ggcggcggag gatctggcgg aggcggttct
1441  ggtggtggcg gatcc

```

**Nucleotide sequence of Anti-4-1BB light chain variable region**

```

1  gcggccgcca tgaatttttg actgaggctg attttccttg tgctgaccct gaaaggcgctc
61  cagtgtgaga tcgtgctgac ccagtctcct gccaccctgt ctctgagccc tggcgagaga
121  gctaccctgt cctgccgtgc ctcccaatcc gtgtcctctt acctggcctg gtatcagcaa
181  aagccccggc aggtctcccc gctgctgata tacgatgcct ccaatagagc caccggcatc
241  cctgccagat tctccggctc tggtctctgg accgacttta cctgacctat ctctctctg
301  gaacccgagg acttcgccgt gtactactgc cagcagcggg ccaactggcc tccgcctctg
361  acatttggcg gaggcaccaa ggtggaaatc aagcggaccg tggcggcgcc c

```

**Figure 21**



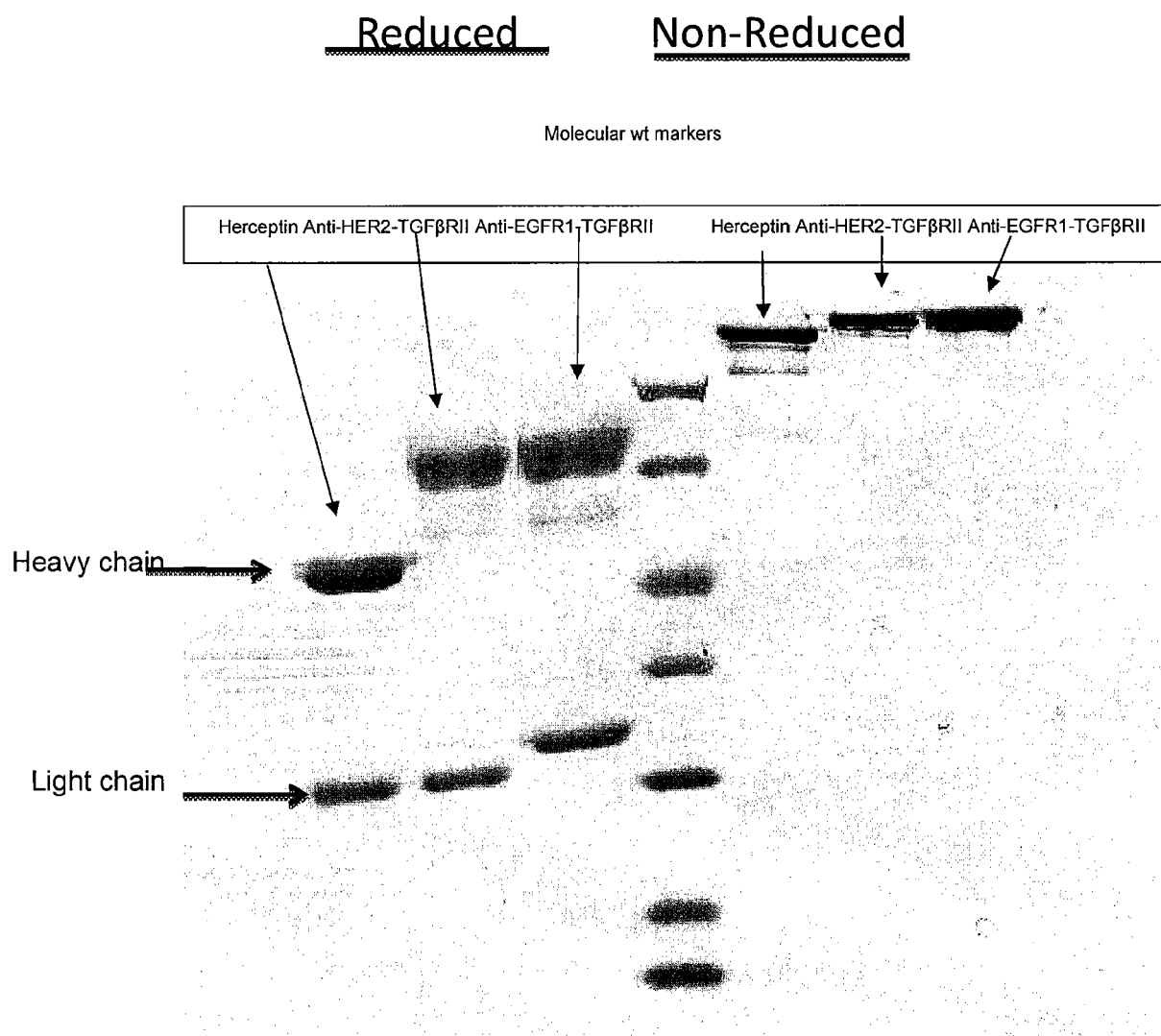


Figure 22

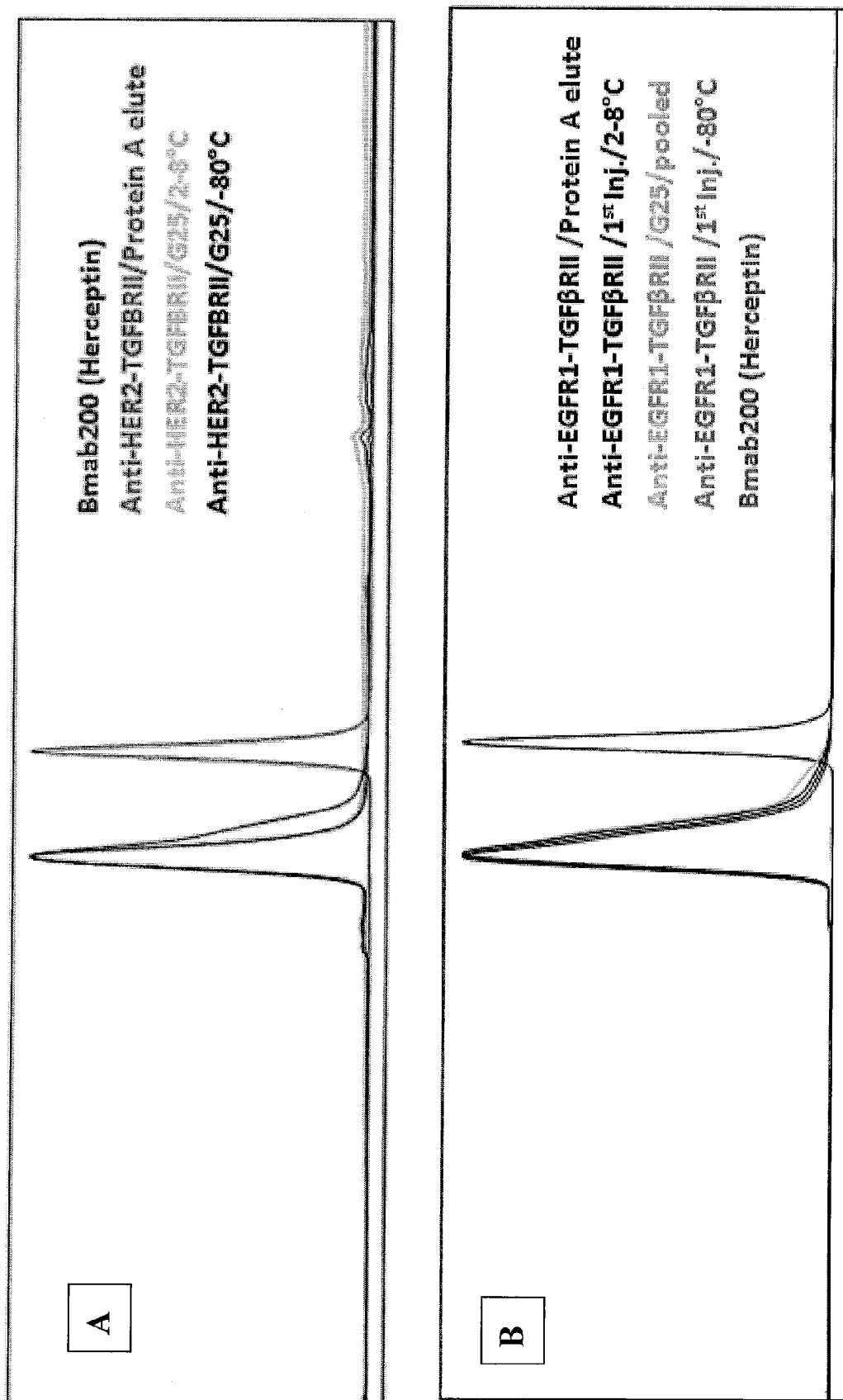
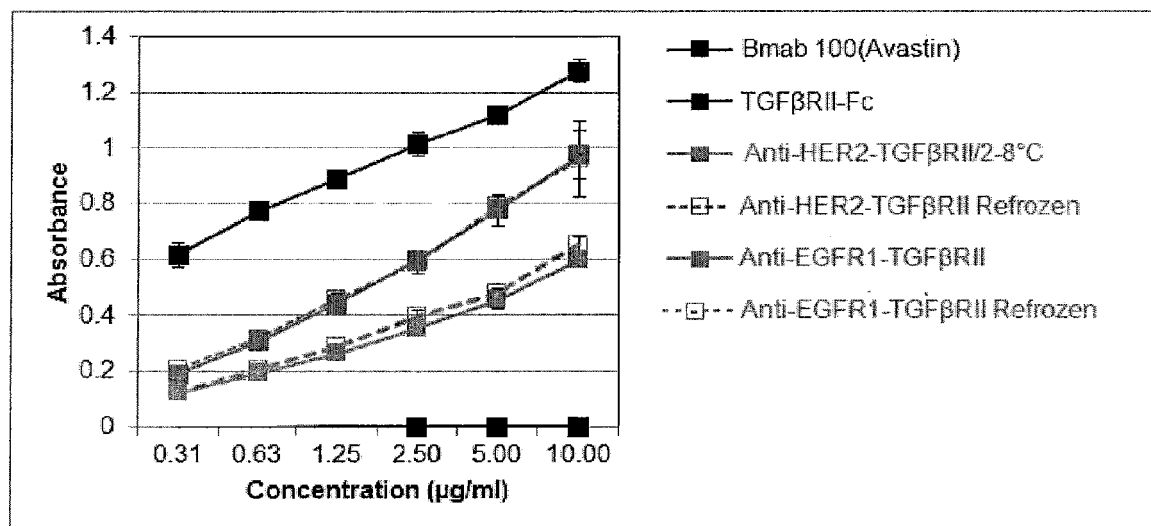


Figure 23

A



B

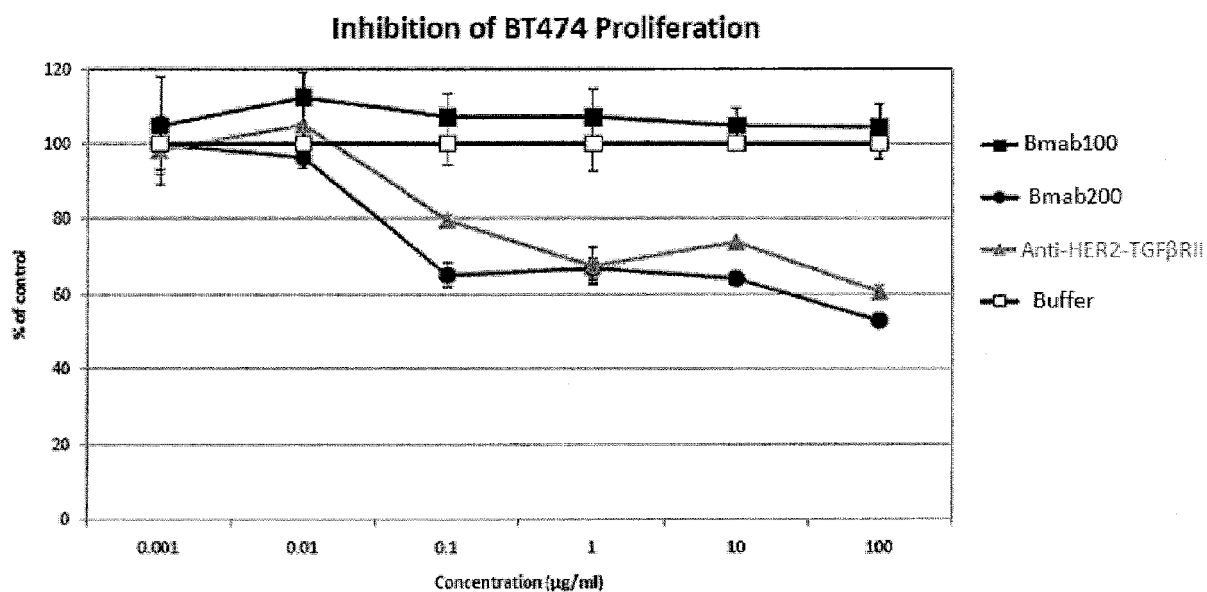


Figure 24

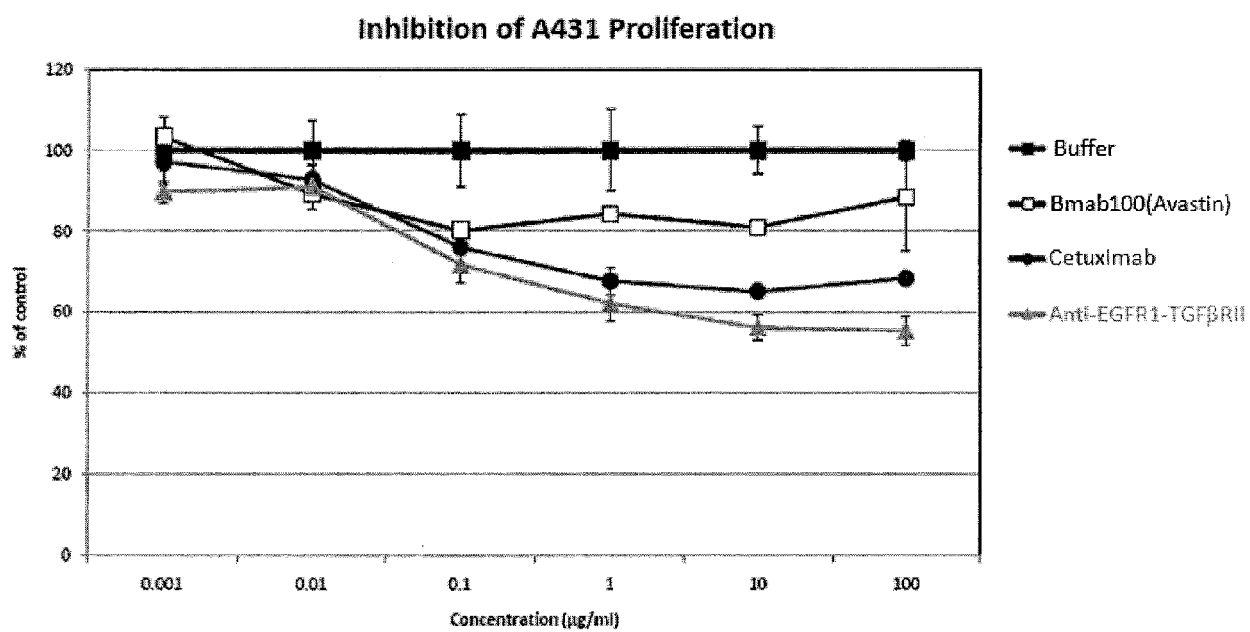


Figure 25

# ADCC Activity of Anti-HER2-TGFβRII on BT474 Cells

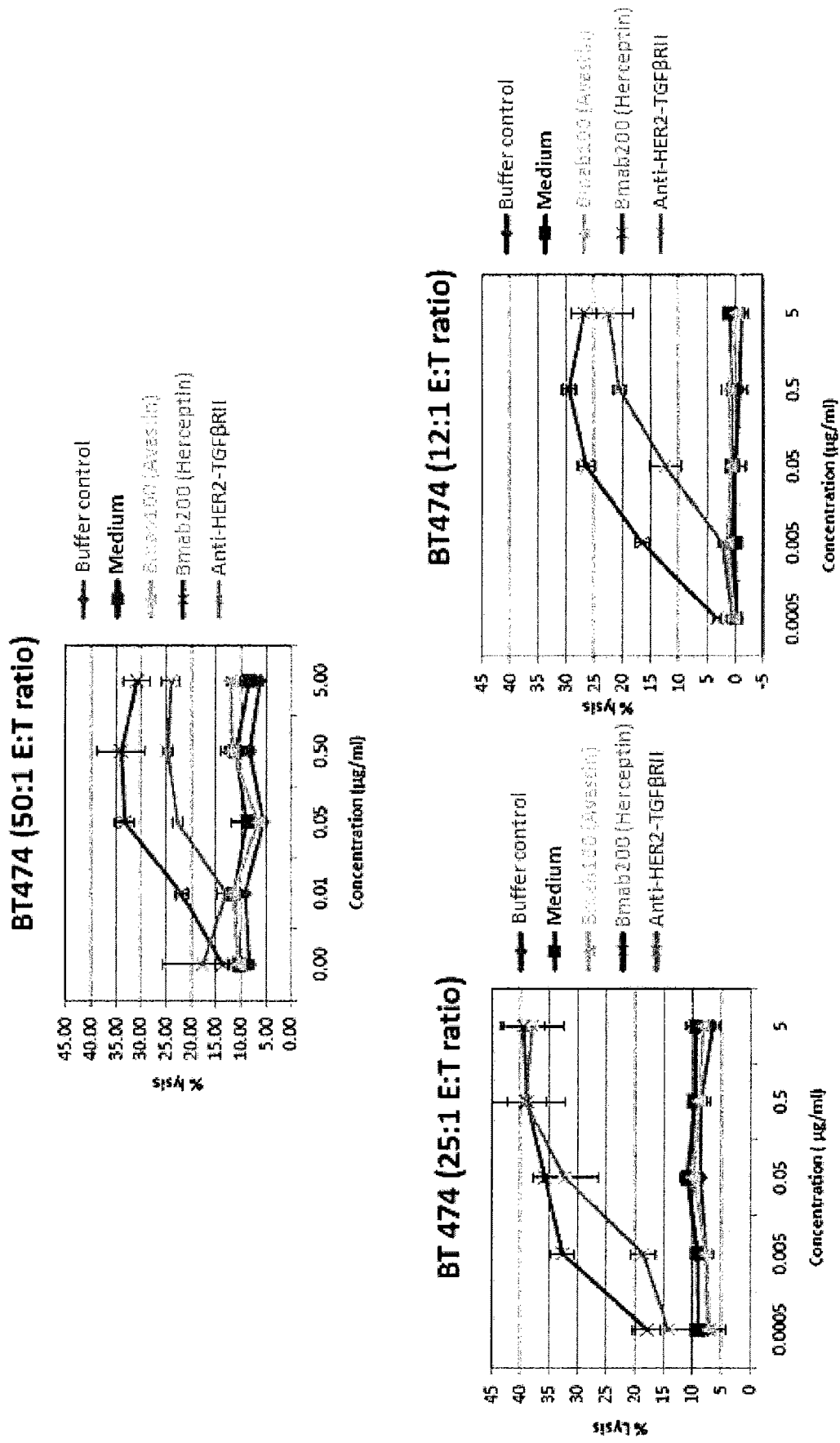


Figure 26

# ADCC Activity of Anti-EGFR1-TGFβRII on A431 Cells

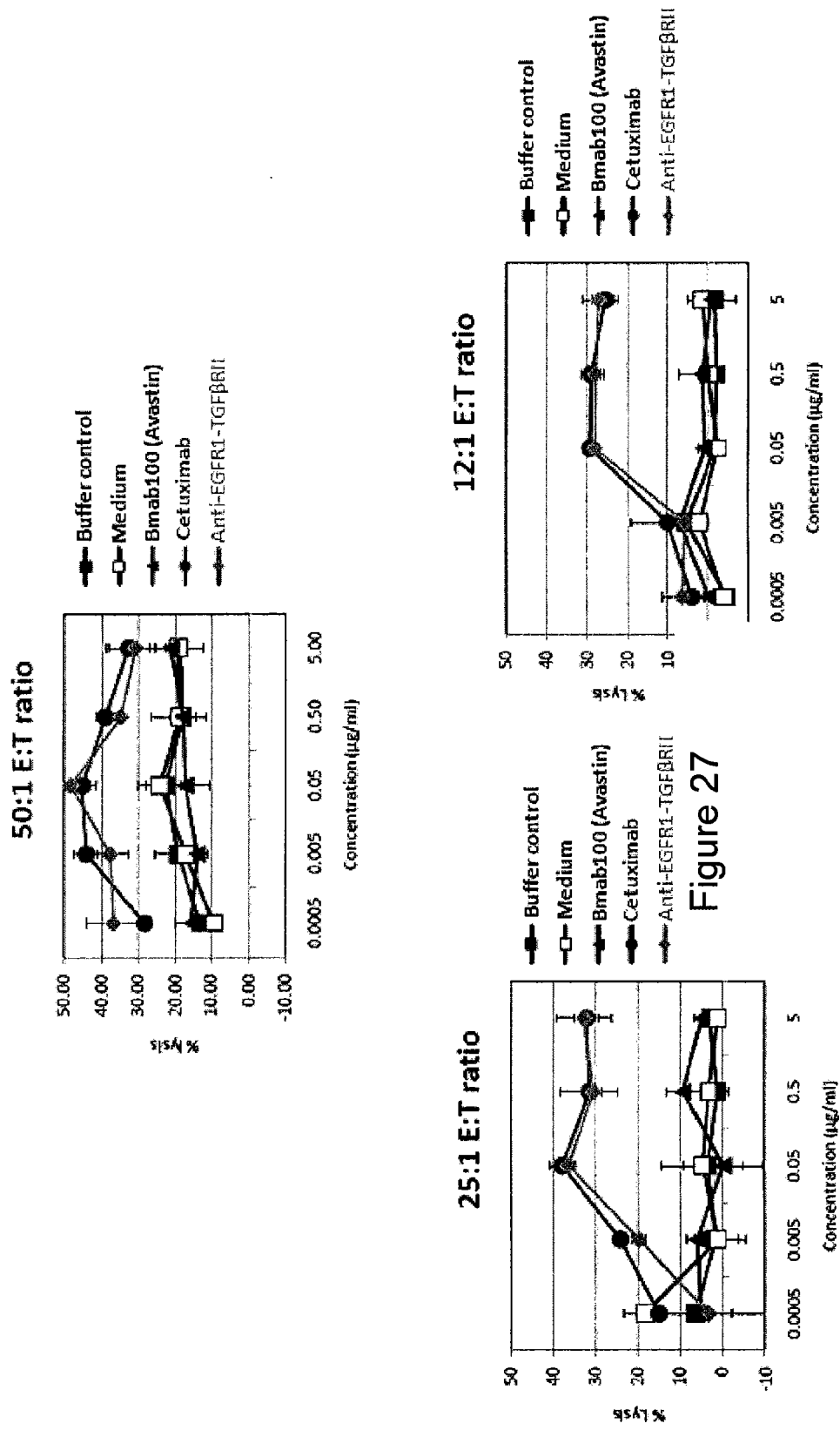


FIGURE 27

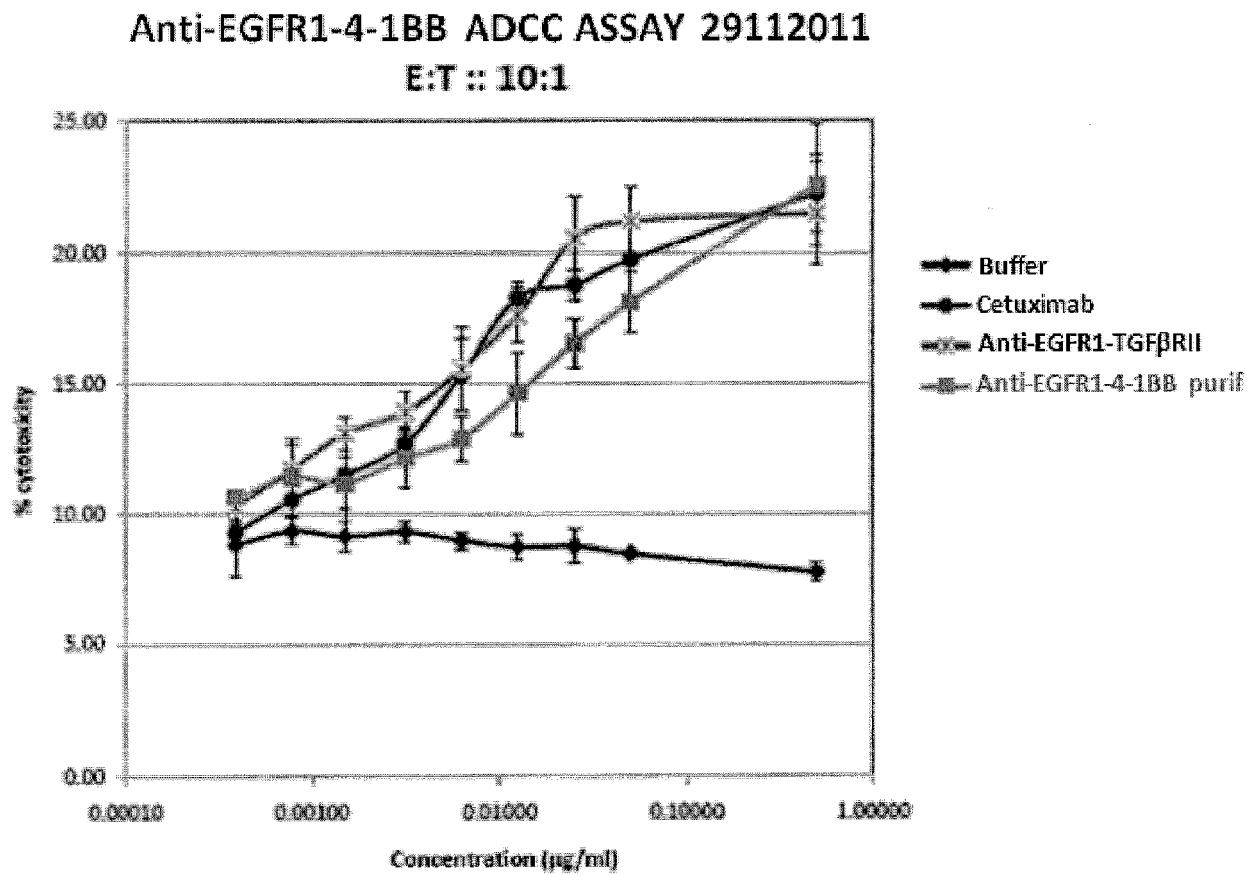


Figure 28

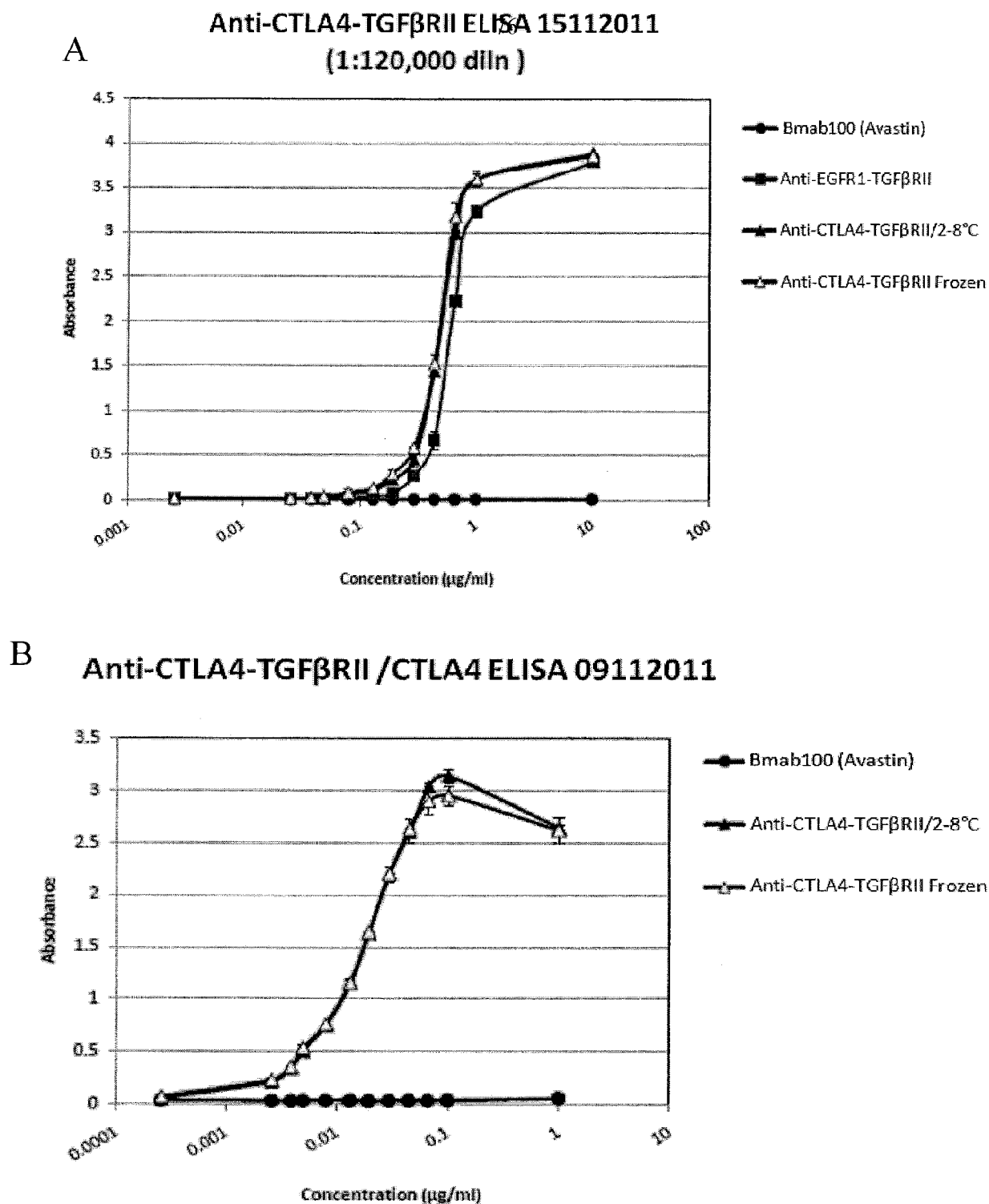


Figure 29



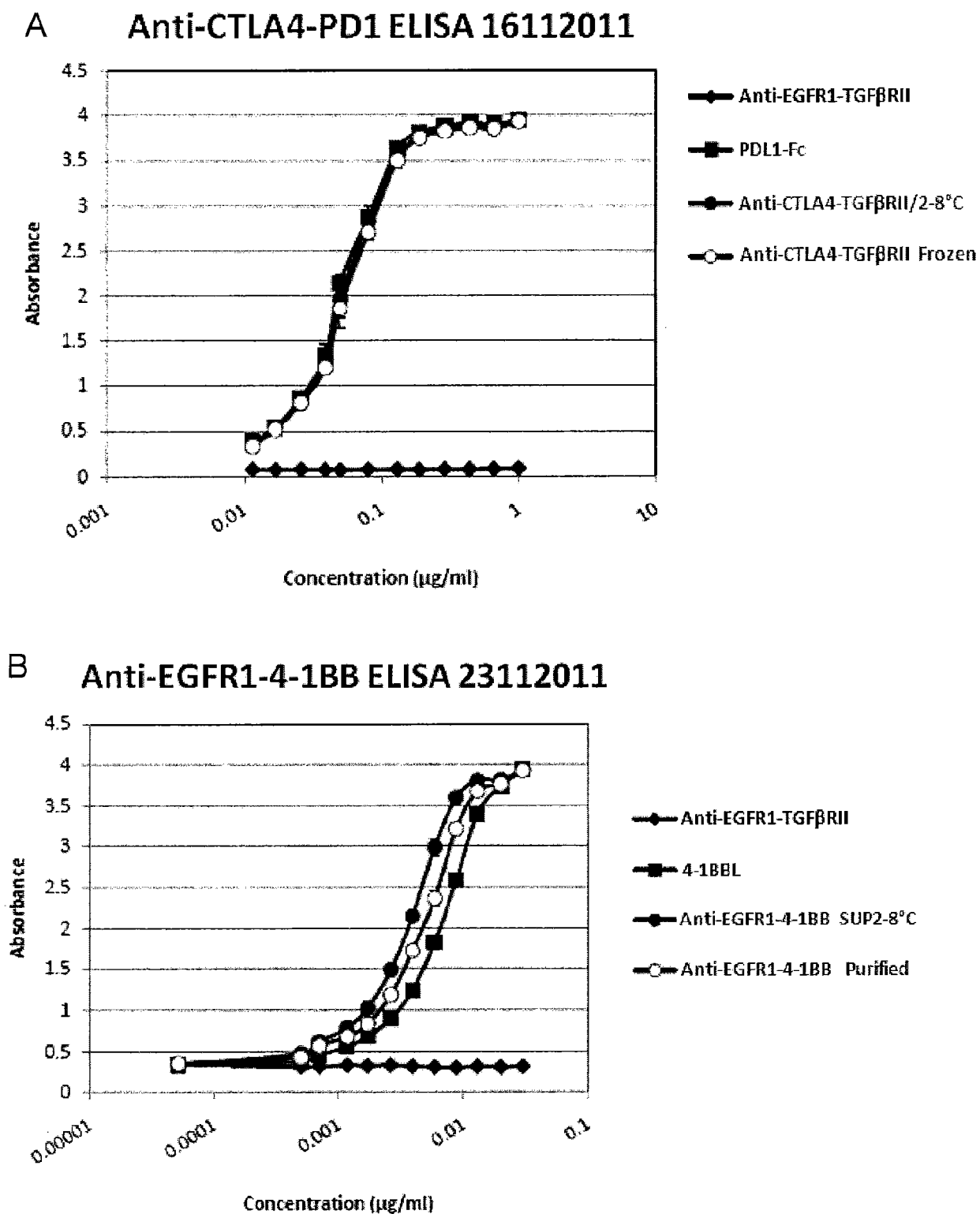
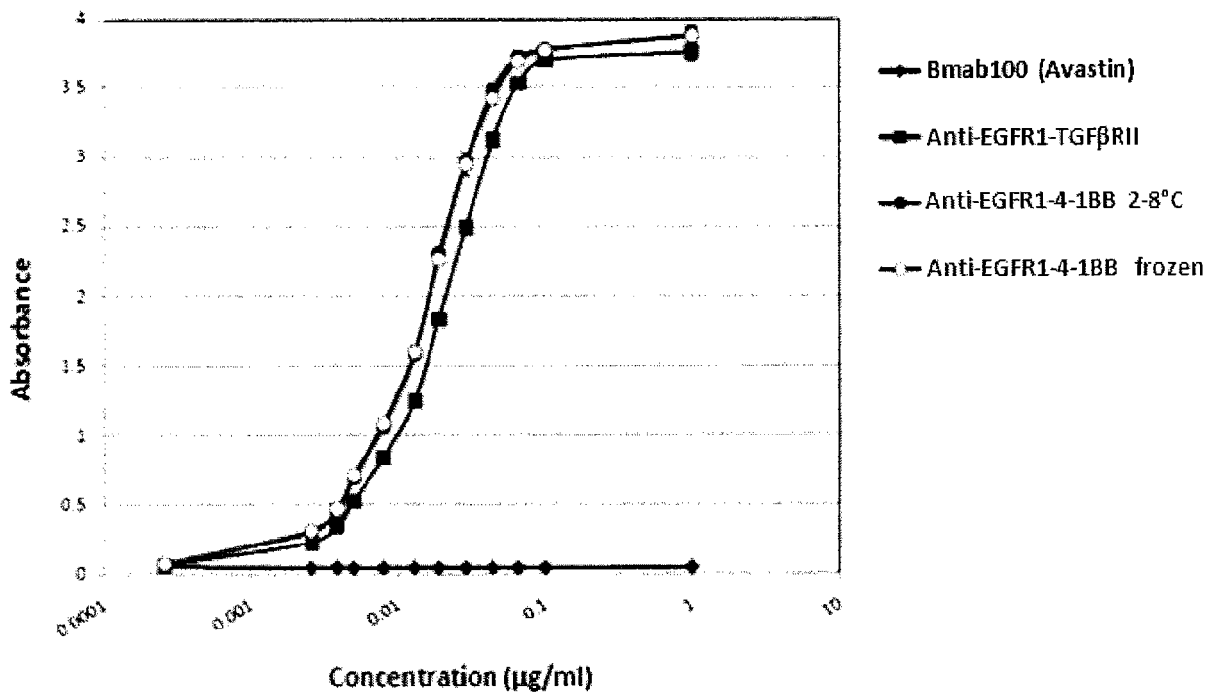


Figure 30

# A Anti-EGFR1-4-1BB SUPERNATANT EGFR ELISA



# B PD1-Fc-4-1BB PDL1 ELISA 08122011

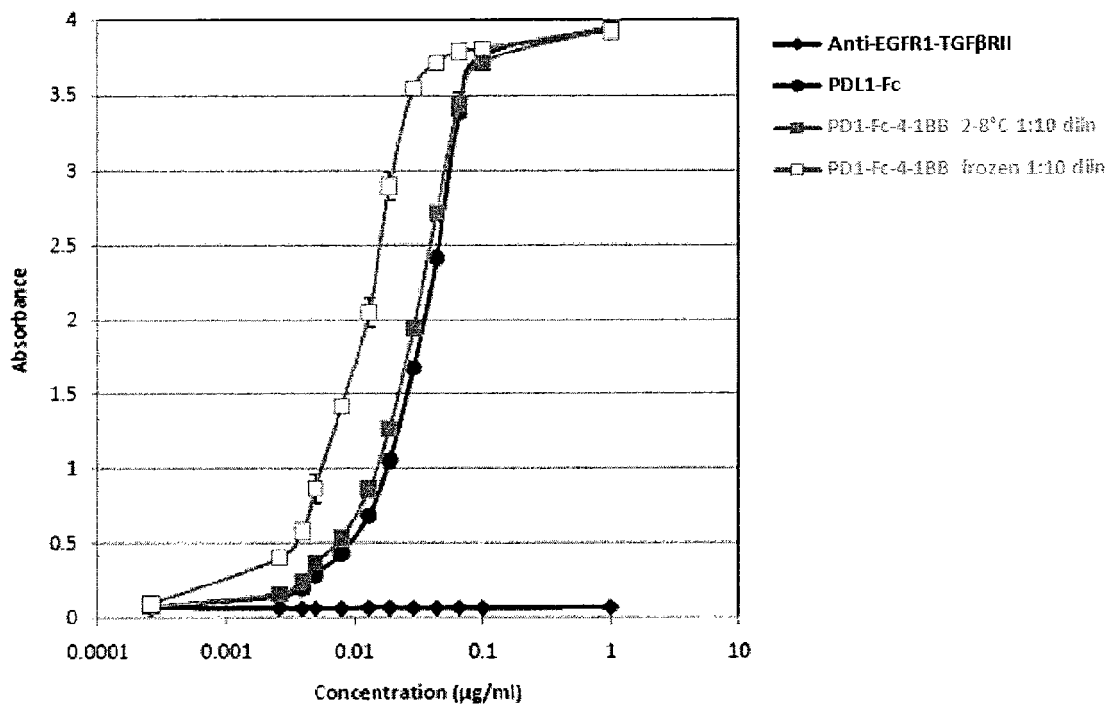


Figure 31

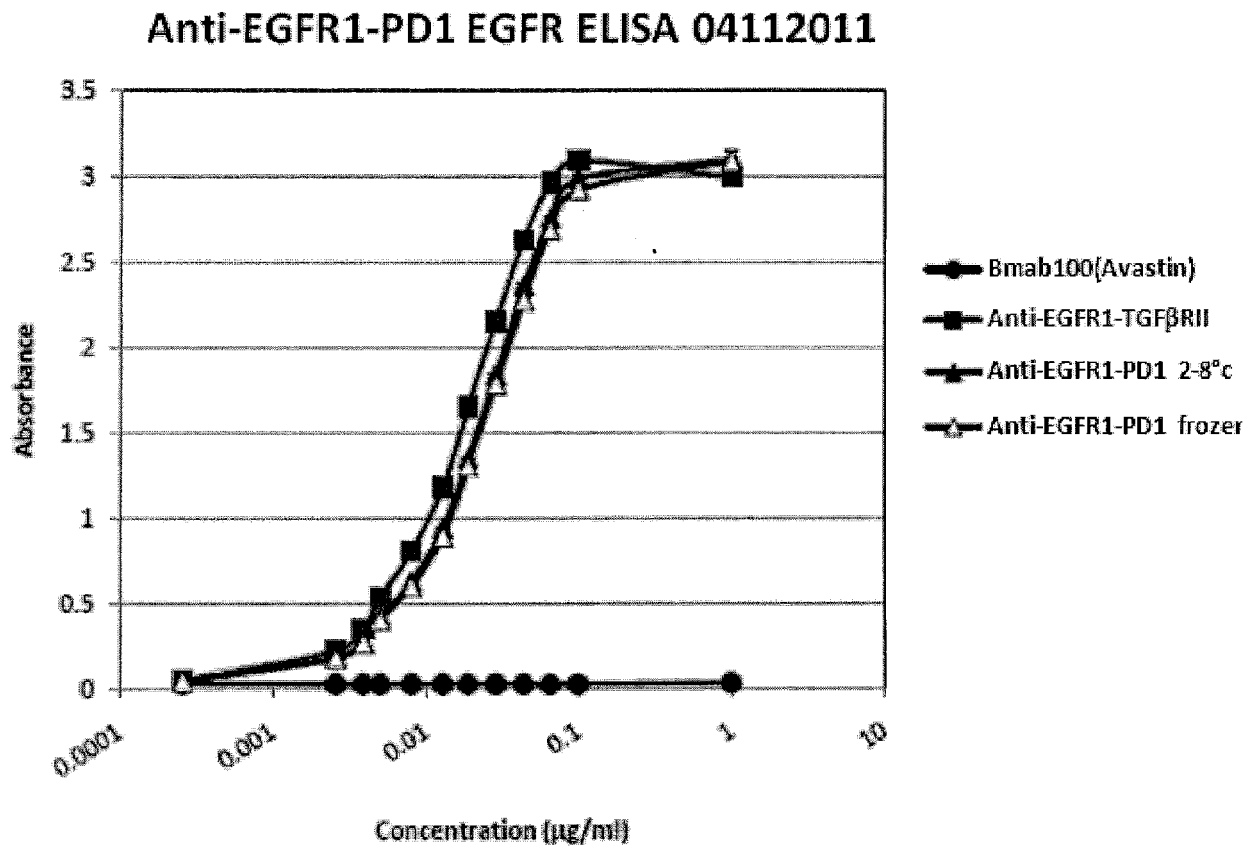


Figure 32

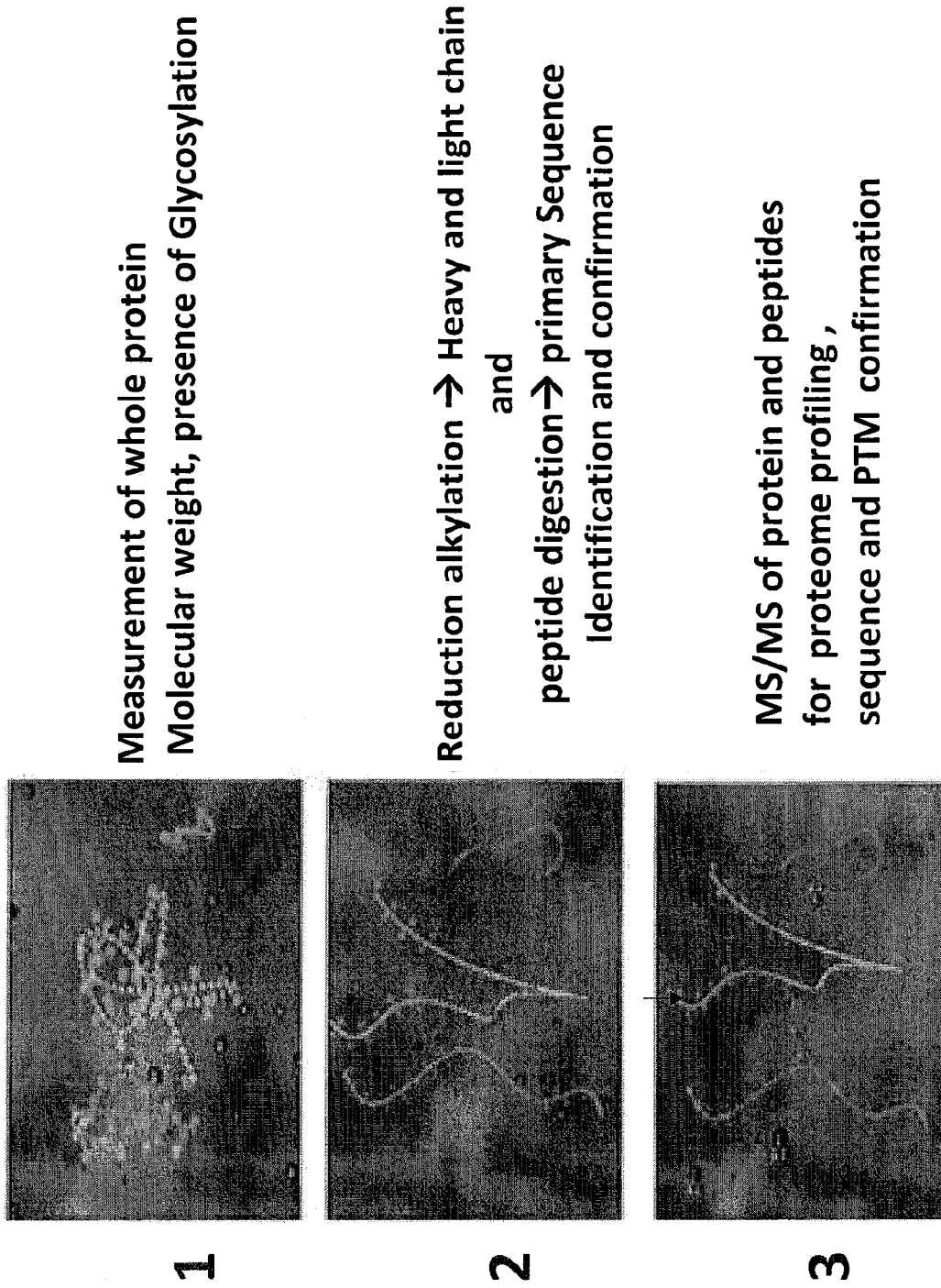
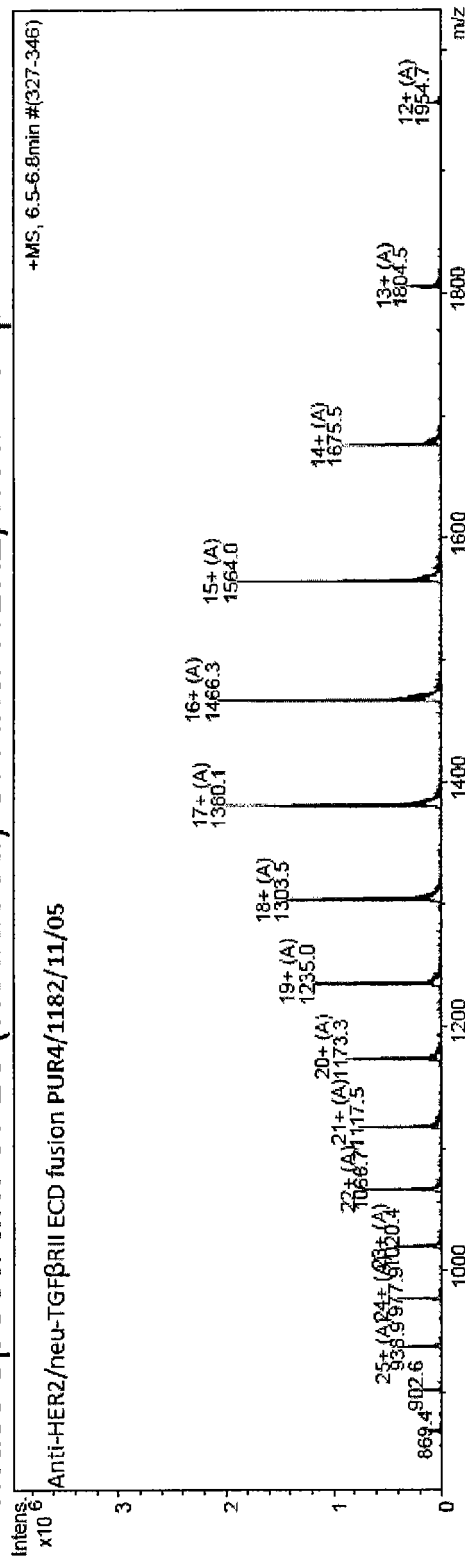


Figure 33

# Mass Spectrum of LC (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion



## Deconvoluted Mass Spectrum of LC (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion

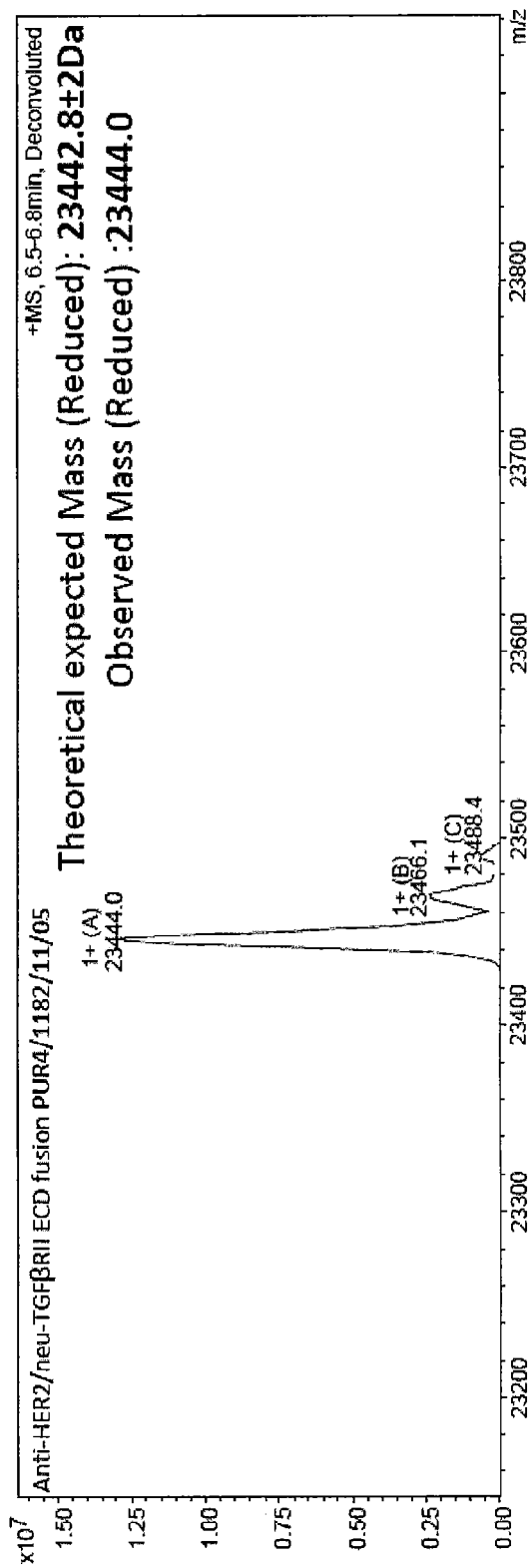


Figure 34

# Mass Spectrum of HC (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion

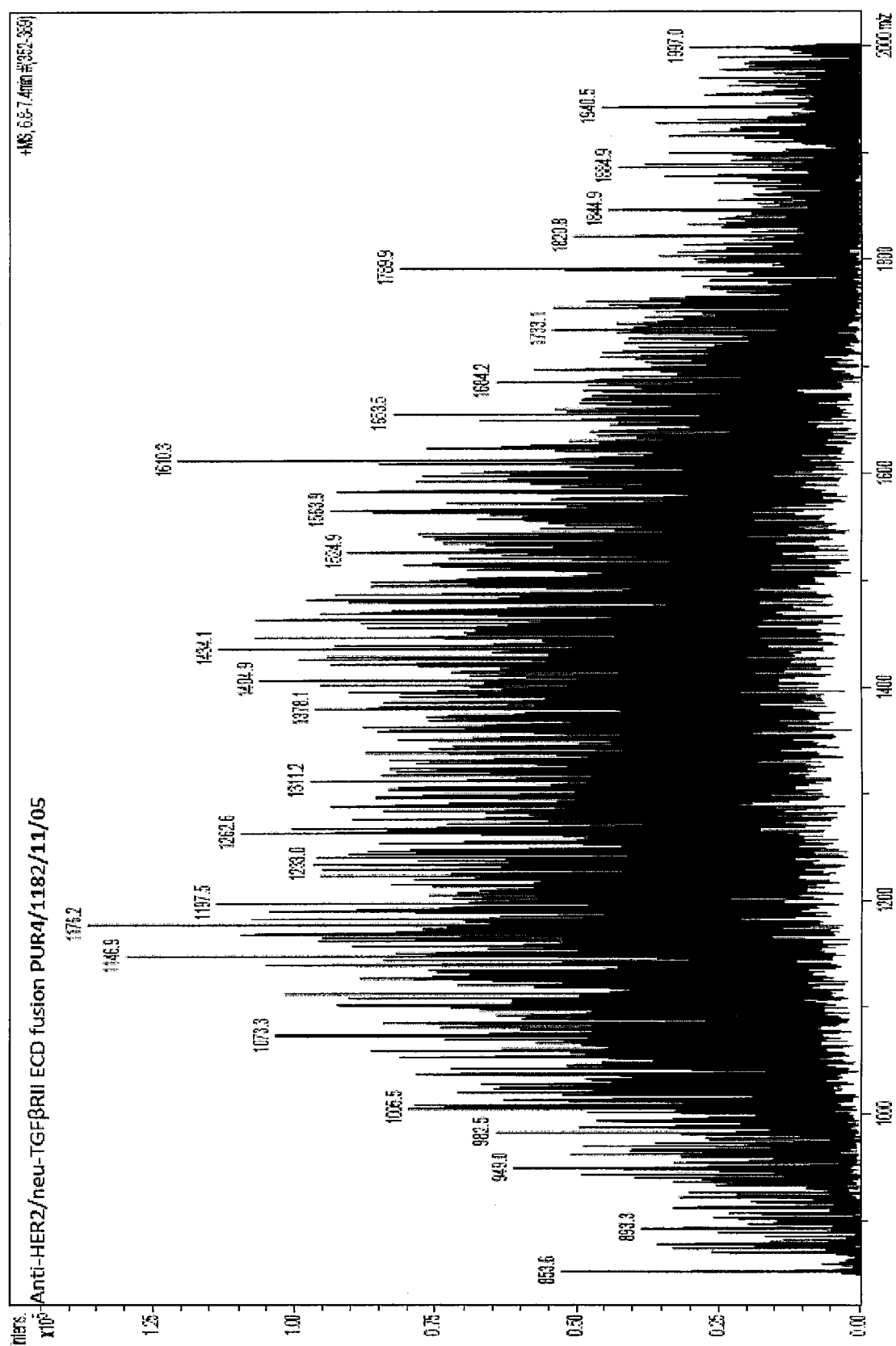


Figure 35

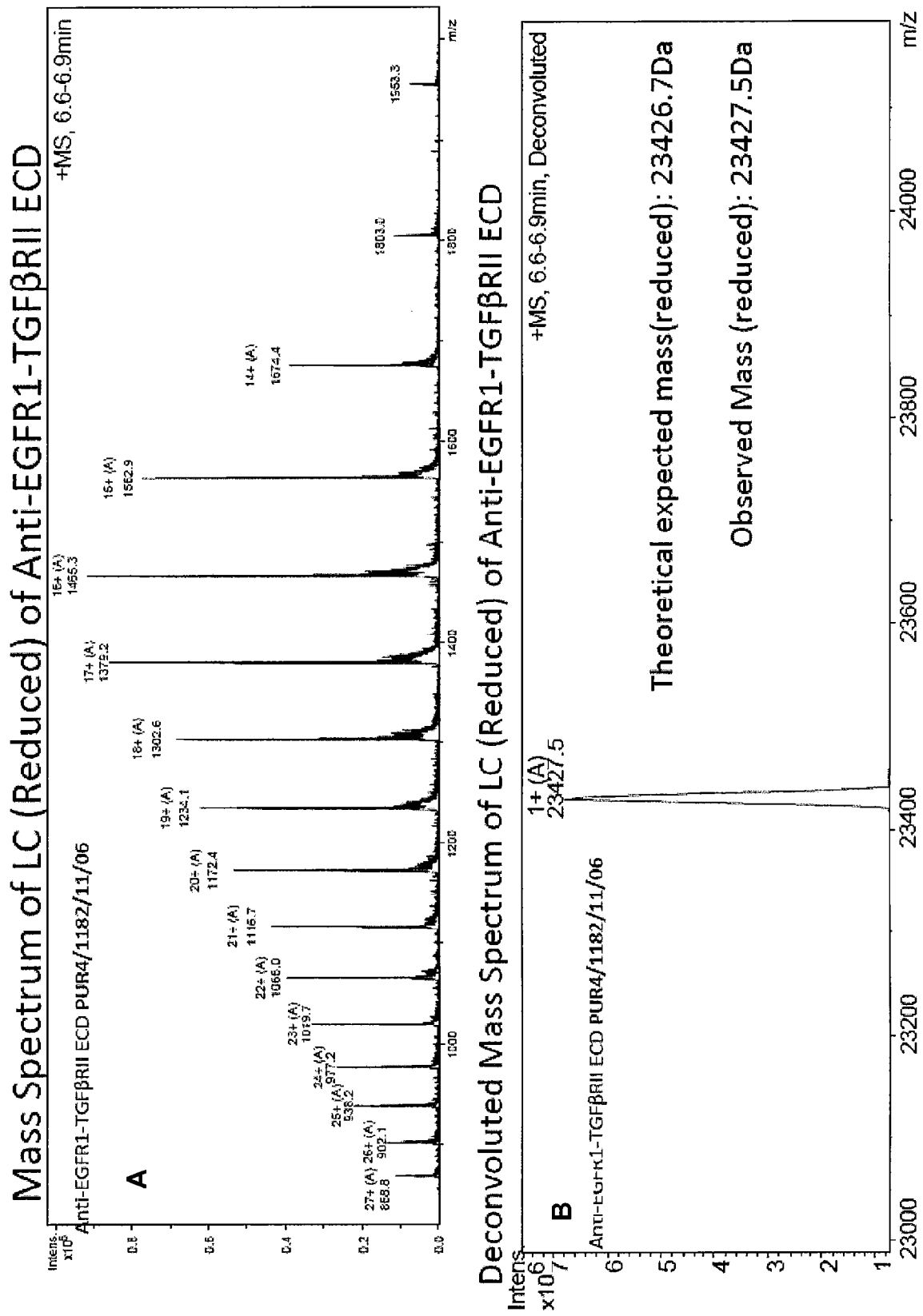


Figure 36

# Mass Spectrum of HC (Reduced) of Anti-EGFR1-TGFβRII ECD

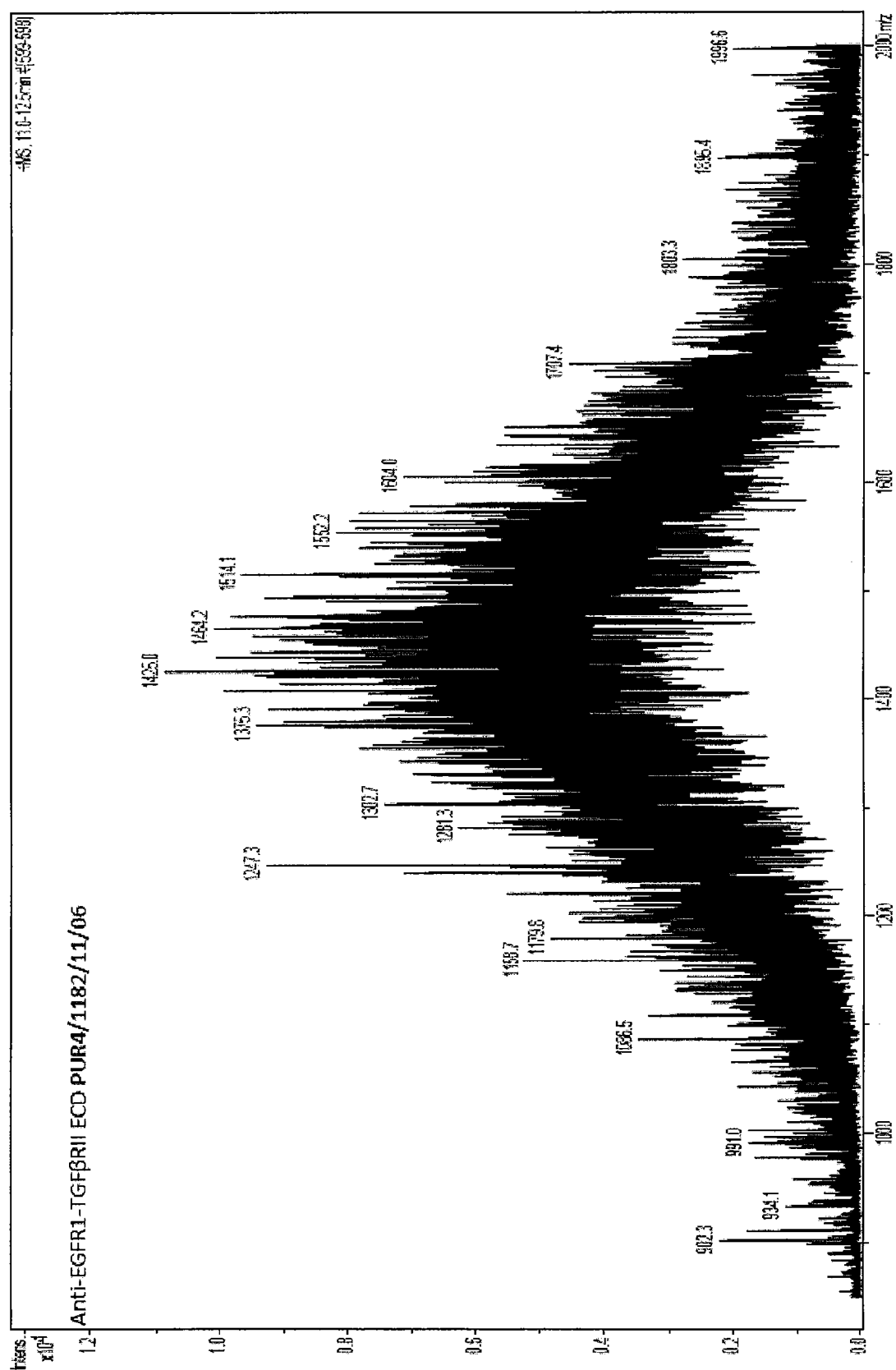


Figure 37



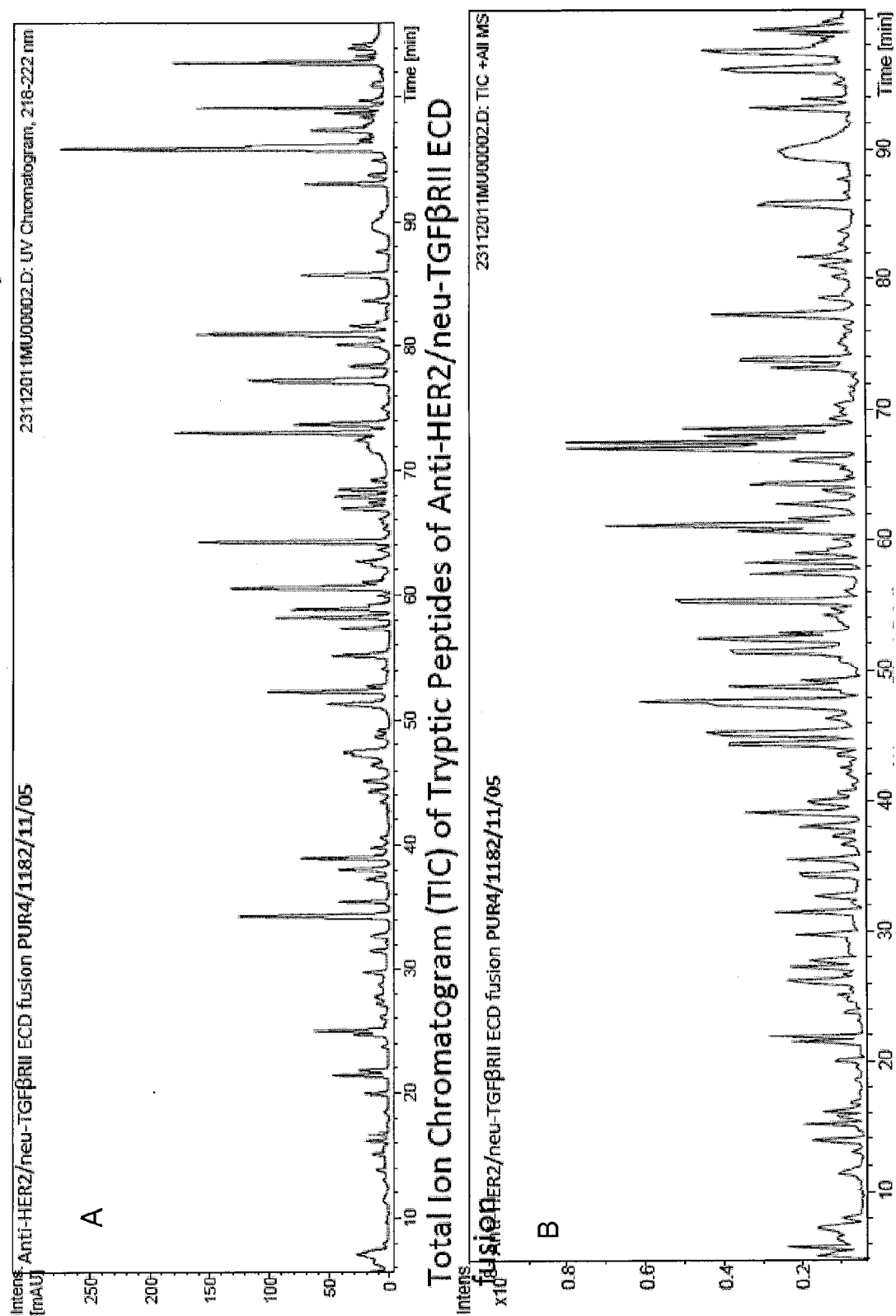
UV Chromatogram of Tryptic Peptides of Anti-HER2/neu-TGF $\beta$ RII ECD fusion

Figure 38

List of Expected/Observed Tryptic Peptide of LC of Anti-HER2/neu-TGFβRII ECD fusion

S.No	Range	Sequence	Expected Mass (M+H) <sup>+</sup>	Observed Mass (M+H) <sup>+</sup>	RT(min)	MS/MS
1	LC(1-18)	DIQMTQSPSSLSASVGDR	1878.9	1879.2, 940.1+2	57.3	Yes
2	LC(19-24)	VTITCR	749.3	749.4	31.7	Yes
3	LC(25-42)	ASQDVNTAVAWYQKPGK	1990.9	1991.2, 996.1+2	58.1	Yes
4	LC(43-45)	APK	314.9	315.3	5.9	Yes
5	LC(46-61)	ILIYSASFYSGVPSR	1772.9	1773.1	93.1	Yes
6	LC(62-66)	FSGSR	553.2	553.3	16.3	Yes
7	LC(67-103)	SGTDFLTITSSLOPEDFATYYCQHHY	4187.9	4188.1	95.7	NO
8	LC(104-107)	VEIK	488.3	488.3	26.2	Yes
9	LC(108-108)	R	175.1	Out of detection range		
10	LC(109-126)	TVAAPSVFIFPPSDEQLK	1946.02	1946.1	89.1	Yes
11	LC(127-142)	SGTASVWCLLNFFPR	1797.8	1797.9	97.4	Yes
12	LC(143-145)	EAK	347.1	Out of detection range		
13	LC(146-149)	VQWK	560.3	560.4	34.3	Yes
14	LC(150-169)	VDNALQSGNSQESVTEQDSK	2135.9	2136.2	35.6	Yes
15	LC(170-183)	DSTYISSTLTLSK	1502.7	1502.9	67.8	Yes
16	LC(184-188)	ADYEK	625.2	625.4	6.5	Yes
17	LC(189-190)	HK	284.1	Out of detection range		
18	LC(191-207)	VYACEVTHQGLSSPVTK	1875.9	1876.1	52.3	Yes
19	LC(208-211)	SFNR	523.2	523.3	52.3	Yes
20	LC(212-214)	GEC	365.1	Out of detection range		

Figure 39

## List of Expected/Observed Tryptic Peptide of HC of Anti-HER2/neu-TGFβRII ECD fusior

S.No	Range	Sequence	Expected Mass (M+H) <sup>+</sup>	Observed Mass (M+H) <sup>+</sup>	RT (min)	MS/MS
1	HC(1-19)	EVQLVESGGGLVQPGGSLR	1881.9	1882.2, 941.6+2	68.5	YES
2	HC(20-30)	LSCAASGFNIK	1167.5	1167.8, 584.4+2	55.2	YES
3	HC(31-38)	DTYIHVWR	1089.5	1089.6, 545.3+2	64.2	YES
4	HC(39-43)	OAPGK	500.2	500.3	7.4	YES
5	HC(44-50)	GLEWVAR	830.4	830.5	60.6	YES
6	HC(51-59)	IYPTNGYTR	1084.5	1084.6, 542.8+2	38.1	YES
7	HC(60-65)	YADSVK	682.3	682.4	21.6	YES
8	HC(66-67)	GR	232.1	Out of detection range		
9	HC(68-76)	FTISADTSK	969.4	969.5, 485.3+2	45.3	YES
10	HC(77-87)	NTAYLQMNLSR	1310.6	1310.6, 655.8+2	61.4	YES
11	HC(88-98)	AEDTAUVYCSR	1334.6	1334.8, 667.9+2	38.9	YES
12	HC(99-124)	WGSDGPMADYWGQGLTVSSASTK	2784.2	2784.3, 1393.2+2	95.7	YES
13	HC(125-136)	GPSVFPLAPSSK	1186.6	1186.8, 593.9+2	67.1	YES
14	HC(137-150)	STSGGTAALGCLVK	1321.6	1321.8, 661.4+2	61.1	YES
15	HC(151-213)	DYFPEPTVSWNSGALTS....IGTQTYIC	6713.3	6, 1679.7+4, 1343.8+5, 1120	102.6	N/D
16	HC(214-216)	VOK	361.2	Out of detection range		
17	HC(217-217)	K	147.1	Out of detection range		
18	HC(218-221)	VEPK	472.2	472.3	11.5	YES
19	HC(222-225)	SCDK	509.1	509.4	5.2	YES
20	HC(226-251)	THTCPPCPAPELLGGPSVFLFPKPK	2844.4	2844.6, 949.4+3	95.9	YES
21	HC(252-258)	DTLMISR	835.4	835.5	48.7	YES
22	HC(259-277)	TPEVTCVVVDVSHEDPEVK	2139.1	2139.2, 1070.1+2, 713.8+2	73.6	YES
23	HC(278-291)	FNWYVDGVEVHNAK	1677.7	1677.8, 839.5+2	73.1	YES
24	HC(292-295)	TKPR	501.3	501.3	7.2	YES
25	HC(296-304)	EEQYNSTYR (GP)	1189.5+1444	2633.5, 1317.6+2	25.1	YES
26	HC(305-320)	VVSVLTVLHQDWLNGK	1807.9	1808.1, 904.5+2	99.1	YES
27	HC(321-323)	EYK	439.2	439.3	5.2	YES
28	HC(324-325)	CK	307.1	Out of detection range		
29	HC(326-329)	VSNIK	447.2	447.3	5.4	YES
30	HC(330-337)	ALPAPIEK	838.4	838.5	47.5	YES
31	HC(338-341)	TISK	448.2	448.3	13.9	YES
32	HC(342-343)	AK	218.1	Out of detection range		
33	HC(344-347)	GQPR	457.2	457.3	6.9	YES
34	HC(348-358)	EPQVYTLPPSR	1286.6	1286.7, 643.9+2	52.8	YES
35	HC(359-363)	EEMTK	637.2	637.4	6.2	YES
36	HC(364-373)	NQVSLTCLVK	1161.6	1161.7, 581.4+2	67.5	YES
37	HC(374-395)	GFYPDSIAVEWESNGQPPENNYK	2545.1	2545.1, 1273.1+2, 849.1+3	81.1	YES
38	HC(396-412)	TTTPVLDSDGSFFLYSK	1873.9	1874.2, 937.6+2	85.8	YES
39	HC(413-417)	LTVDK	575.3	575.4	27.4	YES
40	HC(418-419)	SR	262.1	Out of detection range		
41	HC(420-442)	WQQGNVVFSCVMHEALHNHYTQK	2801.2	2801.4, 1401.2+2, 934.5+3	77.4	YES
42	HC(443-450)	SLSLSPGK	788.4	788.5	44.3	YES

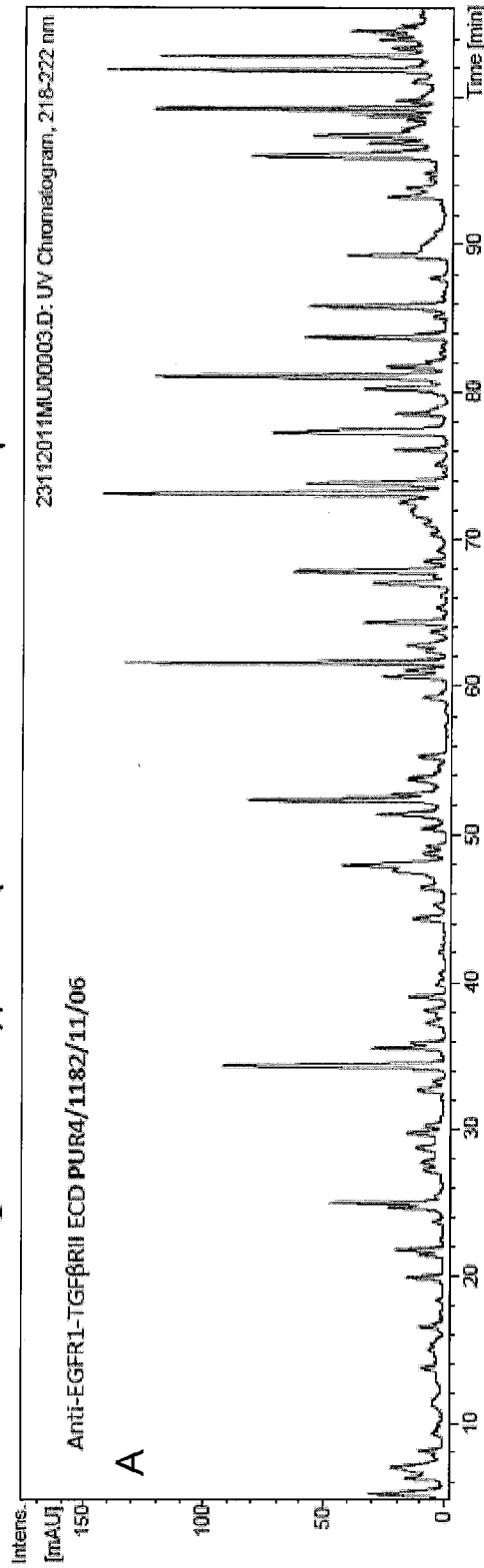
Figure 40

List of Expected/Observed Tryptic Peptide of LM\* of Anti-HER2/neu-TGFβRII ECD fusion

S.No	Range	Sequence	Expected Mass (M+H) <sup>+</sup>	Observed Mass (M+H) <sup>+</sup>	RT (min)	MS/MS
1	LM(1-23)	GGGGSGGGGGGGSTIPPHVQK	1864.8	1864.2, 932.6+2,	76.1	NO
2	LM(24-39)	SVNNDMIVTDNNGAVK	1690.7	1690.8, 846.1+2	49.3	YES
3	LM(40-45)	FPQLCK	792.4	792.5	51.3	YES
4	LM(46-50)	FCDVR	696.3	696.4	29.8	YES
5	LM(51-58)	FSTCDNQK	999.4	999.5	22.1	YES
6	LM(59-82)	SCMSNCSTICEKPKQEVCAVWR	2901.2	Not Detected		
7	LM(83-83)	K	147.1	Out of detection range		
8	LM(84-98)	NDENITLETVCHDPK	1784.8	1785.1	54.2	YES
9	LM(99-113)	LPYHDFILEDAAAPK	1715.8	1715.9	77.2	YES
10	LM(114-117)	CIMK	551.2	551.3	27.8	YES
11	LM(118-119)	EK	276.1	Out of detection range		
12	LM(120-120)	K	147.1	Out of detection range		
13	LM(121-152)	KPGETFFMCCSSDECNDNIIFSEYNTSNPD	3794.8	Not Detected		

Figure 41

# UV Chromatogram of Tryptic Peptide of Anti-EGFR1-TGFβRII ECD



## Total Ion Chromatogram (TIC) of Tryptic Peptides of Anti-EGFR1-TGFβRII ECD.

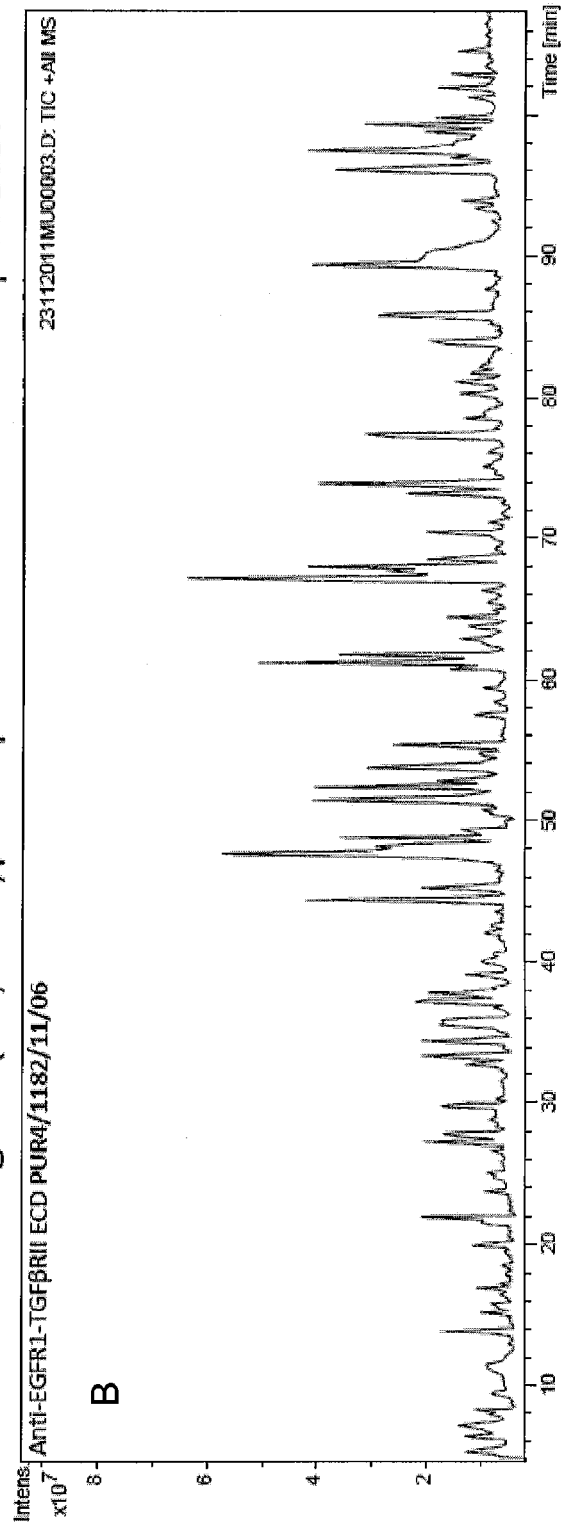


Figure 42

List of expected/observed peptides of Light Chain(LC) of Anti-EGFR1-TGFB $\beta$ R11 ECD.

S.No	Peptide	Sequence	Expected Mss(M+H) <sup>+</sup>	Observed Mass (M+H) <sup>+</sup>	RT(MIN)	MS/MS
1	LC(1-18)	DILLTQSPVILSVSPGER	1924	1924	89.2	Yes
2	LC(19-24)	VSFSCR	755.3	755.4	35.9	Yes
3	LC(25-39)	ASQSIGTNIHWYQQR	1788.8	1789	61.7	Yes
4	LC(40-45)	TNGSPR	631.3	631.4	8.2	Yes
5	LC(46-49)	LLIK	486.3	486.3	47.9	Yes
6	LC(50-61)	YASEISGIPSR	1266.6	1266.6	48	Yes
7	LC(62-103)	FSGSGSGDTFTLSINSEVED	4565.9	4565.9	93.1	NO
8	LC(104-107)	LEIK	502.3	502.4	37.8	Yes
9	LC(108-108)	R	174.1	Out of detection range	Out of detection range	
10	LC(109-126)	TVAAPSVFIFPPSDEQLK	1946	1946.2	89.5	Yes
11	LC(127-142)	SGTASWCLNNFYPR	1797.8	1797.2	97.1	Yes
12	LC(143-145)	EAK	347.1	Out of detection range	Out of detection range	
13	LC(146-149)	VQWK	560.3	560.4	34.3	Yes
14	LC(150-169)	VDNALQSGNSQESVTEQDS	2135.9	2136.2	35.6	Yes
15	LC(170-183)	DSTYSLSLTSLK	1502.7	1502.9	67.8	Yes
16	LC(184-188)	ADYEK	625.2	625.3	7	Yes
17	LC(189-190)	HK	284.1	Out of detection range	Out of detection range	
18	LC(191-207)	VYACEVTHQGLSPVTK	1875.9	1876.2	52.5	Yes
19	LC(208-211)	SFNR	523.2	523.3	20.1	Yes
20	LC(212-214)	GEC	365.1	Out of detection range	Out of detection range	

Figure 43

List of expected/observed peptides of Heavy Chain(HC) of Anti-EGFR1-TGFβRII ECD

S.No	Peptide	Sequence	Expected Mass (M+H) <sup>+</sup>	Observed Mass (M+H) <sup>+</sup>	RT (Min)	M5/MS
1	HC(1-5)	QVQLK	615.3	598.4*	37.1	yes
2	HC(6-38)	QSGPGLVQPSQSLSITCTVSGFSLTNYGVH	3564.9	3564.7	96.8	yes
3	HC(39-43)	QSPGK	516.2	516.3	6.4	yes
4	HC(44-66)	GLEWLGVIWVSGGNTDYNTPPTSR	2571.7	2571.4	101.8	yes
5	HC(67-71)	LSINK	574.3	574.5	33.2	YES
6	HC(72-75)	DNISK	463.2	Not Detected		
7	HC(76-96)	SQVFFMNSLSQSNDAIYCAR	2515.1	Not Detected		
8	HC(97-122)	ALTYDYEFAYWGQGLTVTVSAASTK	2907.1	Not Detected		
9	HC(123-134)	GPSVFPLAPSSK	1186.6	1186.8	66.9	yes
10	HC(135-148)	STSGGTAALGCLVK	1321.6	1321.8	61.1	yes
11	HC(149-211)	DYFPEPVTVSWNSGALTSGVHTFPAVLQ	6716.3	6715	102.6	NO
12	HC(212-214)	VDK	361.2	Not Detected		
13	HC(215-215)	R	175.1	Out of Detection Range		
14	HC(216-219)	VEPK	472.2	472.3	11.2	yes
15	HC(220-223)	SCDK	509.1	Not Detected		
16	HC(224-249)	THTCPPCPAPELLGGPSVFLFPPKPK	2845.3	2845.6	95.8	yes
17	HC(250-256)	DTLMISR	835.4	835.5	48.9	yes
18	HC(257-275)	TPEVTCVVVDVSHEDPEVK	2139	2139.4	73.6	yes
19	HC(276-289)	FNWYVDGVEVHNAK	1677.7	1677.8	73.1	yes
20	HC(290-293)	TKPR	501.3	501.4	7.1	yes
21	HC(294-302)	EEQYNSTYR	1188.5	(+1444)Da 2634.2	25	yes
22	HC(303-318)	VVSVLTVLHQDWLNGK	1809.1	1809.4	99	YES
23	HC(319-321)	EYK	439.2	439.3	5.4	yes
24	HC(322-323)	CK	307.1	Out of Detection Range		
25	HC(324-327)	VSNK	447.2	447.3	5.4	
26	HC(328-335)	ALPAPIEK	838.4	838.5	47.9	yes
27	HC(336-339)	TISK	448.2	448.3	14	yes
28	HC(340-341)	AK	218.1	Out of Detection Range		
29	HC(342-345)	GQPR	457.2	457.3	6.9	yes
30	HC(346-356)	EPQVYTLPPSR	1286.6	1286.7	52.8	YES
31	HC(357-361)	DELTK	605.3	605.4	11.3	yes
32	HC(362-371)	NQVSLTCLVK	1161.6	1161.7	67.4	yes
33	HC(372-393)	GFYPSDIAVEWESNGQPENNYK	2545.6	2545.4	80.8	yes
34	HC(394-410)	TTPPVLDSDGSFFLYSK	1873.9	1874	85.7	yes
35	HC(411-415)	LTVDK	575.3	575.4	27.2	yes
36	HC(416-417)	SR	262.1	Out of Detection Range		
37	HC(418-440)	WQQGNVFSCSVMHEALHNHYTQK	2801.2	2801.4	77.4	NO
38	HC(441-448)	SLSLSPGK	788.4	788.5	44.2	yes

Figure 44



List of expected/observed peptides of Linked Motif (LM) of Anti-EGFR1-TGFBRII ECD

S.No	Peptide	Sequence	Expected Mass (M+H)+	Observed Mass (M+H)+	RT (Min)	MS/MS
1	LM(1-23)	GGGSGGGSGGGSTIPPHVQK	1864.8	1864.2	76.3	NO
2	LM(24-39)	SVNNDMIVTDNNGAVK	1691.7	1691.8	49.2	YES
3	LM(40-45)	FPQLCK	792.4	792.5	51.5	YES
4	LM(46-50)	FCDVR	696.3	696.4	29.8	YES
5	LM(51-58)	FSTCDNQK	999.4	999.5	21.9	YES
6	LM(59-82)	SCMSNCSITSICEK	2903.3	Not Detected		
7	LM(83-83)	K	147.1	Out of Detection Range		
8	LM(84-98)	NDENITLETVCHDPK	1784.8	1784.8	54.3	YES
9	LM(99-113)	LPYHDFILEDAAAPK	1715.8	1715.9	77.1	YES
10	LM(114-117)	CIIMK	551.2	551.3	27.7	YES
11	LM(118-119)	EK	276.1	Out of Detection Range		
12	LM(120-120)	K	147.1	Out of Detection Range		
13	LM(121-152)	KPGTEFFMCSSSCDECDNIIFSEYNTSNPD	3796.9	Not Detected		

Figure 45



**Cantuzumab -TGF $\beta$ RII fusion protein at LC constant region**

Amino acid sequence of Cantuzumab heavy chain:

QVQLVQSGAEVKKPGETVKISCKASDYFTFTYYGMNWVKQAPGQGLKWMGWI  
 DTTTGEPTYAQKFQGRIAFSLETSASTAYLQIKSLKSEDTATYFCARRGPYNWYFD  
 VWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN  
 SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK  
 VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHE  
 DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC  
 KVSNAKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH  
 EALHNHYTQKSLSLSPGK

Amino acid sequence of Cantuzumab light chain fusion protein:

DIVMTQSPLSVPVTPGEPVSISCRSSKSLHSNGNTYLYWFLQRPQGQSPQLLIYR  
 MSNLVSGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGTKLE  
 LKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS  
 QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC  
 GGGGSGGGGSGGGGST**TIPPHVQKSVNNDMIVTDNNGAVKFPQLCK**  
**FCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENI**  
**TLETVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSC**  
**SSDECNDNIIFSEEYNTSNPD**

Figure 46

## Cixutumumab -TGF $\beta$ RII fusion protein at LC constant region

Amino acid sequence of Cixutumumab heavy chain:

EVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPI  
FGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAPLRFLEWSTQ  
DHYYYYYMDVWGKGTITVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF  
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK  
PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC  
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW  
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL  
VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN  
VFSCSVMHEALHNHYTQKSLSLSPGK

Amino acid sequence of Cixutumumab light chain fusion protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYATWYQQKPGQAPILVIYGENKRPS  
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSQGHLVFGGGTKLTVLG  
QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETT  
TPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAECSSGGGG  
**SGGGGSGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCD**  
**VRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLE**  
**TVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSD**  
**ECNDNIIFSEEYNTSNPD**

Figure 47

**Clivatuzumab -TGF $\beta$ RII fusion protein at LC constant region**

Amino acid sequence of Clivatuzumab heavy chain:

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYINP  
YNDGTQTNKKFKGKATLTRDTSINTAYMELSRLRSDDTAVYYCARGFGGSYGFA  
YNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS  
GALTSGVNTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDKRV  
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLNISRTPEVTCVVDVSHEDP  
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV  
EWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVVFSCSVNHEAL  
HNHYTQKSLSLSPGK

Amino acid sequence of Clivatuzumab light chain fusion protein:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPKGKAPKLWIYSTSNL  
ASGVPARFSGSGSGTDFTLTISLQPEDSASYFCHQWNRYPYTFGGGTRLEIKRT  
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAQVQWKVDNALQSGNSQESVTE  
QDSKDSTYLSSTLTLSRKADEYKHKVYACEVTHQGLSSPVTKSFNRGECGGGG  
**SGGGGSGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCD**  
**VRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLE**  
**TVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSD**  
**ECNDNIIFSEEYNTSNPD**

Figure 48

**Pritumumab-TGF $\beta$ RII fusion protein at LC constant region**

Amino acid sequence of Pritumumab heavy chain:

EVQLLESGGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAITP  
 SGGSTNYADSVKGRFTISRDNSTLYLQMNSLRVEDTAVYICGRVPYRSTWYP  
 LYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW  
 NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK  
 KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH  
 EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK  
 CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMH  
 EALHNHYTQKSLSLSPGK

Amino acid sequence of Pritumumab light chain fusion protein:

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASSLH  
 SKVPTQFSGSGSGTDFTLTISLQPEDFATYYCLQYSTYPITFGGGTKVEIKRTVAA  
 PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ  
 DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGG  
**GGSGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRF**  
**STCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVC**  
**HDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECN**  
**DNIIFSEYNTSNPD**

Figure 49

**Cantuzumab HC-4-1BB and LC-TGF $\beta$ RII fusion protein**

Amino acid sequence of heavy chain-4-1BB fusion protein:

QVQLVQSGAEVKKPGETVKISCKASDYFTFTYYGMNWVKQAPGQGLKWMGWI  
 DTTTGEPTYAQKFQGRIAFSLETSAAYLQIKSLKSEDTATYFCARRGPYNWYFD  
 VWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN  
 SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK  
 VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHE  
 DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC  
 KVSNAKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMH  
 EALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS*ACPWAVSGARASPGS*  
*AASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLID*  
*GPLSWYSDPGLAGVSLTGGLSYKEDTKELVAKAGVYVFFQL*  
*ELRRVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPAS*  
*SEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLT*  
*QGATVLGLFRVTPEIPAGLPSPRSE*

Amino acid sequence of Cantuzumab light chain fusion protein:

DIVMTQSPLSVPVTPGEPVSISCRSSKSLHSNGNTYLYWFLQRPQGQSPQLLIYR  
 MSNLVSGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGTKLE  
 LKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS  
 QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC  
 GGGGSGGGGSGGGGS**TIPPHVQKS VNNDMIVTDNNGAVKFPQLCK**  
**FCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENI**  
**TLETVCHDPKLPYHDFILEDAAAPK CIMKEKKKPGETFFMCSC**  
**SSDECNDNIIFSEEYNTSNPD**

Figure 50

### Cixutumumab HC-4-1BB and LC-TGFβRII fusion protein

Amino acid sequence of heavy chain-4-1BB fusion protein:

EVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGGIIP  
FGTANYAQKFQGRVTITADKSTSTAYMELSSLRSED TAVYYCARAPLRFLEWSTQ  
DHYYYYYMDVWGKGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF  
PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHK  
PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC  
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW  
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL  
VKGFYPSDIAVEWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGN  
VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGACPWAVSG  
*ARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVA*  
*QNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVAKAG*  
*VYVFFQLELR RVAGEGSGSVSLALHLQPLRSAAGAAALAL*  
*TVDLP PASSEARN S AFGFQGRLLHLSAGQRLGVHLHTEARA*  
*RHAWOLTQGATVLGLFRVTPEIPAGLP SPRSE*

Amino acid sequence of Cixutumumab light chain fusion protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYATWYQQKPGQAPILVIYGENKRPS  
GIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSQGHLVFGGGTKLTVLG  
QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETT  
TPSKQSNNKYAASSYLSLTPEQWKSHRYSYSCQVTHEGSTVEKTVAPAECSGGGG  
SGGGGSGGGGS**TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCD**  
**VRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLE**  
**TVCHDPKLPYHDFILEDAAAPKCIMKEKKKPGETFFMCSCSSD**  
**ECNDNIIFSEFYNTSNPD**

Figure 51

## Clivatuzumab HC-4-1BB and LC-TGFβRII fusion protein

### Amino acid sequence of heavy chain -4-1BB fusion protein

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYINP  
YNDGTQTNKKFKGKATLTRDTSINTAYMELSRLSDDTAVYYCARGFGGSYGFA  
YNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS  
GALTSGVNTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV  
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPPKPDTLNISRTPEVTCVVDVSHEDP  
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV  
EWESNGQPENNYKTTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVNHEAL  
HNHYTQKSLSLSPGKGGGGSGGGGSGGGGSA CPWAVSGARASPGSAA  
SPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPL  
SWYSDPGLAGVSLTGGLSYKEDTKELVAKAGVYVFFQLELR  
RWAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEA  
RNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQG  
ATVLGLFRVTPEIPAGLPSPRSE

Amino acid sequence of Clivatuzumab light chain fusion protein:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPGKAPKLWIYSTSNL  
ASGVPARFSGSGSGTDFTLTISSLQPEDSASYFCHQWNRYPYTFGGGTRLEIKRT  
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAQVQWKVDNALQSGNSQESVTE  
QDSKIDSTYLSSTLTLSPRKADYEKHKVYACEVTHQGLSPVTKSFNRGECGGGG  
SGGGGSGGGGS**TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCD**  
**VRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITL**  
**TVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSSD**  
**ECNDNIIFSEFYNTSNPD**

Figure 52

**Pritumumab HC-4-1BB and LC-TGF $\beta$ RII fusion protein**

Amino acid sequence of heavy chain-4-1BB fusion protein:

EVQLLESGGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAITP  
 SGGSTNYADSVKGRFTISRDNQNTLYLQMNSLRVEDTAVYICGRVPYRSTWYP  
 LYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW  
 NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK  
 KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH  
 EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK  
 CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMH  
 EALHNHYTQKSLSLSPGKGGGGSGGGSGGGGSACPWAVSGARASPGS  
*AASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLID*  
*GPLSWYSDPGLAGVSLTGGLSYKEDTKELVAKAGVYVFFQL*  
*ELRRVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPAS*  
*SEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLT*  
*QGATVLGLFRVTPEIPAGLPSPRSE*

Amino acid sequence of Pritumumab light chain fusion protein:

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASSLH  
 SKVPTQFSGSGSGTDFTLTISLQPEDFATYYCLQYSTYPITFGGGTKVEIKRTVAA  
 PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ  
 DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGG  
 GGSGGGGST**TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRF**  
**STCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVC**  
**HDPKLPYHDFILEDASPCKIMKEKKKPGETFFMCSCSSDECN**  
**DNIIFSEEYNTSNPD**

Figure 53



**Cantuzumab - HC-PD1 and LC-TGF $\beta$ RII fusion protein**

Amino acid sequence of heavy chain-PD1 fusion protein:

QVQLVQSGAEVKKPGETVKISCKASDYTFYYGMNWVKQAPGQGLKWMGWI  
 DTTTGEPTYAQKFQGRIFALESASTAYLQIKSLKSEDTATYFCARRGPYNWYFD  
 VWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN  
 SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK  
 VEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPPKPDTLMISRTPEVTCVVDVSHE  
 DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC  
 KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH  
 EALHNHYTQKSLSLSPGKGGGGSGGGGGSGGGGGSPGWFLDSPDRPWNPPPT  
*FSPALLVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPPE*  
*DRSQPGQDCRFRTQLPNGRDFHMSVVRARRNDSGTLYCGAISLA*  
*PKAQIKESLRAELRVTERRAEVPTAHPSPPSPRPAGQFQTLV*

Amino acid sequence of Cantuzumab light chain fusion protein:

DIVMTQSPLSVPVTPGEPVSISCRSSKSLHSNGNTYLYWFLQRPQGQSPQLLIYR  
 MSNLVSGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGTKLE  
 LKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS  
 QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC  
 GGGGSGGGGSGGGGSGGGG**TIPPHVQKSVNNDMIVTDNNGAVKFPQLCK**  
**FCDVRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENI**  
**TLETVCHDPKLPYHDFILEDAAAPKCMKEKKKKPGETFFMCSC**  
**SSDECNDNIIFSEYNTSNPD**

Figure 54

**Cixutumumab HC-PD1 and LC-TGF $\beta$ RII fusion protein**

Amino acid sequence of heavy chain-PD1 fusion protein:

.  
 EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIPI  
 FGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAPLRFLEWSTQ  
 DHYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF  
 PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK  
 PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC  
 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW  
 LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL  
 VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN  
 VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSPGWFLDSPD  
*RPWNPPTFSPALLVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQT*  
*DKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGT*  
*LCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPPSPRPAGQFQTL*  
 V

Amino acid sequence of Cixutumumab light chain fusion protein:

SSELTQDPAVSVALGQTVRITCQGDLSRSYYATWYQQKPGQAPILVIYGENKRPS  
 GIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSQHLVFGGGTKLTVLG  
 QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETT  
 TPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAECSSGGGG  
 SGGGGSGGGGS**TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCD**  
**VRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLE**  
**TVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSSSD**  
**ECNDNIIFSEEYNTSNPD**

Figure 55

**Clivatuzumab HC-PD1 and LC-TGF $\beta$ RII-fusion protein**

Amino acid Amino acid sequence of heavy chain-PD1 fusion protein:

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYINP  
YNDGTQTNKKFKGKATLTRDTSINTAYMELSRLRSDDTAVYYCARGFGGSYGFA  
YNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS  
GALTSGVNTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDKRV  
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLNISRTPEVTCVVDVSHEDP  
EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV  
EWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVNHEAL  
HNHYTQKSLSLSPGKGGGGSGGGGGSGGGGGSPGWFLDSPDRPWNPPPTFSP  
*ALLVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRS*  
*QPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKA*  
*QIKESLRAELRVTERRAEVPTAHPSPPSPRPAGQFQTLV*

Amino acid sequence of Clivatuzumab light chain fusion protein:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPGKAPKLWIYSTNL  
ASGVPARFSGSGSGTDFTLTISLQPEDSASYFCHQWNRYPYTFGGGTRLEIKRT  
VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAQVQWKVDNALQSGNSQESVTE  
QDSKDSTYLSSTLTLSPRKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGG  
*SGGGGGSGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCD*  
**VRFSTCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLE**  
**TVCHDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSD**  
**ECNDNIIFSEEYNTSNPD**

Figure 56

**Pritumumab HC-PD1 and LC-TGF $\beta$ RII fusion protein**

Amino acid Amino acid sequence of heavy chain-PD1 fusion protein:

EVQLLESGGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAITP  
 SGGSTNYADSVKGRFTISRDNQNTLYLQMNSLRVEDTAVYICGRVPYRSTWYP  
 LYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW  
 NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK  
 KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH  
 EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK  
 CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMH  
 EALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSPGWFLDSPDRPWNPPPT  
*FSPALLVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAPPE*  
*DRSQPGQDCRFRTQLPNGRDFHMSVVRARRNDSTYLCGAISLA*  
*PKAQIKESLRAELRVTERRAEVPTAHPSPPSPRPAGQFQTLV*

Amino acid sequence of Pritumumab light chain fusion protein:.

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASSLH  
 SKVPTQFSGSGSGTDFTLTISLQPEDFATYYCLQYSTYPITFGGGTKVEIKRTVAA  
 PSVFIFPPSDEQLKSGTASVCLNNFYFPREAKVQWKVDNALQSGNSQESVTEQ  
 DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECGGGGSGG  
 GGGGGGGSTIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRF  
**STCDNQKSCMSNCSITSICEKPQEVCAVWRKNDENITLETVC**  
**HDPKLPYHDFILEDAAAPKCMKEKKKPGETFFMCSCSSDECN**  
**DNIIFSEEYNTSNPD**

Figure 57

**Cantuzumab HC-TGF $\beta$ RII-4-1BB fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-4-1BB fusion protein:

QVQLVQSGAEVKKPGETVKISCKASDYFTYYGMNWVKQAPGQGLKWMGWI  
 DTTTGEPTYAQKFQGRIAFSLETSASTAYLQIKSLKSEDTATYFCARRGPYNWYFD  
 VWGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN  
 SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK  
 VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSHE  
 DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC  
 KVSNAKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCSSVMH  
 EALHNHYTQKSLSLSPGKGGGGSGGGGSGGGG**STIPPHVQKSVNNDMIV**  
**TDNNGAVKFPQLCKFCDFRFSTCDNQKSCMSNCSITSICEKPQ**  
**EVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMK**  
**EKKKPGETFFMCSSSDECNDNIIFSEEYNTSNPDepkscdkAC**  
*PWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMF*  
*AQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV*  
*VAKAGVYYVFFQLELRRVWAGEGSGSVSLALHLQPLRSAAGA*  
*AALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLH*  
*TEARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE*

Amino acid sequence of light chain

DIVMTQSPLSVPVTPGEPVSISCRSSKSLLSNGNTYLYWFLQRPQGQSPQLLIYR  
 MSNLVSGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGTKLE  
 LKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS  
 QESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 58

**Cixutumumab HC-TGF $\beta$ RII-4-1BB fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-4-1BB fusion protein:

EVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPI  
 FGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAPLRFLEWSTQ  
 DHYYYYYMDVWGKGTITVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF  
 PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK  
 PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC  
 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW  
 LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL  
 VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN  
 VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS**TIPPHVQKS**  
**VNNDMIVTDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCST**  
**TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAA**  
**SPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD***ep*  
*kscdk*ACPWAVSGARASPGSAASPRLREGPELSPDDPAGLLD  
 LRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYK  
 EDTKELVAKAGVYVFFQLELRRVWAGEGSGSVSLALHLQP  
 LRSAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQ  
 RLGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPSP  
 RSE

Amino acid sequence of light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYATWYQQKPGQAPILVIYGENKRPS  
 GIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSQGHLVFGGGTKLTVLG  
 QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETT  
 TPSKQSNKYAASSYLSLTPEQWVKSHRSYSCQVTHEGSTVEKTVAPAEC

Figure 59

**Clivatuzumab HC-TGF $\beta$ RII-4-1BB fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-4-1BB fusion protein:

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYINP  
 YNDGTQTNKKFKGKATLTRDTSINTAYMELSRLRSDDTAVYYCARGFGGSYGFA  
 YNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS  
 GALTSGVNTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV  
 EPKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLNISRTPEVTCVVDVSHEDP  
 EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
 SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV  
 EWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVNHEAL  
 HNHYTQKSLSLSPGKGGGGSGGGGSGGGGS**TIPPHVQKSVNNDMIVTD**  
**NNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEV**  
**CVAVWRKNDENITLETVCHDPKLPYHDFILEDASPCKIMKEK**  
**KKPGETFFMCSCSSDECNDNIIFSEYNTSNPDepkscdkACPW**  
*AVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQ*  
*LVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVA*  
*KAGVYYVFFQLELRRVAGEGSGSVSLALHLQPLRSAAGAAA*  
*LALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTE*  
*ARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE*

Amino acid sequence of light chain:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPGKAPKLWIYSTSNL  
 ASGVPARFSGSGSGTDFTLTISLQPEDSASYFCHQWNRYPYTFGGGTRLEIKRT  
 VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAKVQWKVDNALQSGNSQESVTE  
 QDSKDSTYLSSTLTLSPRKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 60

**Pritumumab HC-TGF $\beta$ RII-4-1BB fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-4-1BB fusion protein:

EVQLLES~~GGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAITP~~  
~~SGGSTNYADSVKGRFTISRDN~~SQNTLYLQMNSLRVEDTAVYICGRVPYRSTWYP  
 LYWGQGT~~LVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP~~TVTSW  
 NSGALTSGVHTFPAVLQSSGLYSLSSVTV~~PSSSLGTQTYICNVNHKPSNTKVDK~~  
 KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH  
 EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK  
 CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMH  
 EALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS**TIPPHVQKSVNNDMIV**  
**TDNNGAVKFPQLCKFC**DVRFSTCDNQKSCMSNCSITSICEKPQ  
**EVCVAVWRKNDENITLET**VCHDPKLPYHDFILEDAA**SPKCIMK**  
**EKKKPGETFFMCSSSDECNDNIIFSEEYNTSNP***DepkscdkAC*  
*PWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMF*  
*AQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELV*  
*VAKAGVYYVFFQLELRRVWAGEGSGSVSLALHLQPLRSAAGA*  
*AALALTVDLPPASSEARNSAFGFQGRLLHLSAGQRLGVHLH*  
*TEARARHAWQLTQGATVLGLFRVTPEIPAGLPSPRSE*

Amino acid sequence of light chain:

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASSLH  
 SKVPTQFSGSGSGTDFTLTIS~~SLQPEDFATYYCLQYSTYPITFGGGTKVEIKRTVAA~~  
 PSVFIFPPSDEQLKSGTASVVCLLNNFYPR**EAKVQWKVDNALQSGNSQESVTEQ**  
 DSKDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 61



**Cantuzumab HC-TGFβRII-PD1 fusion protein**

Amino acid sequence of heavy chain-TGFβRII-PD1 fusion protein:

QVQLVQSGAEVKKPGETVKISCKASDYFTFTYYGMNWKQAPGQGLKWMGWI  
 DTTTGEPTYAQKFQGRIFASLETASTAYLQIKSLKSEDTATYFCARRGPYNWYFD  
 VWGQGTTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN  
 SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK  
 VEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHE  
 DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC  
 KVSNAKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMH  
 EALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS**TIPPHVQKSVNNDMIV**  
**TDNNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQ**  
**EVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAAASPKCIMK**  
**EKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD***epkscdkPG*  
*WFLDSPDRPWNPPTFSPALLVTEGDNATFTCSFSNTSESFVLNWYR*  
*MSPSNQTDKLAAPFEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR*  
*RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPPRP*  
*AGQFQTLV*

Amino acid sequence of light chain:

DIVMTQSPLSVPVTPGEPVSISCRSSKSLHSNGNTYLYWFLQRPQGQSPQLLIYR  
 MSNLVSGVPDRFSGSGGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGTKLE  
 LKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNS  
 QESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 62

**Cixutumumab HC-TGF $\beta$ RII-PD1 fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-PD1 fusion protein:

EVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLEWMGGIPI  
 FGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAPLRFLEWSTQ  
 DHYYYYYMDVWGKGTITVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF  
 PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHK  
 PSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC  
 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW  
 LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL  
 VKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGN  
 VFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGG**STIPPHVQKS**  
**VNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSI**  
**TSICEKPQEVCAVWRKNDENITLETVCHDPKLPYHDFILEDAA**  
**SPKCIMKEKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD***ep*  
*kscdk**PGWFLDSPDRPWNPPTFSPALLVTEGDNATFTCSFSNTSESF*  
*VLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHM*  
*SVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAH*  
*PSPSPRPAGQFQTLV*

Amino acid sequence of light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYATWYQQKPGQAPILVIYGENKRPS  
 GIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSQGHLVFGGGTKLTVLG  
 QPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETT  
 TPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPAEC

Figure 63

**Clivatuzumab HC-TGF $\beta$ RII-PD1 fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-PD1 fusion protein:

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYINP  
 YNDGTQTNKKFKGKATLTRDTSINTAYMELSRLRSDDTAVYYCARGFGGSYGFA  
 YNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS  
 GALTSGVNTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDKRV  
 EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLNISRTPEVTCVVDVSHEDP  
 EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV  
 SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAV  
 EWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVNHEAL  
 HNHYTQKSLSLSPGKGGGGSGGGGSGGGGS**TIPPHVQKSVNNDMIVTD**  
**NNGAVKFPQLCKFCDFRSTCDNQKSCMSNCSITSICEKPQEV**  
**CVAVWRKNDENITLETVCHDPKLPYHDFILEDAAAPKCMKEK**  
**KKPGETFFMCSCSSDECNDNIIFSEYNTSNPDepkscdkPGWFL**  
*DSPDRPWNPPTFSPALLVTEGDNATFTCSFSNTSESVLNWYRMSPS*  
*NQTDKLAAFPEDRSQPGQDCRFRTQLPNGRDFHMSVVRARRND*  
*SGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSRPAGQ*  
*FQTLV*

Amino acid sequence of light chain:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPGKAPKLWIYSTSNL  
 ASGVPARFSGSGSGTDFTLTISLQPEDSASYFCHQWNRYPYTFGGGTRLEIKRT  
 VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAKVQWKVDNALQSGNSQESVTE  
 QDSKDSTYLSSTLTLSRKADEYKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 64

**Pritumumab HC-TGF $\beta$ RII-PD1 fusion protein**

Amino acid sequence of heavy chain-TGF $\beta$ RII-PD1 fusion protein:

EVQLLESGGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAITP  
 SGGSTNYADSVKGRFTISRDNQNTLYLQMNSLRVEDTAVYICGRVPYRSTWYP  
 LYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSW  
 NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK  
 KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH  
 EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK  
 CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI  
 AVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSSVMH  
 EALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGS**TIPPHVQKSVNNDMIV**  
**TDNNGAVKFPQLCKFC****DVRFSTCDNQKSCMSNCSITSICEKPQ**  
**EVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAA****SPKCIMK**  
**EKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD***epkscdkPG*  
*WFLDSPDRPWNPPTFSPALLVTEGDNATFTCSFSNTSESFVLNWYR*  
*MSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRAR*  
*RNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPPRP*  
*AGQFQTLV*

Amino acid sequence of light chain:

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASSLH  
 SKVPTQFSGSGSGTDFTLTISLQPEDFATYYCLQYSTYPITFGGGTKVEIKRTVAA  
 PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ  
 DSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 65

## REFERENCES CITED IN THE DESCRIPTION

*This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.*

### Patent documents cited in the description

- WO 2011109789 A2 [0003]
- WO 2009027471 A1 [0003]
- US 2011104734 A1 [0003]

### Non-patent literature cited in the description

- Birch J Retal: Advanced drug delivery reviews, elsevier. 07 August 2006, vol. 58, 671-685 [0003]
- **KALWY S et al.** Molecular Biotechnology. Humana press, Inc, 01 October 2006, vol. 34, 151-156 [0003]
- **SAMBROOK et al.** MOLECULAR CLONING: A LABORATORY MANUAL. 1989 [0030]
- CURRENT PROTOCOLS IN MOLECULAR BIOLOGY. 1987 [0030]
- METHODS IN ENZYMOLOGY. Academic Press, Inc, 1995 [0030]
- ANTIBODIES, A LABORATORY MANUAL, and ANIMAL CELL CULTURE. 1987 [0030]