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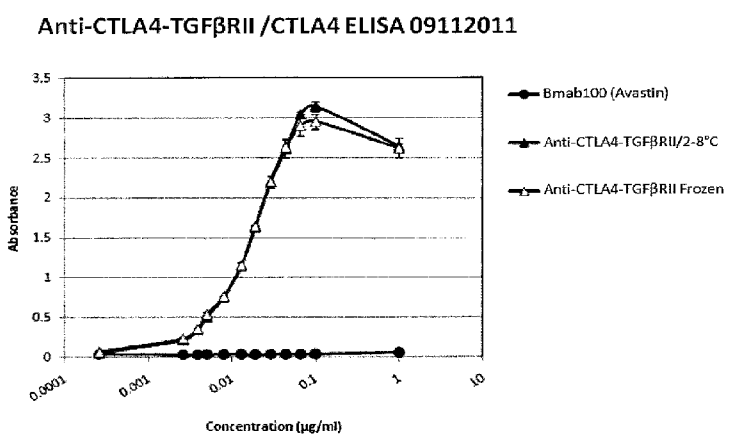


Figure 29

(57) Abstract: The present invention relates generally to the field of generating fusion proteins to be used in cancer therapy, and more specifically, to nucleotide sequences encoding the fusion proteins, wherein the chimeric fusion proteins comprises at least one targeting moiety and at least one immunomodulatory moiety that counteracts the immune tolerance of cancer cells.

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TARGETED/IMMUNOMODULATORY FUSION PROTEINS AND METHODS FOR MAKING SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[001] The application claims priority to Indian Patent Application No. 1689/CHE/2012 filed on April 30, 2012 and Indian Patent Application No. 1690/CHE/2012 filed on April 30, 2012, the contents of both is hereby incorporated by reference herein for all purposes.

BACKGROUND OF THE INVENTION

[002] Technical Field

[003] The present invention relates generally to the field of generating fusion proteins to be used in cancer therapy, and more specifically, to nucleotide sequences encoding the fusion proteins, wherein the fusion or chimeric polypeptides comprises at least one targeting moiety and at least one immunomodulatory moiety that counteracts the immune tolerance of cancer cells.

[004] Related Art

[005] 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.

SUMMARY OF THE INVENTION

[006] The present invention provides polynucleotides, as well as polypeptides encoded thereby, that are expressed in cancer cells. These polynucleotides and expressed polypeptides are useful in a variety of therapeutic methods for the treatment of cancer. The present invention further provides methods of reducing growth of cancer cells by counteracting immune tolerance of cancer cells, wherein T cell remain active and inhibit the recruitment of T-regulatory that are known to suppress the immune system's response to the tumor. Thus the chimeric polypeptides generated by the polynucleotides sequences of the present invention are useful for treating cancer because of the expressed fusion or chimeric polypeptides.

[007] In one aspect, the present 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 invention are useful for binding to a cancer cell receptor and reducing the ability of cancer cells to avoid an immune response.

[008] 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-β)) and regulatory T cells and/or immunosuppressive myeloid dendritic cells (DCs). By counteracting tumor-induced immune tolerance, the present invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment. The present invention 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.

[009] In another aspect, the present invention provides a molecule 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-β): (ii) Programmed death- 1 ligand 1 (PD-Ll) 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-κB (RANK).

[0010] In a further aspect, 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.

[0011] In a still further aspect, there is provided a chimeric polypeptide that comprised a tumor targeting moiety and an immunomodulatory moiety comprising a molecule that binds transforming growth factor beta (TGF-β), 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, ritubximab, ipilimumab, tremelimumab, muromonab-CD3, abciximab, daclizumab, basiliximab, palivizumab, infliximab. gemtuzumab ozogamicin, alemtuzumab, ibritumomab tiuxetan, adalimumab, omalizumab, tositumomab, I-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 Idel), 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 (Biogent 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), ranibizimumab (Genetech), mepolizumab (GSK), TNX-355 (Tanox), or MYO-029 (Wyeth).

[0012] In an another aspect, 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.

[0013] In yet another aspect, the targeting moiety is a molecule that specifically binds epidermal growth factor receptor (EGFR1, Erb-B l), HER2/neu (Erb-B2), CD20, cytotoxic T-lymphocyte antigen-4 (CTLA-4) which is essential for Treg function (CD 152); H-1and Interleukin- 6 (IL-6).

[0014] In a still further aspect, the targeting moiety specifically binds a component of a regulatory T cell (treg), myeloid suppressor cell, or dendritic cell. In another aspect, the targeting moiety specifically binds one of the following molecules: (i) CD4; (ii) CD25 (IL-2ct receptor; IL-2aR); (iii) Transforming growth factor-beta receptor (TGF-pR); (vi) Transforming growth factor-beta (TGF-β): (vii) Programmed Death- 1 (PD-1); (viii) Programmed death- 1 ligand (PD-LI or PD-L2.

[0015] In another aspect, the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β): (ii) Programmed death-1 ligand (PD-Ll or PD-L2); or 4-1BB receptor.

[0016] In yet another aspect, the immunomodulatory moiety includes a molecule that binds TGF-β and inhibits the function thereof. Specifically the immunomodulatory moiety includes an extracellular ligand-binding domain of Transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb, or TGF-βRIII. In another aspect the immunomodulatory moiety includes an extracellular ligand-binding domain (ECD) of TGF-βRII. Still further the immunomodulatory moiety may include H-4-1BB ligand which binds to the 4-1BB receptor to stimulate T-cells to help eradiate tumor.

[0017] In a still further aspect, 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-βRII.

[0018] In yet another aspect, the immunomodulatory moiety includes a molecule that specifically binds to and inhibit the activity of Programmed death- 1 ligand 1 (PD-L l) or Programmed death- 1 ligand 2 (PD-L2). In another aspect, the immunomodulatory moiety includes an extracellular ligand-binding domain or ectodomain of Programmed Death- 1 (PD-1).

[0019] In a further aspect, the targeting moiety includes an antibody, antibody fragment, or polypeptide that specifically binds to HER2/neu, EGFRl, CD20, cytotoxic T-lymphocyte antigen-4 (CTLA-4), CD25 (lL-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).

[0020] In a still further aspect, the targeting moiety includes an antibody or antibody fragment that specifically binds to CD20, and the immunomodulatory moiety includes a sequence from transforming growth factor-β (TGF-β).

[0021] In one aspect, the present invention 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.

[0022] In another aspect, the present invention 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. 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.

[0023] In yet another aspect, the present invention provides for a method of treating cancer in a subject, the method comprising:

a. providing at least one recombinant vector comprising nucleotide sequences that encode at least one targeting moiety and at least one immunomodulatory moiety selected from SEQ ID NOs: 12 to 28; and

b. administering the recombinant vector to the subject under conditions such that said nucleotide sequences are expressed at a level which produces a therapeutically effective amount of the encoded fusion proteins in the subject.

[0024] In an alternative aspect, the present invention 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.

[0025] In yet another aspect, the present invention provides a recombinant host cell transfected with a polynucleotide that encodes a fusion protein peptide of the present invention.

[0026] In a still further aspect, the present invention contemplates a process of preparing a fusion protein of the present invention comprising:

a. transfecting a host cell with polynucleotide sequences that encode chimeric fusion proteins to produce a transformed host cell, wherein the polynucleotide sequences encode at least one targeting moiety and at least one immunomodulatory moiety selected from SEQ ID NOs: 12 to 28; and

b. maintaining the transformed host cell under biological conditions sufficient for expression of the peptide.

[0027] In another aspect, the present invention relates to the use of a chimeric fusion protein, as shown in Figures 1 to 15, in the use of a medicament for the treatment of cancer. Preferably, the fusion protein is expressed in a host cell and such expressed proteins are administered in a therapeutic amount to reduce the effects of cancer in a subject in need thereof.

[0028] In a still further aspect, the present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more fusion proteins of the invention, in various aspects, the subject is administered one or more molecule of the invention in combination with another anticancer therapy, in one aspect, the anticancer therapy includes a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent or cytotoxic agent. The anticancer therapy may also include ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[0029] In yet another aspect, the present invention provides for a method of preparing therapeutically active antibody-peptide fusion proteins, the method comprising;

a. preparing a codon optimized sequence of the said fusion protein;

b. cloning the optimized sequence of said fusion protein in a host cell capable of transient or continued expression;

c. growing the host cell in a media under suitable conditions for growing and allowing the host cell to express the cloned protein; and

d. subjecting the expressed protein to purification and optionally checking the bi-specific binding capabilities of the protein to its targets.

[0030] In a preferred embodiment the therapeutically active antibody-peptide fusion proteins is a targeting antibody fused to one or more immunomodulating moiety that counteracts immune tolerance of a cancer cell. In one aspect, the immunomodulating moiety may be linked by an amino acid spacer of sufficient length to allow bi-specific binding of the molecule. The immunomodulating moiety may be bound to either the C-terminus of the heavy or light chain of the antibody

[0031] In a preferred method as described above, the immunomodulating moiety is (i) Transforming growth factor-beta (TGF-β), (ii) Programmed death-1 (PD-1), (iii) CTLA-4 or

(iv) 4-1BB or parts thereof and the targeting antibody binds epidermal growth factor receptor (EGFR1, Erb-B 1), HER2/neu (Erb-B2), CD20, CD6, CTLA-4, Mucin 1(MUC-1), Interleukin-2 (IL-2) or Interleukin- 6 (IL-6).

[0032] 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

[0033] Once the fusion peptides are expressed, they are preferably subjected to purification and 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.

[0034] 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 are previously disclosed, such as a mammalian cell line, including but not limited to HEK293, CHO or NSO.

[0035] In a further aspect, the culture medium can be improved by additions to such medium. For example, the culture medium may 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. A desirable transitional metallic salt includes a zinc ion and the addition of the metal ion may be carried out during different phases of the production.

[0036] Other features and advantages of the invention will be apparent from the following detailed description, drawings and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] Figure 1 shows the amino acid sequences of with the amino acid sequence of Anti-HER2/neu-TGFβRII 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-βRII (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-βRII and shown in italics.

[0038] Figure 2 shows the amino acid sequences of Anti-EGFR1-TGFβRII 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-βRII (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-βRII and shown in italics.

[0039] Figure 3 shows the amino acid sequences of Anti-CTLA4-TGFβRII 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-βRII (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-βRII and shown in italics.

[0040] Figure 4 shows the amino acid sequences of Anti-HER2/neu HC-4-1BB and LC-TGFβRII 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-βRII (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-βRII and shown in italics.

[0041] Figure 5 shows the amino acid sequence of Anti-EGFR1 HC-4-1BB and LC-TGFβRII 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0042] Figure 6 shows the amino acid sequence of Anti-CTLA4 HC-4-1BB and LC-TGFβRII 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0043] Figure 7 shows the amino acid sequence of Anti-HER2/neu HC-PD1 and LC-TGFβRII fusion protein with amino acid sequence of Anti-HER2/neu heavy chain-PD1 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0044] Figure 8 shows the amino acid sequence of Anti-EGFR1 HC-PD1 and LC-TGFβRII fusion protein with amino acid sequence of Anti-EGFR1 heavy chain-PD1 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0045] Figure 9 shows the amino acid sequence of Anti-CTLA4 HC-PD1 and LC-TGFβRII fusion protein with amino acid sequence of Anti-CTLA4 heavy chain-PD1 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0046] Figure 10 shows the amino acid sequence of Anti-HER2/neu HC-TGFβRII-4-1BB fusion protein with amino acid sequence of Anti-HER2/neu heavy chain-TGFβRII-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 TGFβRII (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).

[0047] Figure 11 shows the amino acid sequence of Anti-EGFR1 HC-TGFβRII-4-1BB fusion protein with amino acid sequence of Anti-EGFR1 heavy chain-TGFβRII-4-1BB fusion protein wherein the amino acid sequence for Anti-EGFR1 heavy chain (SEQ ID NO: 5) sequence 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 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).

[0048] Figure 12 shows the amino acid sequence of Anti-CTLA4 HC-TGFβRII-4-1BB fusion protein with amino acid sequence of Anti-CTLA4 heavy chain-TGFβRII-4-1BB 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 TGFβRII (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).

[0049] Figure 13 shows the amino acid sequence of Anti-HER2/neu HC-TGFβRII-PD1 fusion protein with amino acid sequence of Anti-HER2/neu heavy chain-TGFβRII-PD1 fusion protein wherein the amino acid sequence 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 TGFβRII (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).

[0050] Figure 14 shows the amino acid sequence of Anti-EGFR1 HC-TGFβRII-PD1 fusion protein with amino acid sequence of Anti-EGFR1 heavy chain-TGFβRII-PD1 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 TGFβRII (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).

[0051] Figure 15 shows the of Anti-CTLA4 HC-TGFβRII-PD1 fusion protein with amino acid sequence of Anti-CTLA4 heavy chain-TGFβRII-PD1 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 TGFβRII (immunomodulatory moiety) (SEQ ID NO: 4) is identified in bold letters and the amino acid sequence for PD-1 (immunomodulatory moiety) (SEQ ID NO: 8) 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).

[0052] Figure 16 shows the nucleotide sequence of Anti-HER2/neu heavy chain constant region with linker (SEQ ID NO: 12) and TGFβRII ECD (SEQ ID NO: 13) that have been codon optimized for expression in CHO cell.

[0053] 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.

[0054] 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.

[0055] 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.

[0056] 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.

[0057] 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.

[0058] Figure 22 shows the analysis of Protein A purified Anit-HER2/neu-TGFβRII and Anti-EGFR1- TGFβRII at 12 % PAGE

[0059] Figure 23 A shows Anti-HER2/neu-TGFβRII samples analyzed by Protein A/SEC Chromtography and B Anti-EGFR1-TGFβRII samples analyzed by Protein A/SEC Chromtography.

[0060] 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).

[0061] Figure 25 shows that Anti-EGFR1-TGFβRII–inhibits the proliferation of A431 cell line similar to the Cetuximab.

[0062] Figure 26 shows the ADCC activity of Anti-HER2-TGFβRII on BT474 cells is similar to that of Bmab200 (Herceptin).

[0063] Figure 27 shows the ADCC activity of Anti-EGFR1-TGFβRII on A431 cells wherein the ADCC activities are similar to that of Cetuximab.

[0064] Figure 28 shows the ADCC activity of ADCC activity of Anti-EGFR1-4-1BB in comparison with Anti-EGFR1-TGFβRIIand cetuximab.

[0065] 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.

[0066] 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-EGRF1-4-1BB to determine the binding of 4-1BBL.

[0067] Figure 31 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.

[0068] Figure 32 shows the binding activity of Anti-EGFR1-PD1 to EGFR and PD1.

[0069] Figure 33 shows photographs of expressed proteins and reduction alkylation thereof.

[0070] 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.

[0071] Figure 35 shows the Mass Spectrum of heavy chain (HC) (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion.

[0072] Figure 36A 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.

[0073] Figure 37 shows the Mass Spectrum of HC (Reduced) of Anti-EGFR1-TGFβRII ECD.

[0074] Figure 38 A shows the UV Chromatogram of Tryptic Peptides of Anti-HER2/neu-TGFβRII ECD fusion protein and B shows the Total Ion Chromatogram (TIC) of Tryptic Peptides of Anti-HER2/neu-TGFβRII ECD fusion protein.

[0075] 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.

[0076] 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.

[0077] Figure 43 provides a list of expected/observed tryptic peptide of the light chain of the Anti-EGFR1-TGFβRII ECD fusion protein.

[0078] Figure 44 shows the list of expected/observed tryptic peptide of the heavy chain of the Anti-EGFR1-TGFβRII ECD fusion protein.

[0079] Figure 45 shows the list of expected/observed tryptic peptide of the heavy chain of the Anti-EGFR1-TGFβRII ECD fusion protein.

[0080] 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.

[0081] 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.

[0082] 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.

[0083] 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.

[0084] 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.

[0085] 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.

[0086] 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.

[0087] 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0088] Figure 54 shows the amino acid sequence of Cantuzumab - HC-PD1 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) 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0089] Figure 55 shows the amino acid sequence of Cixutumumab - HC-PD1 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 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0090] Figure 56 shows the amino acid sequence of Clivatuzumab - HC-PD1 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 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0091] Figure 57 shows the amino acid sequence of Pritumumab - HC-PD1 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 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-βRII (immunomodulatory moiety) (SEQ ID NO: 4) identified in bold letters with a linker (SEQ ID NO: 3) therebetween.

[0092] Figure 58 shows the amino acid sequence of Cantuzumab HC-TGFβRII-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 TGFβRII (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).

[0093] Figure 59 shows the amino acid sequence of Cixutumumab HC-TGFβRII-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 TGFβRII (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).

[0094] Figure 60 shows the amino acid sequence of Clivatuzumab HC-TGFβRII-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 TGFβRII (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).

[0095] Figure 61 shows the amino acid sequence of Pritumumab HC-TGFβRII-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 TGFβRII (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).

[0096] Figure 62 shows the amino acid sequence of Cantuzumab HC-TGFβRII-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 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 Cantuzumab light chain (SEQ ID NO: 30).

[0097] 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).

[0098] 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).

[0099] 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

[00100] 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)).

[00101] Definitions

[00102] 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. The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. 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:

[00103] 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 of 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 of the present invention can be prepared using standard techniques well known to one of skill in the art.

[00104] 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 the production cell or organism, generally a eukaryotic cell, for example, a cell of Pichia, a cell of Trichoderma, a Chinese Hamster Ovary cell (CHO) or a human cell. 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; however optimized expression of these sequences in other eukaryotic cells is also envisioned herein. 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.

[00105] 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.

[00106] 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.

[00107] 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 invention.

[00108] 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. Preferably the host cell is a transient cell line or a stable cell line and more preferably a mammalian host cell and selected from the group consisting of HEK293, CHO and NSO.

[00109] The tern "subject," as used herein means a human or vertebrate animal including a dog, cat, horse, cow, pig, sheep, goat, chicken, monkey, rat, and mouse.

[00110] 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.

[00111] 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.

[00112] 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.

[00113] 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.

[00114] 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.

[00115] 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.

[00116] 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 according to the present invention; 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.

[00117] Although chemotherapeutic agents can induce "immunogenic" tumor cell death and facilitate cross-presentation of antigens by dendritic ceils, 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 invention provides strategies 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.

[00118] The present invention 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 invention provides effective compositions and methods for cancer treatment, optional in combination with another existing cancer treatment.

[00119] The present invention 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. 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+ 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 cytoreduction and reinforces adaptive antitumor immunity.

[00120] In addition, the fusion proteins of the present 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.

[00121] The targeted immunostimulatory antibodies and/or fusion proteins of the 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-β); (ii) Programmed death- 1 ligand 1 (PD-L1 ; B7-H1); (iii) Vascular endothelial growth factor (VEGF); and (iv) Interleukin-10 (lL-10).

[00122] In addition to blocking dendritic cell (DC) maturation, these molecules promote the development of specialized subsets of immunosuppressive $CD4^+$ T cells (regulatory T cells; Treg cells) and myeloid-derived suppressor cells (MDSC). Tregs are a minority sub-population of $CD4^+$ T cells that constitutively express CD25 [the interleukin-2 (IL-2) receptor cc-chain] and the forkhead box P3 (FOXP3) transcription factor. Tregs (CD4+CD25+FoxP3+ cells) maintain immune tolerance by restraining the activation, proliferation, and effector functions of a wide range of immune cells, including CD4 and

CDS T cells, natural killer (NK) and NKT cells, B cells and antigen presenting cells (APCs) in vitro and in vivo.

[00123] 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-β; PD-L1) and tumor-infiltrating Tregs is correlated with a reduction of survival of patients with diverse types of cancers. The fusion proteins of the present 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.

[00124] The present invention provides a method of preventing or treating a neoplastic disease. The method includes administration to a subject in need thereof one or more fusion proteins of the present invention in combination with another anticancer therapy, wherein the anticancer therapy is a chemotherapeutic molecule, antibody, small molecule kinase inhibitor, hormonal agent, cytotoxic agent, targeted therapeutic agent, anti-angiogenic agent, ionizing radiation, ultraviolet radiation, cryoablation, thermal ablation, or radiofrequency ablation.

[00125] 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.

[00126] Examples of antibodies which can be incorporated into compositions and methods disclosed herein include, but are not limited, to antibodies such as trastuzumab (anti-HER2/neu antibody); Pertuzumab (anti-HER2 mAb); cetuximab (chimeric monoclonal antibody to epidermal growth factor receptor EGFR): panitumumab (anti-EGFR antibody); nimotuzumab (anti-EGFR antibody); Zalutumumab (anti-EGFR mAb); Necitumumab (anti-EGFR mAb); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-210 (humanized anti-HER-2 bispecific antibody); MDX-447 (humanized anti-EGF receptor bispecific antibody); Rituximab (chimeric murine/human anti-CD20 mAb); Obinutuzumab (anti-CD20 mAb); Ofatumumab (anti-CD20 mAb); Tositumumab-1131 (anti-CD20 mAb); ibritumomab tiuxetan (anti-CD20 mAb); Bevacizumab (anti-VEGF mAb); Ramucirumab (anti-VEGFR2 mAb); Ranibizumab (anti-VEGF mAb); Aflibercept (extracellular domains of YEGFR1 and VEGFR2 fused to IgGl Fc): AMG386 (angiopoietin-1 and -2 binding peptide fused to IgGl Fc); Dalotuzumab (anti-lGF-lR mAb): Gemtuzumab ozogamicin (anti-CD33 mAb); Alemtuzumab (anti-Campath-l/CD52 mAb); Brentuximab vedotin (anti-CD30 mAb); Catumaxomab (bispecific mAb that targets epithelial cell adhesion molecule and CD3); Naptumomab (anti-5T4 mAb); Girentuximab (anti-Carbonic anhydrase ix): or Farletuzumab (anti-folate receptor). Other examples include antibodies such as Panorex™ (17-1 A) (murine monoclonal antibody); Panorex (@ (17-1 A) (chimeric murine monoclonal antibody); BEC2 (ami-idiotypic mAb, mimics the GD epitope) (with BCG): Oncolym (Lym-1 monoclonal antibody); SMART M l 95 Ab, humanized 13' 1 LYM-1 (Oncolym), Ovarex (B43.13, anti-idiotypic mouse mAb); 3622W94 mAb that binds to EGP40 (17- 1 A) pancarcinoma antigen on adenocarcinomas; Zenapax (SMART Anti-Tac (IL-2 receptor); SMART Ml 95 Ab, humanized Ab, humanized); NovoMAb-G2 (pancarcinoma specific Ab): TNT (chimeric mAb to histone antigens); TNT (chimeric mAb to histone antigens); GJiomab-H (Monoclonals— Humanized Abs); GN1-250 Mab; EMD-72000 (chimeric-EGF antagonist); LymphoCide (humanized IL.L.2 antibody); and MDX-260 bispecific, targets GD-2, ANA Ab, SMART lDiO Ab, SMART ABL 364 Ab or ImmuRAIT-CEA.

[00127] 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.

[00128] In one embodiment, a hybridoma can produce a targeted fusion protein comprising a targeting moiety and an immunomodulatory moiety. In one embodiment, a targeting moiety comprising an antibody, antibody fragment, or polypeptide is linked or fused to an immunomodulatory moiety consisting of a polypeptide, with a linker or without a linker. The linker can be an amino acid linker. In one embodiment, a linker is (GGGGS)n wherein n is 1, 2, 3, 4, 5, 6, 7, or 8. For example, GGGGSGGGGSGGGGS (SEQ ID NO: 4). In another embodiment, a linker is EPKSCDK (SEQ ID NO: 11). In various aspects, the length of the linker may be modified to optimize binding of the target moiety or the function of the immunomodulatory moiety. In various aspects, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the Fc region of the heavy chain of a targeting antibody or Fc-containing fusion protein. In another aspect, the immunomodulatory moiety is a polypeptide that is fused to the C-terminus of the light chain of a targeting antibody.

[00129] 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')2, 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, CHI, 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 tor any other modified Fc (e.g. glycosylation or other engineered Fc).

[00130] The fusion proteins of the present invention may be synthesized by conventional techniques known in the art, for example, by chemical synthesis such as solid phase peptide synthesis. Such methods are known to those skilled in the art. In general, these methods employ either solid or solution phase synthesis methods, well known in the art. Specifically, the methods comprise the sequential addition of one or more amino acids or suitably protected amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then be either attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complementary (amino or carboxyl) group suitably protected, under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining protecting groups and any solid support are removed either sequentially or concurrently to afford the final polypeptide. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under condition that do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide.

[00131] Typical protecting groups include t-butyloxycarbonyl (Boc), 9-fluorenylmethoxycarbonyl (Fmoc), benxyloxycarbonyl (Cbz), p-toluenesulfonyl (Tos); 2,4-dinitrophenyl, benzyl (Bzl), biphenylisopropyloxy-carboxycarbonyl, cyclohexyl, isopropyl, acetyl, o-nitrophenylsulfonyl, and the like. Of these, Boc and Fmoc are preferred.

[00132] Typical solid supports are generally cross-linked polymeric materials. These include divinylbenzene cross-linked styrene-based polymers, for example, divinylbenzene-hydroxymethylstyrene copolymers, divinylbenzene-chloromethylstyrene copolymers, and divinylbenzene-benzhydrylaminopolystyrene copolymers. The divinylbenzene-benzhydrylaminopolystyrene copolymers, as illustrated herein using p-methyl-benzhydrylamine resin, offers the advantage of directly introducing a terminal amide functional group into the peptide chain, which function is retained by the chain when the chain is cleaved from the support.

[00133] In one method, the polypeptides are prepared by conventional solid phase chemical synthesis on, for example, an Applied Biosystems, Inc. (ABI) 430A peptide synthesizer using a resin that permits the synthesis of the amide peptide form and using t-Boc amino acid derivatives (Peninsula Laboratories, Inc.) with standard solvents and reagents. Polypeptides containing either L- or D-amino acids may be synthesized in this manner. Polypeptide composition is confirmed by quantitative amino acid analysis and the specific sequence of each peptide may be determined by sequence analysis.

[00134] Preferably, the polypeptides can be produced by recombinant DNA techniques by synthesizing DNA encoding the desired polypeptide. Once coding sequences for the desired polypeptides have been synthesized or isolated, they can be 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.

[00135] 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.

[00136] The expression vector may then used to transform an appropriate host cell. A number of mammalian cell lines are known in the art and include immortalized cell lines available from the American Type Culture Collection (ATCC), such as, but not limited to,

Chinese hamster ovary (CHO) cells, HeLa cells, HEK293, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), Madin-Darby bovine kidney ("MDBK") cells, NOS cells derived from carcinoma cells, such as sarcoma, as well as others. Similarly, bacterial hosts such as E. coli, Bacillus subtilis, and Streptococcus spp., will find use with the present expression constructs. Yeast hosts useful in the present invention include inter alia, Saccharomyces cerevisiae, Candida albicans, Candida maltosa, Hansenula polymorpha, Kluyveromyces fragilis, Kluyveromyces lactis, Pichia guillerimondii, Pichia pastoris, Schizosaccharomyces pombe and Yarrowia lipolytica. Insect cells for use with baculovirus expression vectors include, inter alia, Aedes aegypti, Autographa californica, Bombyx mori, Drosophila melanogaster, Spodoptera frugiperda, and Trichoplusia ni. The proteins may also be expressed in Trypanosomes.

[00137] Depending on the expression system and host selected, the proteins of the present invention are produced by growing host cells transformed by an expression vector described above under conditions whereby the protein of interest is expressed. The protein is then isolated from the host cells and 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 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.

[00138] Once synthesized or otherwise 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.

[00139] The fusion proteins of the present invention can be formulated into therapeutic compositions in a variety of dosage forms such as, but not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the particular cancer type targeted. The compositions also preferably include pharmaceutically acceptable vehicles, carriers or adjuvants, well known in the art,

such as human serum albumin, ion exchangers, alumina, lecithin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, and salts or electrolytes such as protamine sulfate. Suitable vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. Actual methods of preparing such compositions are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 18th edition, 1990.

[00140] The above compositions can be administered using conventional modes of delivery including, but not limited to, intravenous, intraperitoneal, oral, intralymphatic, or subcutaneous administration. Local administration to a tumor in question, or to a site of inflammation, e.g., direct injection into an arthritic joint, will also find use with the present invention.

[00141] Therapeutically effective doses will be easily determined by one of skill in the art and will depend on the severity and course of the disease, the patient's health and response to treatment, and the judgment of the treating physician.

[00142] Experimental

[00143] 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.

[00144] Example 1

[00145] 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

[00146] The expressed protein were characterized by using SDS PAGE and the expressed fusion proteins Anit-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-HER2/neu-TGFβRII & Anti-EGFR1-TGFβRII heavy chains mass are higher because of the TGFβRII. Also Anti-HER2/neu-TGFβRII heavy chain has four N-glycosylation sites while Anti-EGFR1-TGFβRII has five N-glycosylation sites.

[00147] Example 2

[00148] Protein A/SEC chromatography. The Anti-HER2/neu-TGFβRII 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.

[00149] Example 3

[00150] Functional assays for the Fusion proteins. ELISA experiment was carried out to check the binding ability of Anti-HER2/neu-TGFβRII and Anti-EGFR1-TGFβRII to TGFβ. 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. Figure 24 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.

[00151] Example 4

[00152] Antibody dependent cellular cytotoxicity ADCC activity for Anti-HER2/neu-TGFβRII 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-TGFβRII on BT474 cells is similar to that of Bmab200(Herceptin). Figure 27 shows 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.

[00153] Example 5

[00154] 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-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-EGRF1-4-1BB 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.

[00155] Example 6

[00156] 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.

[00157] Mass Spectrometry Analysis of Anti-HER2/neu-TGFβRII and Anti-EGFR1-TGFβRII. 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-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.

[00158] The fusion protein shown in Figure 2 was expressed and tested. 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.

[00159] Example 7

[00160] 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 Anti-HER2/neu-TGFβRII ECD fusion protein and B shows the Total Ion Chromatogram (TIC) of Tryptic Peptides of Anti-HER2/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. 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 βRII).

[00161] 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. 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-TGFβRII 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 βRII).

[00162] Example 8

[00163] 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:

[00164] 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.

[00165] The light chain or the light chain fusion protein coding nucleic acid sequences are cloned into the restriction enzyme sites AvrII and BstZl7 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.

[00166] 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.

[00167] Example 9

[00168] 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 $0.3\text{-}0.45\text{x}10^6$ cells/ml at 37 ± 1°C, the first 3-4 days are dedicated to grow the cells in batch phase. Next step involves lowering the temperature to 31±1 °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.

[00169] Example 10

[00170] 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 $0.3\text{-}0.45\text{x}10^6$ cells/nil at 37 ± 1° C, the first 3-4 days are dedicated to growing the cells in batch phase. Next step involves lowering the temperature to 31+1-1 °C and continuing the run till 7th day.

[00171] Example 11

[00172] 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°C or at 2-8°C.

[00173] Example 12

[00174] Differentiating Trastuzumab from Trastuzumab-TGF βRII receptor fusion molecule

[00175] 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 β and specific cytokines involved in the subdued immune response. A novel molecule is generated wherein Trastuzumab (anti ErbB2) is fused with the TGF βRII 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 TGFβRII receptor will sequester TGFβ 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 TGFβ, cytotoxic CD8 positive cells and NK cells. While Trastuzumab will be ineffective in inducing cytotoxicity Trastuzumab TGFβRII receptor fusion molecule will sequester the TGFβ thereby preventing the inhibition of cytotoxic CD8 and NK cells. This

will lead to enhanced cytotoxicity observed in Trastuzumab -TGFβRII 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.

[00176] Although the invention has been described with reference to the above example, it will be understood that modifications and variations are encompassed within the spirit and scope of the invention. Accordingly, the invention is limited only by the following claims.

Claims

That which is claimed is:

1. A polynucleotide sequence comprising a nucleic acid sequence encoding a chimeric fusion protein comprising: at least one targeting moiety to target a cancer cell and at least one immunomodulating moiety that counteracts immune tolerance, wherein the targeting moiety and the immunomodulating moiety are linked by an amino acid spacer of sufficient length of amino acid residues so that both moieties can successfully bind to their individual target.

2. The polynucleotide sequence of claim 1, wherein the immunomodulatory moiety specifically binds one of the following molecules: (i) Transforming growth factor-beta (TGF-β), (ii) Programmed death-1 (PD-1) ligand (iii) 4-1BB, (iv) insulin-like growth factor 1 (IGF-1) (v) or Interleukin- 6 (IL-6).

3. The polynucleotide sequence of claim I, wherein the targeting moiety is an antibody or a fragment thereof that binds epidermal growth factor receptor (EGFR1, Erb-B 1), HER2/neu (Erb-B2), CD20, CTLA-4, Mucin 1 (MUC-1), IGF-1, Epithelial cell adhesion molecule (EpCAM), Interleukin-2 (IL-2), Interleukin- 6 (IL-6) receptor, PD-L1 and 41BB-L1 or parts thereof.

4. The polynucleotide sequence of claim 1, wherein the nucleic acid sequence encoding at least one targeting moiety is selected from the group consisting of SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 25 and SEQ ID NO: 26.

5. The polynucleotide sequence of claim 1, wherein the nucleic acid sequence encoding at least one immunomodulatory moiety is selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 27 and SEQ ID NO: 28.

6. The polynucleotide sequence of claim 1, wherein the targeting moiety comprises a heavy and light chain of an antibody that binds a member selected from the group consisting of EGFR1, HER2/neu, CD20, CTLA-4, Interleukin- 6 (IL-6), Mucin 1(MUC-1), insulin-like growth factor I (IGF-1), Epithelial cell adhesion molecule (EpCAM) and Interleukin- 2 (IL-2).

7. The polynucleotide sequence of claim 6, wherein the immunomodulating moiety is fused to the light and/or heavy chain and is a member selected from TGF ECD, Programmed cell death-1 (PD-1) receptor ECD, CTLA-4 receptor ECD, 4-1BB ligand.

8. The polynucleotide sequence of claim 1, wherein the immunomodulatory moiety includes an extracellular ligand-binding domain of transforming growth factor-beta receptor TGF-βRII, TGF-βRIIb. or TGF-βRIII.

9. An optimized gene 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 gene has been modified to increase expression in a human subject and selected from SEQ ID NOs: 12 to 28.

10. A vector comprising optimized genes according to claim 9, wherein the optimized genes have been modified to increase CG sequences.

11. A method of treating cancer in a subject, the method comprising:
providing at least one recombinant vector comprising nucleotide sequences that encode at least one targeting moiety and at least one immunomodulatory moiety selected from SEQ ID NOs: 12 to 28; and
administering the recombinant vector to the subject under conditions such that said nucleotide sequences are expressed at a level which produces a therapeutically effective amount of the encoded fusion proteins in the subject.

12. 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.

13. A recombinant host cell transfected with the expression vector of claim 12.

14. A process of preparing a fusion protein of the present invention comprising:
transfecting a host cell with polynucleotide sequences that encode chimeric fusion proteins to produce a transformed host cell, wherein the polynucleotide sequences encode at least one

targeting moiety and at least one immunomodulatory moiety selected from SEQ ID NOs: 12 to 28; and

maintaining the transformed host cell under biological conditions sufficient for expression of the peptide.

15. A chimeric fusion protein comprising at least one targeting moiety to target a cancer cell and at least one immunomodulating moiety that counteracts immune tolerance, wherein the targeting moiety and the immunomodulating moiety are linked by an amino acid spacer of sufficient length of amino acid residues so that both moieties can successfully bind to their individual target, wherein the immunomodulating moiety is selected from SEQ ID NO: 4, SEQ ID NO: 9 and SEQ ID NO: 10; wherein the amino acid spacer is selected from SEQ ID NO: 3 and SEQ ID NO: 11; and wherein one targeting moiety is an antibody selected from Anti-HER2/Neu (heavy chain SEQ ID NO: 1 and light chain SEQ ID NO: 2); Anti-EGFR1 (heavy chain SEQ ID NO: 5 and light chain SEQ ID NO: 6); anti-CTLA4 (heavy chain SEQ ID NO: 7 and light chain SEQ ID NO: 82); Cantuzumab (heavy chain SEQ ID NO: 29 and light chain SEQ ID NO: 30); Cixutumumab (heavy chain SEQ ID NO: 31 and light chain SEQ ID NO: 32); Clivatuzumab (heavy chain SEQ ID NO: 33 and light chain SEQ ID NO: 34); and Pritumumab (heavy chain SEQ ID NO: 35 and light chain SEQ ID NO: 36).

16. A method of treating a neoplastic disease, the method comprising the administration to a subject in need thereof a chimeric fusion protein according to claim 15.

17. A method of preparing therapeutically active antibody-peptide fusion proteins comprising

a) preparing a codon optimized sequence of the said fusion protein;

b) cloning the optimized sequence of said fusion protein a host cell capable of transient or continued expression;

c) growing the host cell in a media sufficiently and allowing it to express the cloned protein; and

d) subjecting the expressed protein to purification and optionally checking the bi-specific binding capabilities of the protein to its targets.

18. The method of claim 17, wherein the therapeutically active antibody-peptide fusion proteins is a targeting antibody fused to one or more immunomodulating moiety that counteracts immune tolerance of a cancer cell.

19. The method of claim 18, wherein the immunomodulating moiety are linked by an amino acid spacer of sufficient length to allow bi-specific binding of the molecule.

20. The method of claim 19, wherein the immunomodulating moiety is either bound to the C-terminus of the heavy or light chain of the antibody.

21. The method of claim 20, wherein the immunomodulating moiety is extracellular domain (ECD) of (i) Transforming growth factor-beta (TGF-β), (ii) Programmed death-1 (PD-1) ligand (iii) 4-1BB, (iv) insulin-like growth factor 1 (IGF-1) (v) or Interleukin- 6 (IL-6).

22. A method in claim 21, wherein the antibody binds epidermal growth factor receptor (EGFR1, Erb-B l), HER2/neu (Erb-B2), CD20, CD6, CTLA-4, Mucin 1(MUC-1), Interleukin-2 (IL-2) or Interleukin- 6 (IL-6).

23. The method of claim 17, wherein the host cell is a transient cell line or a stable cell line

24. The method of claim 23, wherein transient expression is done by transfecting or transforming the host with vectors carrying the fusion proteins into mammalian host cells

25. The method of claim 24, wherein the transiently expressed fusion peptides are subjected to purification and in-vitro tests to check its bi-specificity

26. The method of claim 25, wherein the in-vitro test are ELISA or NK/T-cell binding assays to validate bi-functional target binding or immune cell stimulation.

27. The method of claim 25, wherein the peptides demonstrating desired bi-specificity are selected for sub-cloning into a stable cell line for larger scale expression and purification.

28. The method of claim 27, wherein the expression levels in stable cell line are comparable to the earlier generations.

29. The method of claim 17, wherein the host is a mammalian cell line.

30. The method of claim 29, wherein the mammalian cell is HEK293, CHO or NSO.

31. The method of claim 30, wherein the media is a chemically defined media.

32. The method of claim 32, wherein divalent transitional metallic salt 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.

Anti-HER2/neu-TGFβRII fusion protein at LC constant region

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAP
GKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQM
NSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG
ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN
HKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN
AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA
LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

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

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPG
KAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFA
TYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNN**
GAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEV
CVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKE
KKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 1

Anti-EGFR1-TGFβRII fusion protein at LC constant region

Amino acid sequence of Anti-EGFR1 heavy chain:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSP
GKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKM
NSLQSNDTAIYYCARALTYYDYEFAYWGQGTLVTVSAASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

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

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGS
PRLLIKYASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYY
CQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGA**
VKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCV
AVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKK
KPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 2

Anti-CTLA4-TGFβRII fusion protein at LC constant region

Amino acid sequence of anti-CTLA4 heavy chain:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQA
PGKGLEWVTFISYDGNNKYYADSVKGRFTISRDNSKNTLYLQ
MNSLRAEDTAIYYCARTGWLGPFDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPK
DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFY
PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS
RWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

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

EIVLTQSPGTLSLSPGERATLSCRASQSVGSSYLAWYQQKP
GQAPRLLIYGAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDF
AVYYCQQYGSSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQL
KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ
DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDN**
NGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQE
VCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMK
EKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 3

Anti-HER2/neu HC-4-1BB and LC-TGFβRII fusion protein:

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEW
VARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY
CSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG*GGGGSGGGGSGGGGSACPWAVSG*
ARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLL
IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLEL
RRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNS
AFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFR
VTPEIPAGLPSPRSE

Amino acid sequence of light chain-TGFβRII fusion protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLI
YSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP
TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC*GGGGSGGGGSGGGGS***TIPPHVQKS**
VNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEK
KKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 4

Anti-EGFR1 HC-4-1BB and LC-TGFβRII fusion protein:

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

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLE
WLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYY
CARALTYYDYEFAYWGQGTLVTVSAASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG*GGGGSGGGGSGGGGSACPWAVSG*
ARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLL
IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLEL
RRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNS
AFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFR
VTPEIPAGLPSPRSE

Amino acid sequence of light chain-TGFβRII fusion protein:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKY
ASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFG
AGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC*GGGGSGGGGSGGGGS***TIPPHVQKSVN**
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKP
QEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKK
KPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 5

Anti-CTLA4 HC-4-1BB and LC-TGFβRII fusion protein:

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

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLE
WVTFISYDGNNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAIY
YCARTGWLGPFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPG*GGGGSGGGGSGGGGS**ACPWAVSGA*
RASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLI
DGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELR
RVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNSA
FGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRV
TPEIPAGLPSPRSE

Amino acid sequence of light chain-TGFβRII fusion protein:

EIVLTQSPGTLSLSPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLL
IYGAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP
WTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC*GGGGSGGGGSGGGGS***TIPPHVQ**
KSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSI
CEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMK
EKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 6

Anti-HER2/neu HC-PD1 and LC-TGFβRII fusion protein:

Amino acid sequence of heavy chain-PD1 fusion protein:

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEW
VARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY
CSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG*GGGGSGGGGSGGGGSPGWFLDSP*
DRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSN
QTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRND
SGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPA
GQFQTLV

Amino acid sequence of light chain-TGFβRII fusion protein:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLI
YSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP
TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC*GGGGSGGGGSGGGGS***TIPPHVQKS**
VNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICE
KPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEK
KKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 7

Anti-EGFR1 HC-PD1 and LC-TGFβRII fusion protein:

Amino acid sequence of heavy chain-PD1 fusion protein:

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLE
WLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYY
CARALTYYDYEFAYWGQGTLVTVSAASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPG*GGGGSGGGGSGGGGSPGWFLDSP*
DRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSN
QTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRND
SGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPA
GQFQTLV

Amino acid sequence of light chain-TGFβRII fusion protein:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKY
ASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFG
AGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC*GGGGSGGGGSGGGGS***TIPPHVQKSVN**
NDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEKP
QEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKK
KPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 8

Anti-CTLA4 HC-PD1 and LC-TGFβRII fusion protein:

Amino acid sequence of heavy chain-PD1 fusion protein:

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLE
WVTFISYDGNNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAIY
YCARTGWLGPFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPG*GGGGSGGGGSGGGGSPGWFLDSPD*
RPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQ
TDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDS
GTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAG
QFQTLV

Amino acid sequence of light chain-TGFβRII fusionprotein:

EIVLTQSPGTLSLSPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLL
IYGAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP
WTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC*GGGGSGGGGSGGGGS***TIPPHVQ**
KSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSI
CEKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMK
EKKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 9

Anti-HER2/neu HC-TGFβRII-4-1BB fusion protein

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEW
VARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY
CSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPPHVQK**
SVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC
EKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKE
KKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD*EPKSCDKACPWAV*
SGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNV
LLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQL
ELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEAR
NSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGL
FRVTPEIPAGLPSPRSE

Amino acid sequence of light chain:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLI
YSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP
TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

Figure 10

Anti-EGFR1 HC-TGFβRII-4-1BB fusion protein

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

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLE
WLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYY
CARALTYYDYEFAYWGQGTLVTVSAASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPPHVQK**
SVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC
EKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKE
KKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD*EPKSCDKACPWAV*
SGARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNV
LLIDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQL
ELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEAR
NSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGL
FRVTPEIPAGLPSPRSE

Amino acid sequence of light chain:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKY
ASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFG
AGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

Figure 11

Anti-CTLA4 HC-TGFβRII-4-1BB fusion protein

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

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLE
WVTFISYDGNNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAIY
YCARTGWLGPFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPPHVQKSV**
NNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEK
PQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKK
KPGETFFMCSCSSDECNDNIIFSEEYNTSNPD*EPKSCDKACPWAVSG*
ARASPGSAASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLL
IDGPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLEL
RRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARNS
AFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFR
VTPEIPAGLPSPRSE

Amino acid sequence of light chain:

EIVLTQSPGTLSLSPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLL
IYGAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP
WTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC

Figure 12

Anti-HER2/neu HC-TGFβRII-PD1 fusion protein

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

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEW
VARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY
CSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVV
TVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPPHVQK**
SVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC
EKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKE
KKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD*EPKSCDKPGWFLD*
SPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSP
SNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARR
NDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPR
PAGQFQTLV

Amino acid sequence of light chain:

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLI
YSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPP
TFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

Figure 13

Anti-EGFR1 HC-TGFβRII-PD1 fusion protein:

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

QVQLKQSGPGLVQPSQSLSITCTVSGFSLTNYGVHWVRQSPGKGLE
WLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDTAIYY
CARALTYYDYEFAYWGQGTLVTVSAASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP
APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPPHVQK**
SVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSIC
EKPQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKE
KKKPGETFFMCSCSSDECNDNIIFSEEYNTSNPD*EPKSCDKPGWFLD*
SPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSP
SNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARR
NDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPR
PAGQFQTLV

Amino acid sequence of light chain:

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKY
ASESISGIPSRFSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFG
AGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

Figure 14

Anti-CTLA4 HC-TGFβRII-PD1 fusion protein

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

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYTMHWVRQAPGKGLE
WVTFISYDGNNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAIY
YCARTGWLGPFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTA
ALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV
HNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPI
EKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS
VMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPPHVQKSV**
NNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKSCMSNCSITSICEK
PQEVCVAVWRKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKK
KPGETFFMCSCSSDECNDNIIFSEEYNTSNPD*EPKSCDKPGWFLDSP*
DRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSN
QTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRND
SGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPA
GQFQTLV

Amino acid sequence of light chain:

EIVLTQSPGTLSLSPGERATLSCRASQSVGSSYLAWYQQKPGQAPRLL
IYGAFSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP
WTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE
AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC

Figure 15

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

1 gctagcacca agggcccctc cgtgttccct ctggcccct ccagcaagtc cacctctggc
61 ggcaccgccg ctctgggctg cctggtcaag gactacttcc ccgagcccgt gaccgtgtcc
121 tggaactctg gcgctctgac ctccggcgtg cacaccttcc ctgccgtgct gcagtcctcc
181 ggcctgtact ccctgtcctc cgtcgtgacc gtgccctcca gctctctggg cacccagacc
241 tacatctgca acgtgaacca caagccctcc aacaccaagg tggacaagaa ggtggaaccc
301 aagtcctgcg acaagaccca cacctgtcccccctgccctg cccctgagct cctgggaggc
361 cctagcgtgt tcctgttccc cccaaagccc aaggacaccc tgatgatctc ccggaccccc
421 gaagtgacct gcgtggtggt ggacgtgtcc cacgaggacc ctgaagtgaa gttcaattgg
481 tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ccagagagga acagtacaac
541 tccacctacc gggtggtgtc cgtgctgacc gtgctgcacc aggactggct gaacggcaaa
601 gagtacaagt gcaaggtgtc caacaaggcc ctgcctgccc ccatcgaaaa gaccatctcc
661 aaggccaagg gccagccccg cgagcctcag gtgtacaccc tgcccctag ccgggaagag
721 atgaccaaga accaggtgtc cctgacctgt ctggtcaagg gcttctaccc ctccgatatc
781 gccgtggaat gggagtccaa cggccagccc gagaacaact acaagaccac ccccctgtg
841 ctggactccg acggctcatt cttcctgtac tccaagctga ccgtggacaa gtcccggtgg
901 cagcagggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc
961 cagaagtccc tgtccctgag cccaggcaaa ggcggaggcg gatctggcgg cggaggatct
....1021 ggtggcg gatcc

Nucleotide sequence of TGFβRII ECD:

1 ggatccacca tccccccaca cgtgcagaaa tccgtgaaca acgacatgat cgtgaccgac
61 aacaacggcg ctgtgaagtt cccccagctg tgcaagttct gcgacgtgcg gttctctacc
121 tgcgacaacc agaaatcctg catgtccaac tgctccatca cctccatctg cgagaagccc
181 caggaagtgt gcgtcgccgt ctggcggaag aacgacgaga acatcaccct ggaaaccgtg
241 tgccacgacc ccaagctgcc ctaccacgac ttcatcctgg aagatgccgc ctcccccaag
301 tgcatcatga aggaaaagaa gaagcccggc gagactttct tcatgtgcag ctgctcctcc
361 gacgagtgca acgacaacat catcttctcc gaagagtaca acacctccaa ccccgactga
421 agctt

Figure 16

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

```
   1 gcggccgcca tgaacttcgg cctgcggctg atcttcctgg tgctgaccct gaagggcgtg
  61 cagtgcgagg tgcagctggt ggaatccggc ggaggcctgg tccagcctgg cggatctctg
 121 agactgtcct gcgccgcctc cggcttcaac atcaaggaca cctacatcca ctgggtccga
 181 caggcccctg gcaagggcct ggaatgggtg gcccggatct accccaccaa cggctacacc
 241 agatacgccg actccgtgaa gggccggttc accatctccg ccgacacctc caagaacacc
 301 gcctacctgc agatgaactc cctgcgggcc gaggacaccg ccgtgtacta ctgctccaga
 361 tggggaggcg acggcttcta cgccatggac tactggggcc agggcaccct ggtcaccgtg
 421   ctccgcta gc
```

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

```
   1 gcggccgcca tggaatccca gacccaggtg ctgatctccc tgctgttctg ggtgtccggc
  61 acctgtggcg acatccagat gacccagtcc ccctccagcc tgtccgcctc tgtgggcgac
 121 agagtgacca tcacctgtcg ggcctcccag gacgtgaaca ccgccgtggc ctggtatcag
 181 cagaagcccg gcaaggcccc caagctgctg atctactccg cctccttcct gtactccggc
 241 gtgccctccc ggttctccgg ctctagatcc ggcaccgact ttaccctgac catctccagc
 301 ctgcagcccg aggacttcgc cacctactac tgccagcagc actacaccac ccccccccacc
 361 tttggccagg gcaccaaggt ggaaatcaag cggaccgtgg ccgctccctc cgtgttcatc
 421   cccaccct ccgacgagca gctg
```

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

```
   1 gctagcacca agggcccctc cgtgtttccc ctggcccccc ccagcaagtc cacctctggc
  61 ggcaccgccg ctctgggctg cctggtcaag gactacttcc ccgagcccgt gaccgtgtcc
 121 tggaactctg gcgctctgac ctccggcgtg cacaccttcc ctgccgtgct gcagtcctcc
 181 ggcctgtact ccctgtcctc cgtcgtgacc gtgccctcca gctctctggg cacccagacc
 241 tacatctgca acgtgaacca caagccctcc aacaccaagg tggacaagcg ggtggaaccc
 301 aagtcctgcg acaagaccca cacctgtccc ccctgccctg cccctgaact gctgggaggc
 361 ccttccgtgt tcctgttccc cccaaagccc aaggacaccc tgatgatctc ccggacccccc
 421 gaagtgacct gcgtggtggt ggacgtgtcc cacgaggacc ctgaagtgaa gttcaattgg
 481 tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ccagagagga acagtacaac
 541 tccacctacc gggtggtgtc cgtgctgacc gtgctgcacc aggactggct gaacggcaaa
 601 gagtacaagt gcaaggtgtc caacaaggcc ctgcctgccc ccatcgaaaa gaccatctcc
 661 aaggccaagg gccagccccg cgagcctcag gtgtacaccc tgcctcccag ccgggacgag
 721 ctgaccaaga accaggtgtc cctgacctgt ctggtcaagg gcttctaccc ctccgatatc
 781 gccgtggaat gggagtccaa cggccagccc gagaacaact acaagaccac ccccctgtg
 841 ctggactccg acggctcatt cttcctgtac tccaagctga ccgtggacaa gtcccggtgg
 901 cagcagggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc
 961 cagaagtccc tgtctctgag ccccggcaaa ggcggcggag gatctggcgg tggcggatca
1021   ggcggag gatcc
```

Figure 17

Nucleotide sequence of Anti-EGFR1 heavy chain variable region

```
  1 gcggccgcca tgaacttcgg cctgcggctg atcttcctgg tgctgaccct gaagggcgtg
 61 cagtgccagg tgcagctgaa gcagtccgga cctggcctgg tgcagccttc ccagtccctg
121 tccatcacct gtaccgtgtc cggcttctcc ctgaccaact acggcgtgca ctgggtccga
181 cagtccccag gcaagggcct ggaatggctg ggagtgattt ggagcggcgg caacaccgac
241 tacaacaccc ccttcacctc ccggctgtcc atcaacaagg acaactccaa gtcccaggtg
301 ttcttcaaga tgaactccct gcagtccaac gacaccgcca tctactactg cgccagagcc
361 ctgacctact atgactacga gttcgcctac tggggacagg gcaccctggt caccgtgtct
421   cgctagc
```

Nucleotide sequence of Anti-EGFR1light chain variable region

```
1 gcggccgcca tggaatccca gacccaggtg ctgatctccc tgctgttctg ggtgtccggc
 61 acctgtggcg acatcctgct gacccagtcc cccgtgatcc tgtccgtgtc tcctggcgag
121 cgggtgtcct tctcctgccg ggcctcccag tccatcggca ccaacatcca ctggtatcag
181 cagcggacca acggctcccc tcggctgctg attaagtacg cctccgagtc tatctccggc
241 atcccctccc ggttctccgg ctctggctcc ggcaccgact tcaccctgtc catcaactcc
301 gtggaatccg aggatatcgc cgactactac tgccagcaga acaacaactg gcccaccacc
361 ttcggcgctg gcaccaagct ggaactgaag cggaccgtgg ccgctccctc cgtgttcatc
421   cccaccct ccgacgagca gctg
```

Nucleotide sequence of Anti-CTLA4 heavy chain variable region

```
  1 gcggccgcca tgaacttcgg cctgcggctg atcttcctgg tgctgaccct gaagggcgtg
 61 cagtgccagg tgcagctggt ggaatccggc ggaggcgtgg tgcagcctgg cagatccctg
121 agactgtcct gcgccgcctc cggcttcacc ttctccagct acaccatgca ctgggtccga
181 caggcccctg gcaagggcct ggaatgggtc accttcatca gctacgacgg caacaacaag
241 tactacgccg actccgtgaa gggccggttc accatctccc gggacaactc caagaacacc
301 ctgtacctgc agatgaactc cctgcgggcc gaggacaccg ccatctacta ctgcgcccgg
361 accggctggc tgggcccttt tgattactgg ggccagggca ccctggtcac cgtgtcctcc
421   tagc
```

Nucleotide sequence of Anti-CTLA4light chain variable region

```
  1 gcggccgcca tggaatccca gacccaggtg ctgatctccc tgctgttctg ggtgtccggc
 61 acctgtggcg agatcgtgct gacccagtcc cccggcaccc tgtctctgag ccctggcgag
121 agagccaccc tgtcctgcag agcctcccag tccgtgggct cctcctacct ggcttggtat
181 cagcagaagc ccggccaggc ccctcggctg ctgatctacg gcgctttctc tcgggccacc
241 ggcatccctg accggttctc tggctccggc tccggcaccg acttcaccct gaccatctcc
301 cggctggaac ccgaggactt cgccgtgtac 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 tgtgtttcct ctggctccct cttccaaatc cacttctggt
  61 ggcactgctg ctctgggatg cctggtgaag gattactttc ctgaacctgt gactgtctca
 121 tggaactctg gtgctctgac ttctggtgtc cacactttcc ctgctgtgct gcagtctagt
 181 ggactgtact ctctgtcatc tgtggtcact gtgccctctt catctctggg aacccagacc
 241 tacatttgta atgtgaacca caaaccatcc aacactaaag tggacaaaaa agccgaaccc
 301 aaatcctgtg acaaaaccca cacctgccca ccttgtcctg cccctgaact gctgggagga
 361 ccttctgtgt ttctgttccc accaaaacca aaagataccc tgatgatctc tagaacccct
 421 gaggtgacat gtgtggtggt ggatgtgtct catgaggacc ctgaggtcaa atttaattgg
 481 tacgtcgatg gagtggaagt ccacaatgcc aaaaccaagc ctagagagga acagtacaat
 541 tcaacctaca gagtcgtcag tgtgctgact gtgctgcatc aggattggct gaatggcaag
 601 gaatacaagt gtaaagtctc aaacaaggcc ctgcctgctc caattgagaa aacaatctca
 661 aaggccaagg gacagcctag ggaaccccag gtctacaccc tgccaccttc acgcgacgaa
 721 ctgaccaaaa accaggtgtc cctgacatgc ctggtcaaag gcttctaccc ttctgacatt
 781 gctgtggagt gggagtcaaa tggacagcct gagaacaact acaaaacaac ccccctgtg
 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 tgaattttgg actgaggctg attttcctgg tgctgaccct gaaaggcgtc
  61 cagtgtcagg tgcagctgca gcagcctggt gccgagctcg tgaaacctgg cgcctccgtg
 121 aagatgtcct gcaaggcctc cggctacacc ttcaccagct acaacatgca ctgggtcaag
 181 cagacccccg gcagaggcct ggaatggatc ggcgctatct accccggcaa cggcgacacc
 241 tcctacaacc agaagttcaa gggcaaggcc accctgaccg ccgacaagtc ctcttccacc
 301 gcctacatgc agctgtcctc cctgacctcc gaggactccg ccgtgtacta ctgcgcccgg
 361 tctacctact acggcggcga ctggtacttc aacgtgtggg gcgctggcac caccgtgacc
 421 gtgtctgctg ctagc
```

Nucleotide sequence of Anti-CD20 light chain variable region

```
   1 gcggccgcca tgaattttgg actgaggctg attttcctgg tgctgaccct gaaaggcgtc
  61 cagtgtcaga tcgtgctgtc ccagtcccct gccatcctgt ctgctagccc tggcgagaaa
 121 gtgacaatga cctgccgggc ctcctcctcc gtgtcctaca tccactggtt ccagcagaag
 181 cccggctcca gccccaagcc ttggatctac gccacctcca acctggcctc tggcgtgcca
 241 gtgcggtttt ccggctctgg ctctggcacc tcctactccc tgaccatctc tcgggtggaa
 301 gccgaggatg ccgccaccta ctactgccag cagtggacca gcaacccccc cacatttggc
 361 ggaggcacca agctggaaat caagcggacc gtggcggcgc cctct
```

Figure 19

Nucleotide sequence of 4-1BB.

```
   1  ggatccgcct gtccttgggc cgtgtccggc gctagagcct ctcctggctc tgccgcctcc
  61  cccagactga gagagggccc tgagctgtcc cctgacgatc ctgccggcct gctggacctg
 121  agacagggca tgtttgccca gctggtggcc cagaacgtgc tgctgatcga cggccccctg
 181  tcctggtact ctgatcctgg cctggccggc gtgtccctga ccggcggact gtcctacaaa
 241  gaggacacca aagaactggt ggtggccaag gctggcgtgt actacgtgtt ctttcagctg
 301  gaactgcggc gggtggtggc cggcgagggc tctggatctg tgtccctggc cctgcatctg
 361  cagcccctga gatctgccgc tggcgccgct gctctggccc tgacagtgga tctgcctcct
 421  gcctcctccg aggcccggaa ctccgcattc gggtttcagg gccggctgct gcacctgtct
 481  gctggccaga gactgggagt gcatctgcac 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 attttcctgg 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 cttcctctaa atctacctct
 481 ggcggcaccg ccgctctggg ctgcctcgtg aaggactact tccccgagcc cgtgacagtg
 541 tcttggaact ctggcgccct gacctccggc gtgcacacct ttccagctgt gctgcagtcc
 601 tccggcctgt actccctgtc cagcgtcgtg actgtgccct cctcatctct gggcacccag
 661 acctacatct gcaacgtgaa ccacaagccc tccaacacca aggtggacaa gaaggtggaa
 721 cccaagtcct gcgacaagac ccacacctgt ccccttgtc ctgcccctga actgctgggc
 781 ggaccctctg tgttcctgtt cccaccaaaa ccgaaagaca ccctgatgat ctcccggacc
 841 cccgaagtga cctgcgtggt ggtggatgtg tcccacgagg accctgaagt gaagttcaat
 901 tggtacgtgg acggcgtgga agtgcacaac gccaagacca agcctagaga ggaacagtac
 961 aactccacct accgggtggt gtccgtgctg accgtgctgc accaggattg gctgaacggc
1021 aaagagtaca agtgcaaggt gtccaacaag gccctgcctg cccccatcga aaagaccatc
1081 tccaaggcca agggccagcc acgggaaccc caggtgtaca cactgccccc tagccgcgac
1141 gagctgacca agaatcaggt gtccctgaca tgcctcgtga aaggcttcta cccctccgat
1201 atcgccgtgg aatgggagtc caacggccag cctgagaaca actacaagac cacccccct
1261 gtgctggact ccgacggctc attcttcctg tactcaaagc tgacagtgga caagtcccgg
1321 tggcagcagg gcaacgtgtt ctcctgctcc gtgatgcacg aggccctgca caaccactac
1381 acccagaagt ccctgtccct gagccccggg aaaggcggcg gaggatctgg cggaggcggt
1441 tctggtggtg gcggatcc
```

Figure 20

Nucleotide sequence of Anti-IL6R light chain variable region:

```
   1 gcggccgcca tgaattttgg actgaggctg attttcctgg tgctgaccct gaaaggcgtc
  61 cagtgtgaca tccagatgac ccagtccccc tccagcctgt ctgcctctgt gggcgacaga
 121 gtgaccatca cctgtcgggc ctcccaggac atctcctcct acctgaactg gtatcagcag
 181 aagcccggca aggcccccaa gctgctgatc tactacacct cccggctgca ctccggcgtg
 241 ccctctagat ttccggctc tggctccggc 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 tgaattttgg actgaggctg attttcctgg tgctgaccct gaaaggcgtc
  61 cagtgtcagg tgcagctgca gcagtgggga gctggactgc tgaagccctc cgagacactg
 121 tctctgacct gcgctgtgta cggcggctcc ttctccggct actactggtc ctggattcgg
 181 cagtcccctg agaagggcct ggaatggatc ggcgagatca accacggcgg ctacgtgacc
 241 tacaacccca gcctggaatc cagagtgacc atctccgtgg acacctccaa gaaccagttc
 301 tccctgaagc tgtcctccgt gaccgccgct gataccgccg tgtactactg cgccagagac
 361 tacggccctg gcaactacga ctggtacttc gacctgtggg gcagaggcac cctcgtgacc
 421 gtgtcctctg ctagcaccaa gggcccctcc gtgtttcctc tggccccttg ctcacgctcc
 481 acctccgaat ctaccgccgc tctgggctgc ctcgtgaagg actacttccc cgagcccgtg
 541 actgtgtctt ggaactctgg cgccctgacc tccggcgtgc acaccttcc agctgtgctg
 601 cagtcctccg gcctgtactc cctgtccagc gtcgtgacag tgccctccag ctctctgggc
 661 accaagacct acacctgtaa cgtggaccac aagccctcca acaccaaggt ggacaagcgg
 721 gtggaatcta aatacggccc tccctgccct ccttgcccag cccctgaatt tctgggcgga
 781 ccttccgtgt tcctgttccc cccaaaaccc aaggacaccc tgatgatctc ccggacccct
 841 gaagtgacct gcgtggtggt ggatgtgtcc caggaagatc ccgaggtgca gttcaattgg
 901 tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ctagagagga acagttcaac
 961 tccacctacc gggtggtgtc cgtgctgacc gtgctgcacc aggattggct gaacggcaaa
1021 gagtacaagt gcaaggtgtc caacaagggc ctgcccagct ccatcgaaaa gaccatcagc
1081 aaggccaagg gccagccccg ggaaccccag gtgtacacac tgcctccaag ccaggaagag
1141 atgaccaaga atcaggtgtc cctgacctgt ctcgtgaaag gcttctaccc ctccgatatc
1201 gccgtggaat gggagtccaa cggccagcct gagaacaact acaagaccac ccccctgtg
1261 ctggactccg acggcagctt cttcctgtac tctcgcctga ccgtggacaa gtcccggtgg
1321 caggaaggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc
1381 cagaagtccc tgtccctgtc tctggggaaa ggcggcggag gatctggcgg aggcggttct
1441 ggtggtggcg gatcc
```

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

```
   1 gcggccgcca tgaattttgg actgaggctg attttcctgg tgctgaccct gaaaggcgtc
  61 cagtgtgaga tcgtgctgac ccagtctcct gccaccctgt ctctgagccc tggcgagaga
 121 gctaccctgt cctgccgtgc ctcccaatcc gtgtcctctt acctggcctg gtatcagcaa
 181 aagcccggcc aggctccccg gctgctgatc tacgatgcct ccaatagagc caccggcatc
 241 cctgccagat tctccggctc tggctctggc accgacttta ccctgaccat ctcctctctg
 301 gaacccgagg acttcgccgt gtactactgc cagcagcggt ccaactggcc tcccgccctg
 361 acatttggcg gaggcaccaa ggtggaaatc aagcggaccg tggcggcgcc c
```

Figure 21

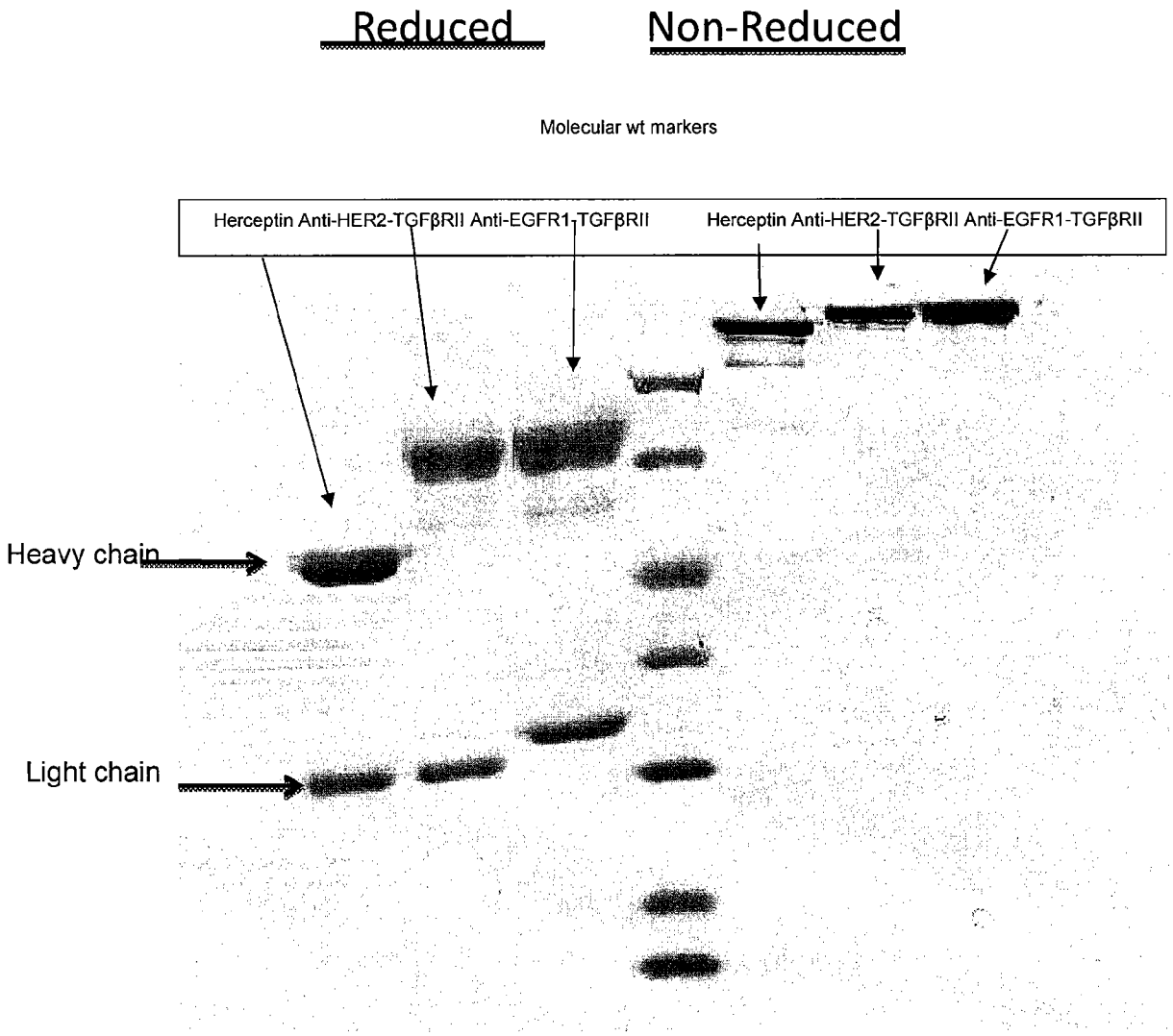

Reduced
Non-Reduced
Molecular wt markers
Herceptin
Anti-HER2-TGFβRII
Anti-EGFR1-TGFβRII
Herceptin
Anti-HER2-TGFβRII
Anti-EGFR1-TGFβRII
Heavy chain
Light chain

Figure 22

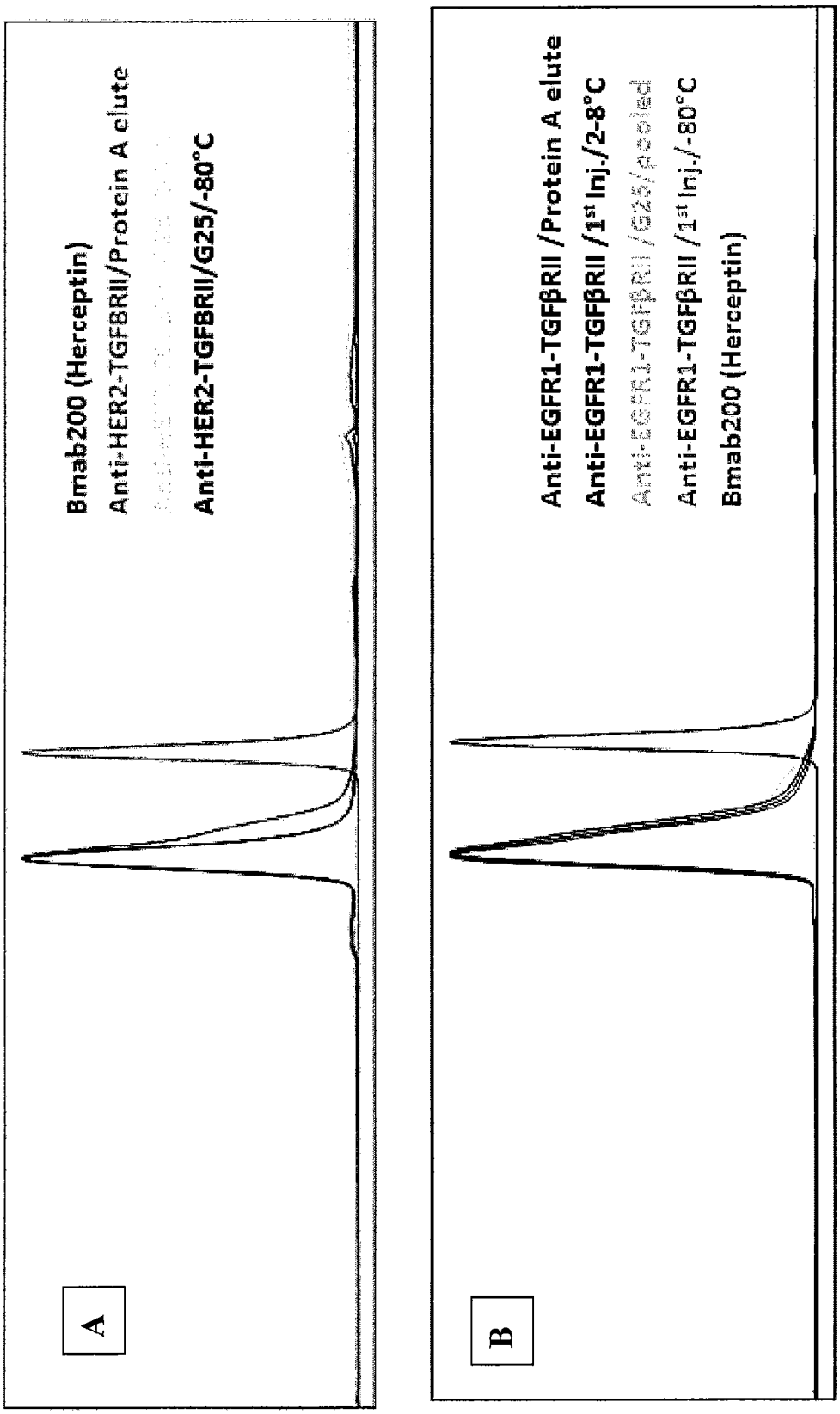
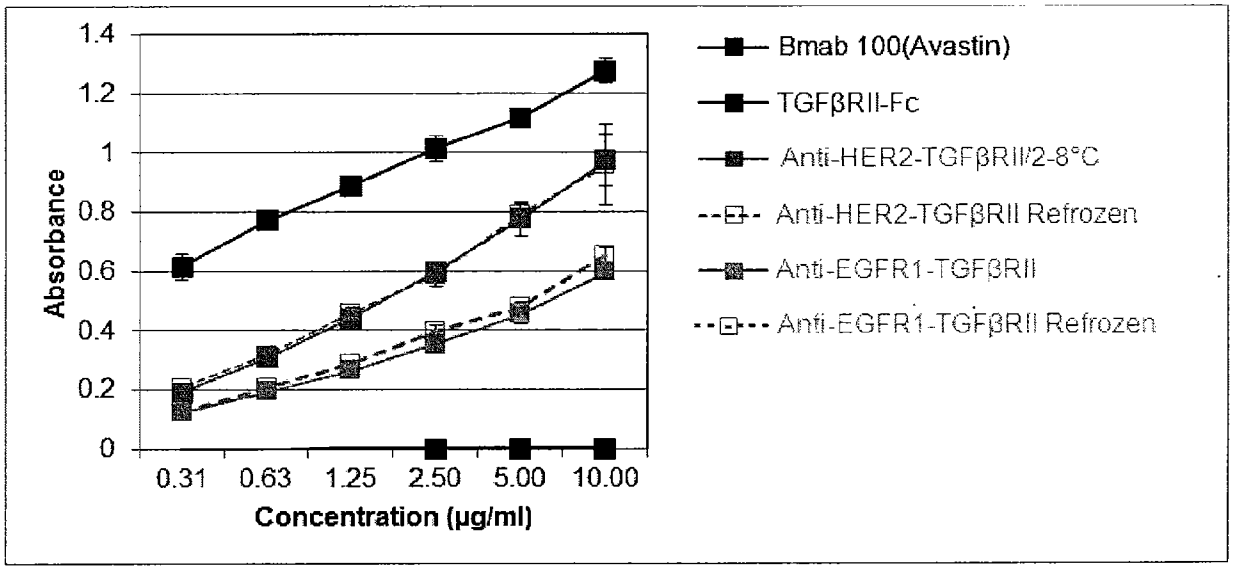

A
Bmab200 (Herceptin)
Anti-HER2-TGFBRII/Protein A elute
Anti-HER2-TGFBRII/G25/-80°C
B
Anti-EGFR1-TGFβRII /Protein A elute
Anti-EGFR1-TGFβRII /1st Inj./2-8°C
Anti-EGFR1-TGFβRII /G25/pooled
Anti-EGFR1-TGFβRII /1st Inj./-80°C
Bmab200 (Herceptin)

Figure 23

A


1.4
1.2
1
0.8
0.6
0.4
0.2
0
Absorbance
0.31
0.63
1.25
2.50
5.00
10.00
Concentration (µg/ml)
Bmab 100(Avastin)
TGFβRII-Fc
Anti-HER2-TGFβRII/2-8°C
Anti-HER2-TGFβRII Refrozen
Anti-EGFR1-TGFβRII
Anti-EGFR1-TGFβRII Refrozen

B

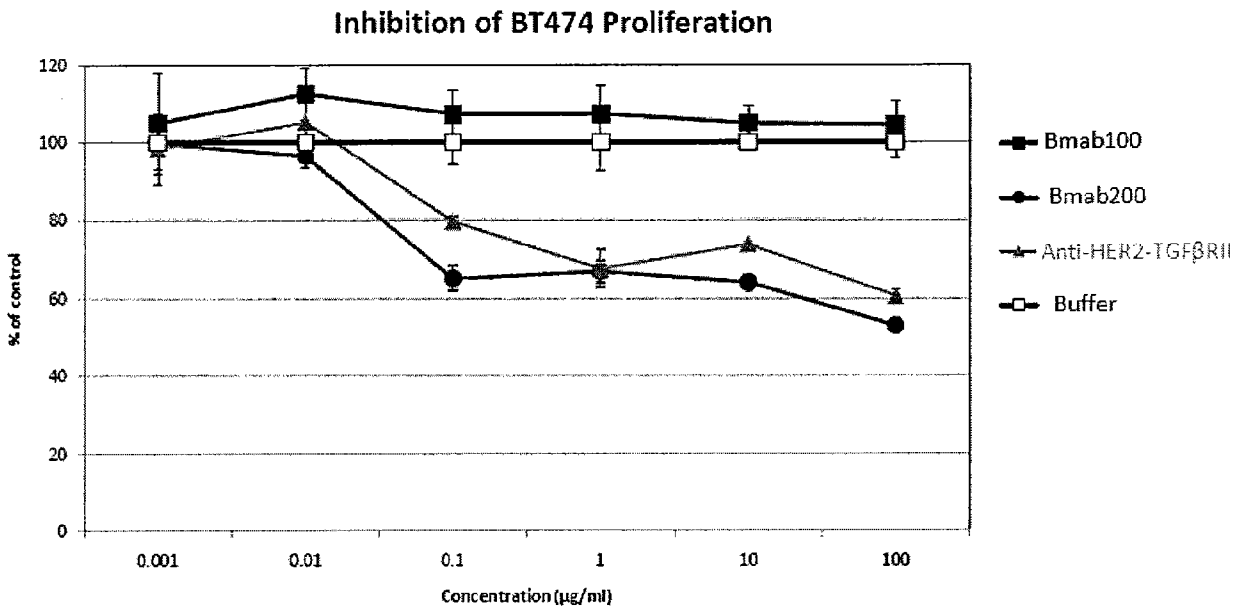

Inhibition of BT474 Proliferation
120
100
80
60
40
20
0
% of control
0.001
0.01
0.1
1
10
100
Concentration (µg/ml)
Bmab100
Bmab200
Anti-HER2-TGFβRII
Buffer

Figure 24

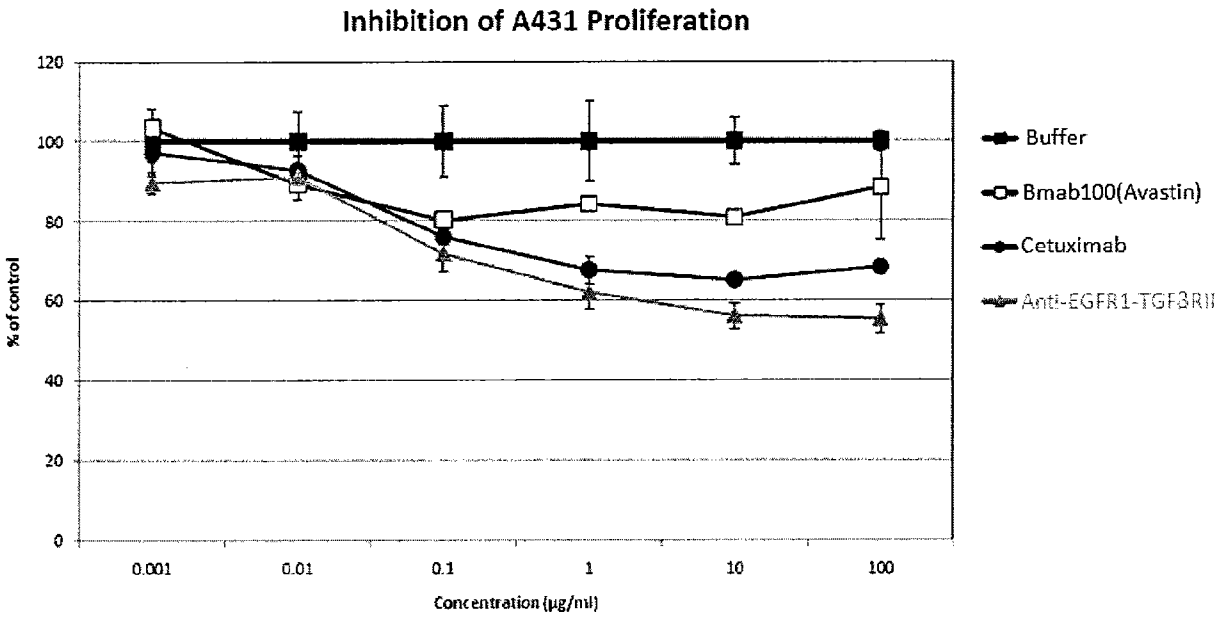

Inhibition of A431 Proliferation
120
100
80
60
40
20
0
% of control
0.001
0.01
0.1
1
10
100
Concentration (µg/ml)
Buffer
Bmab100(Avastin)
Cetuximab
Anti-EGFR1-TGF3RII

Figure 25

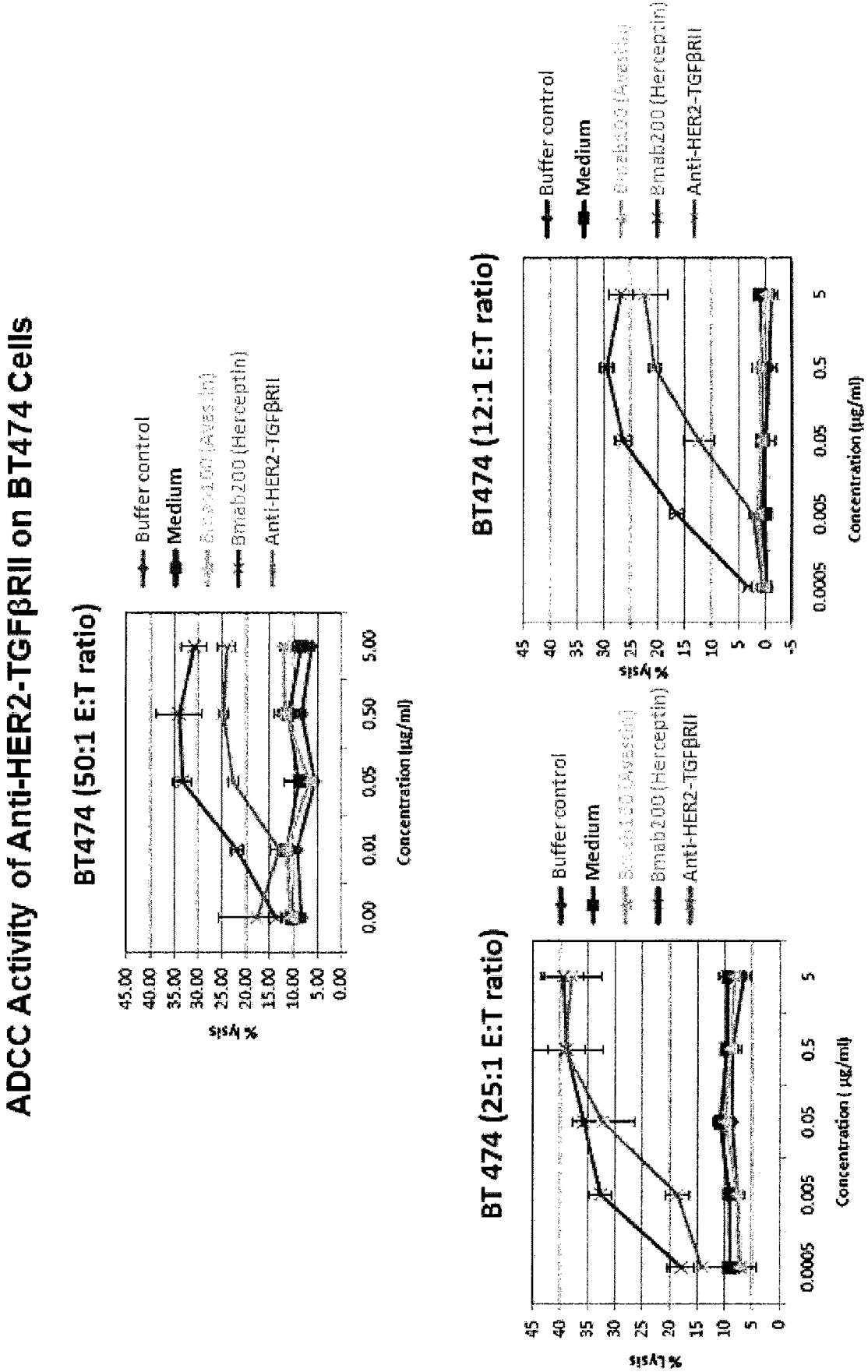

ADCC Activity of Anti-HER2-TGFβRII on BT474 Cells
BT474 (50:1 E:T ratio)
BT474 (12:1 E:T ratio)
BT 474 (25:1 E:T ratio)
Buffer control
Medium
Bmab200 (Herceptin)
Anti-HER2-TGFβRII
% lysis
Concentration (µg/ml)

Figure 26

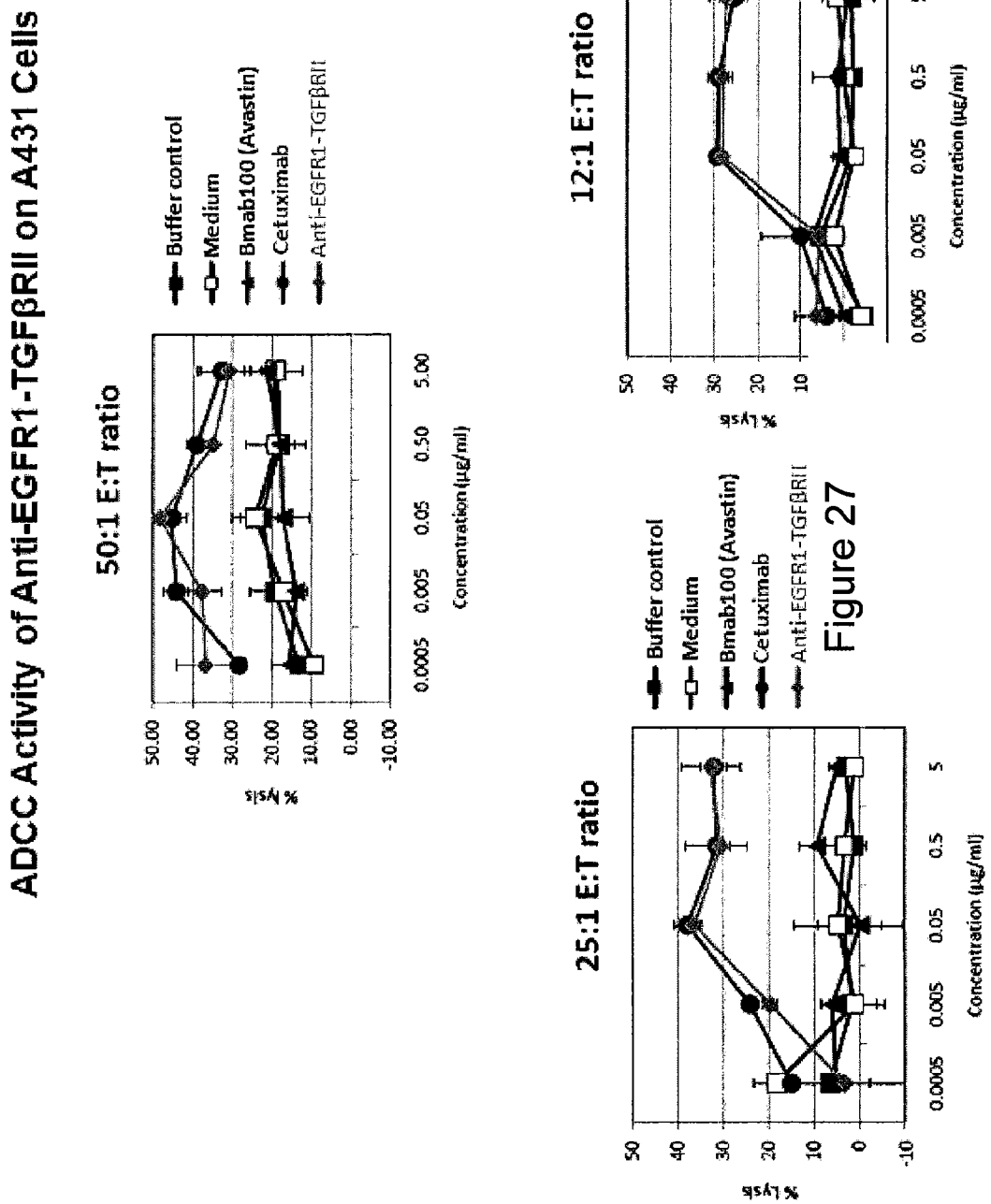


Figure 27

FIGURE 27

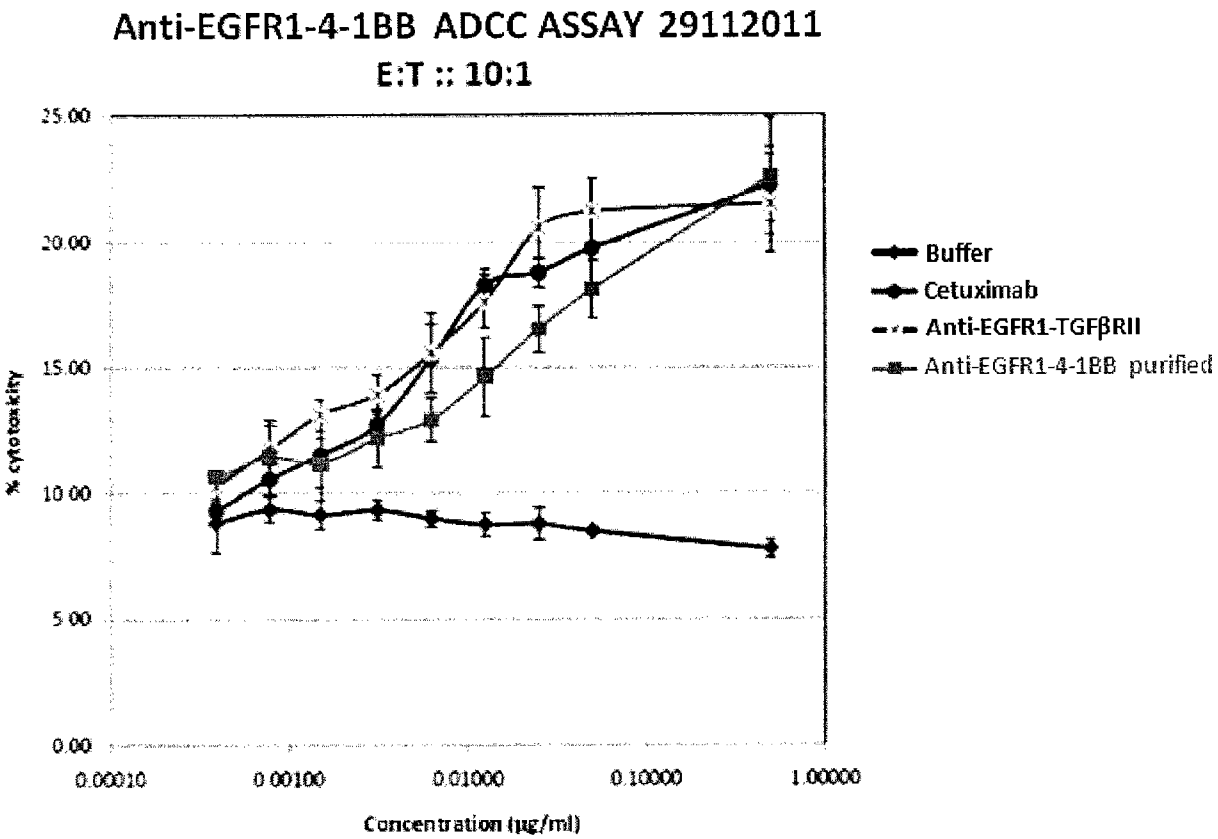
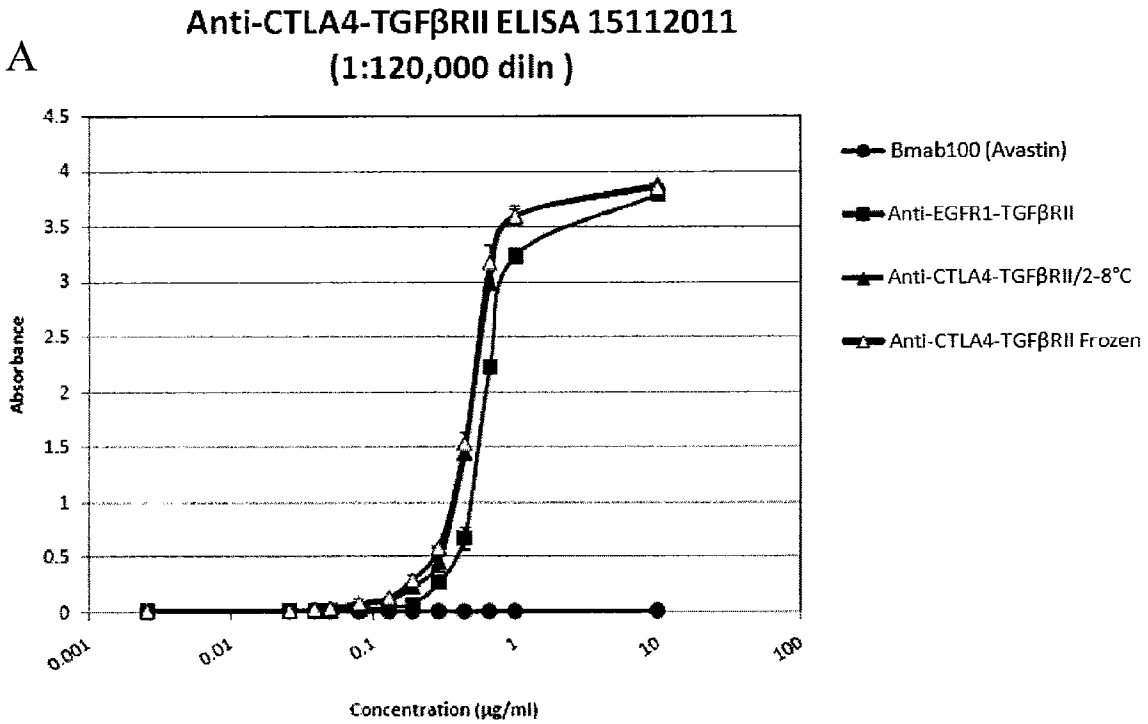

Anti-EGFR1-4-1BB ADCC ASSAY 29112011
E:T :: 10:1
25.00
20.00
15.00
10.00
5.00
0.00
% cytotoxicity
0.00010
0.00100
0.01000
0.10000
1.00000
Concentration (µg/ml)
Buffer
Cetuximab
Anti-EGFR1-TGFβRII
Anti-EGFR1-4-1BB purified

Figure 28

A


Anti-CTLA4-TGFβRII ELISA 15112011
(1:120,000 diln)
Absorbance
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.001
0.01
0.1
1
10
100
Concentration (µg/ml)
Bmab100 (Avastin)
Anti-EGFR1-TGFβRII
Anti-CTLA4-TGFβRII/2-8°C
Anti-CTLA4-TGFβRII Frozen

B

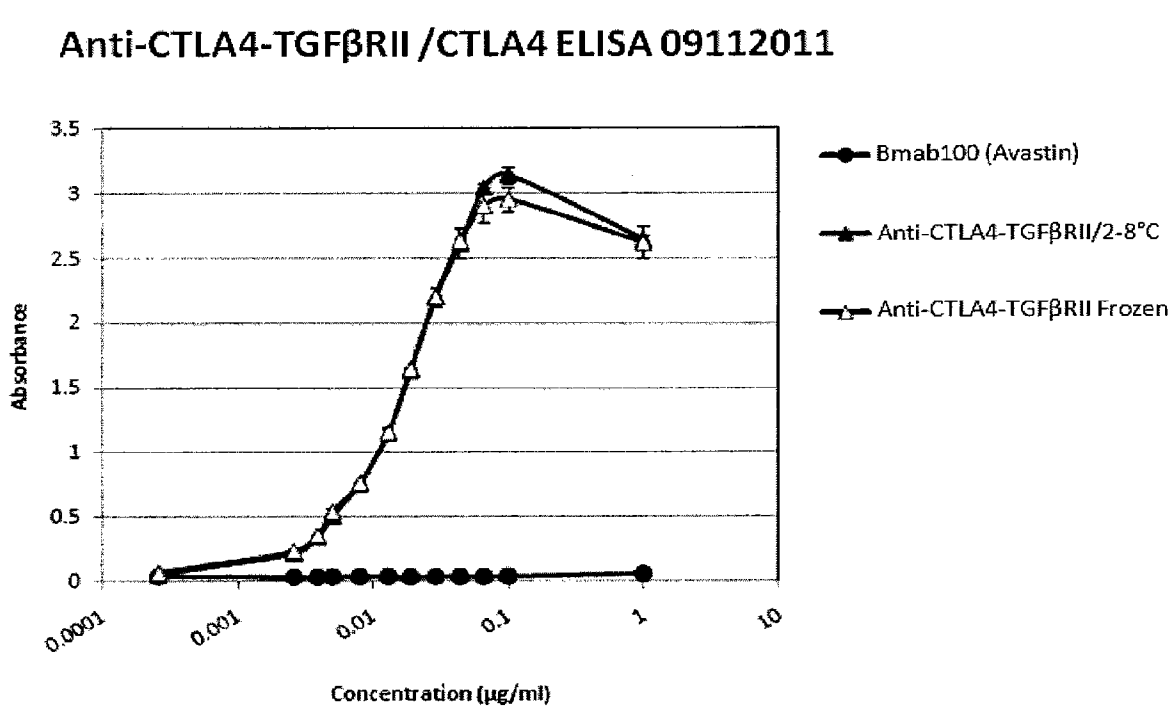
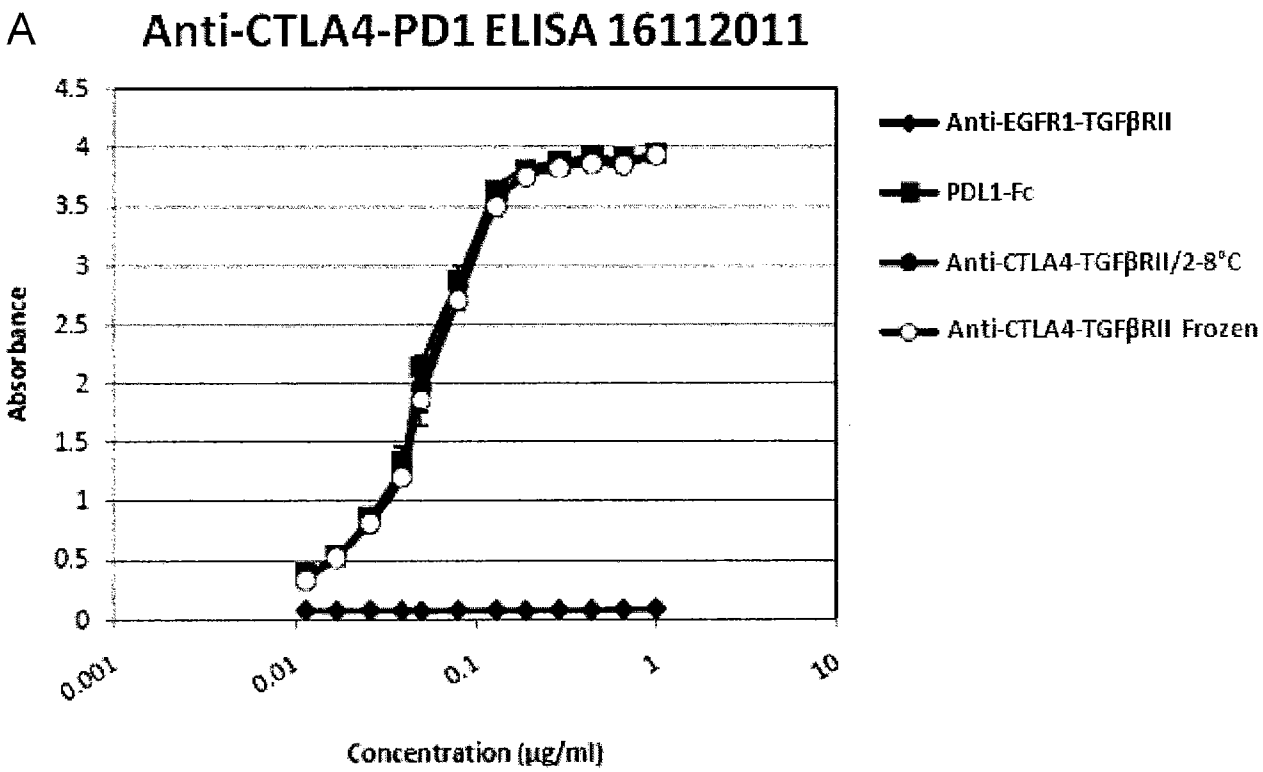

Anti-CTLA4-TGFβRII /CTLA4 ELISA 09112011
Absorbance
3.5
3
2.5
2
1.5
1
0.5
0
0.0001
0.001
0.01
0.1
1
10
Concentration (µg/ml)
Bmab100 (Avastin)
Anti-CTLA4-TGFβRII/2-8°C
Anti-CTLA4-TGFβRII Frozen

Figure 29

A


Anti-CTLA4-PD1 ELISA 16112011
Absorbance
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.001
0.01
0.1
1
10
Concentration (µg/ml)
Anti-EGFR1-TGFβRII
PDL1-Fc
Anti-CTLA4-TGFβRII/2-8°C
Anti-CTLA4-TGFβRII Frozen

B

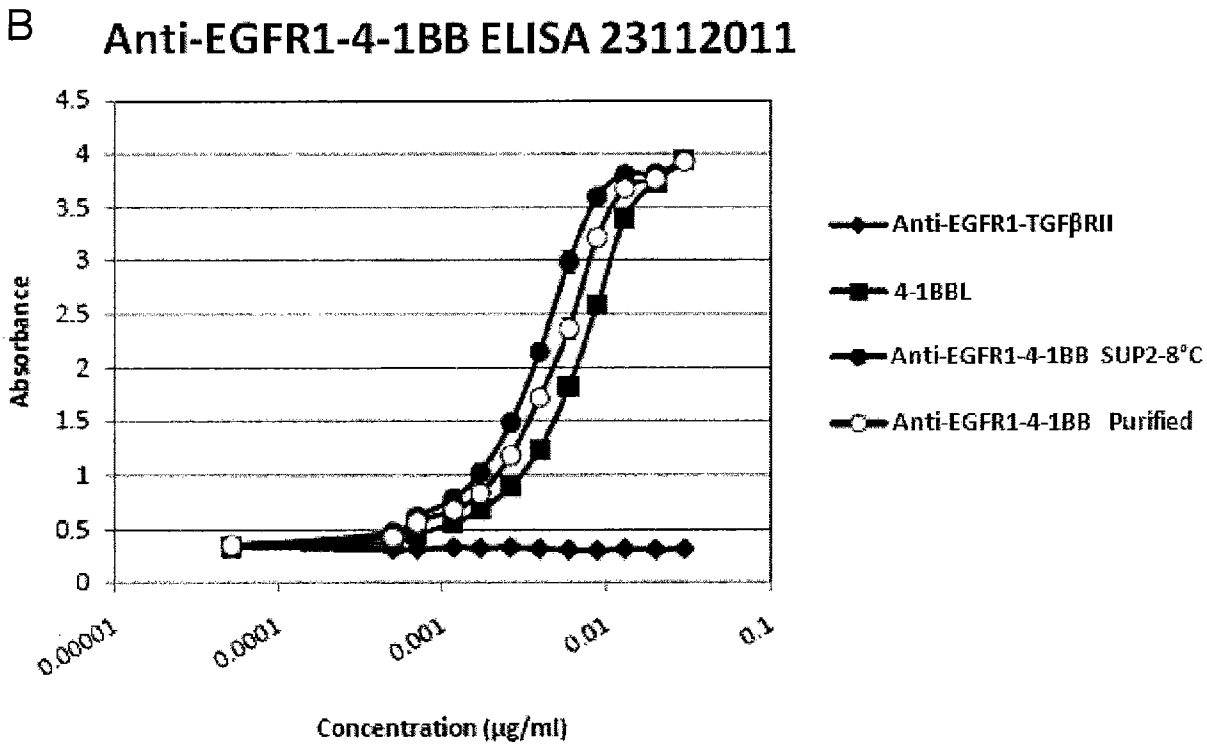
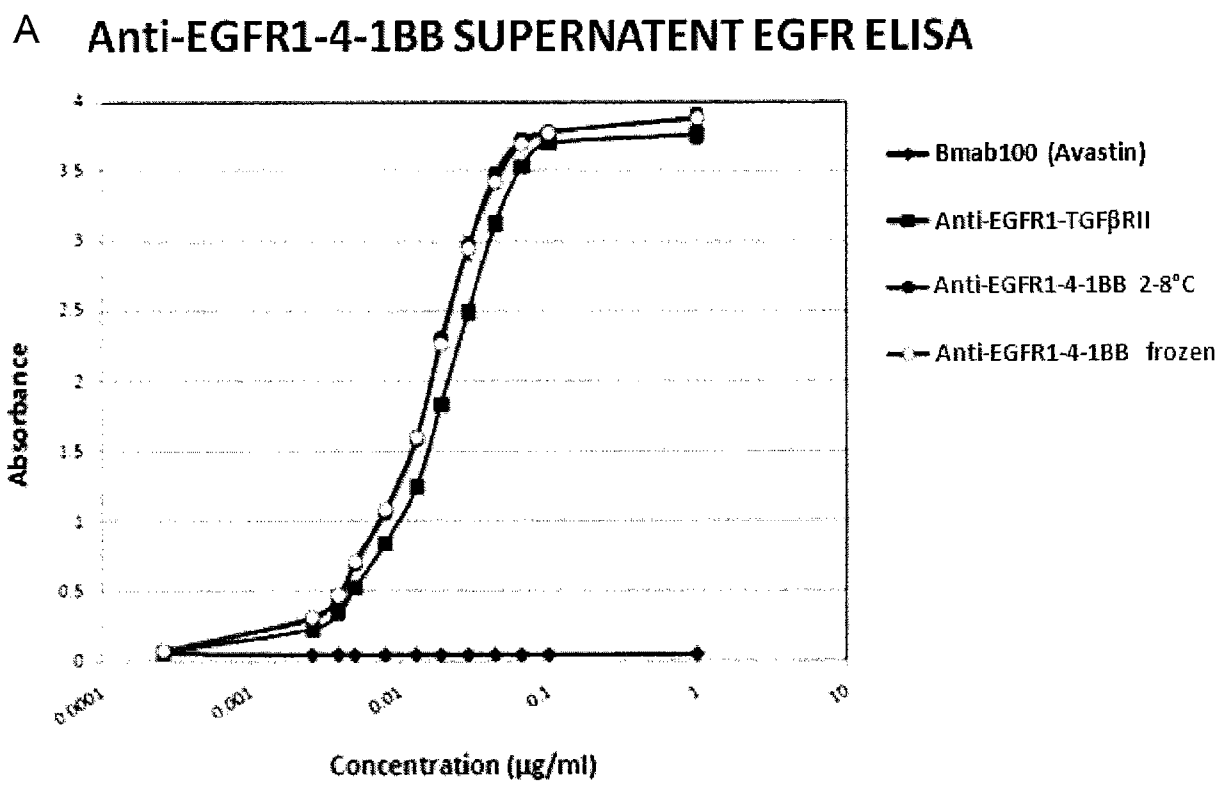

Anti-EGFR1-4-1BB ELISA 23112011
Absorbance
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.00001
0.0001
0.001
0.01
0.1
Concentration (µg/ml)
Anti-EGFR1-TGFβRII
4-1BBL
Anti-EGFR1-4-1BB SUP2-8°C
Anti-EGFR1-4-1BB Purified

Figure 30

A


Anti-EGFR1-4-1BB SUPERNATENT EGFR ELISA
Absorbance
Concentration (µg/ml)
Bmab100 (Avastin)
Anti-EGFR1-TGFβRII
Anti-EGFR1-4-1BB 2-8°C
Anti-EGFR1-4-1BB frozen

B

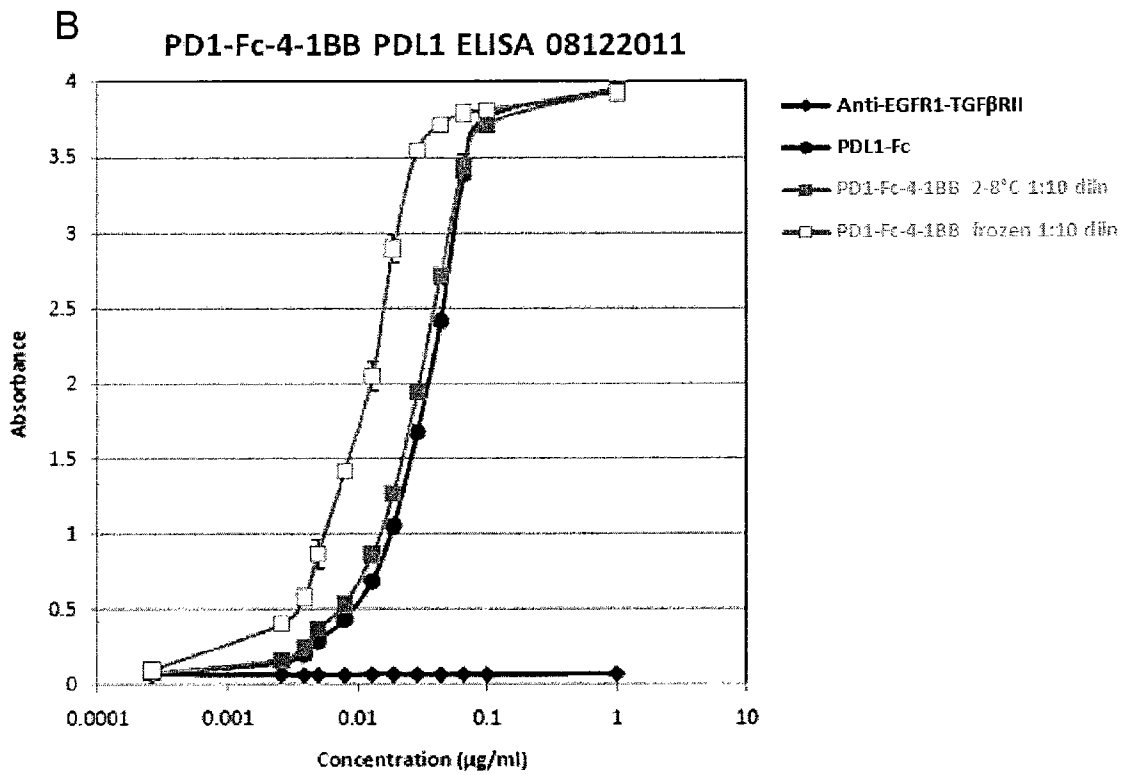

PD1-Fc-4-1BB PDL1 ELISA 08122011
Absorbance
Concentration (µg/ml)
Anti-EGFR1-TGFβRII
PDL1-Fc
PD1-Fc-4-1BB 2-8°C 1:10 dilln
PD1-Fc-4-1BB frozen 1:10 dilln

Figure 31

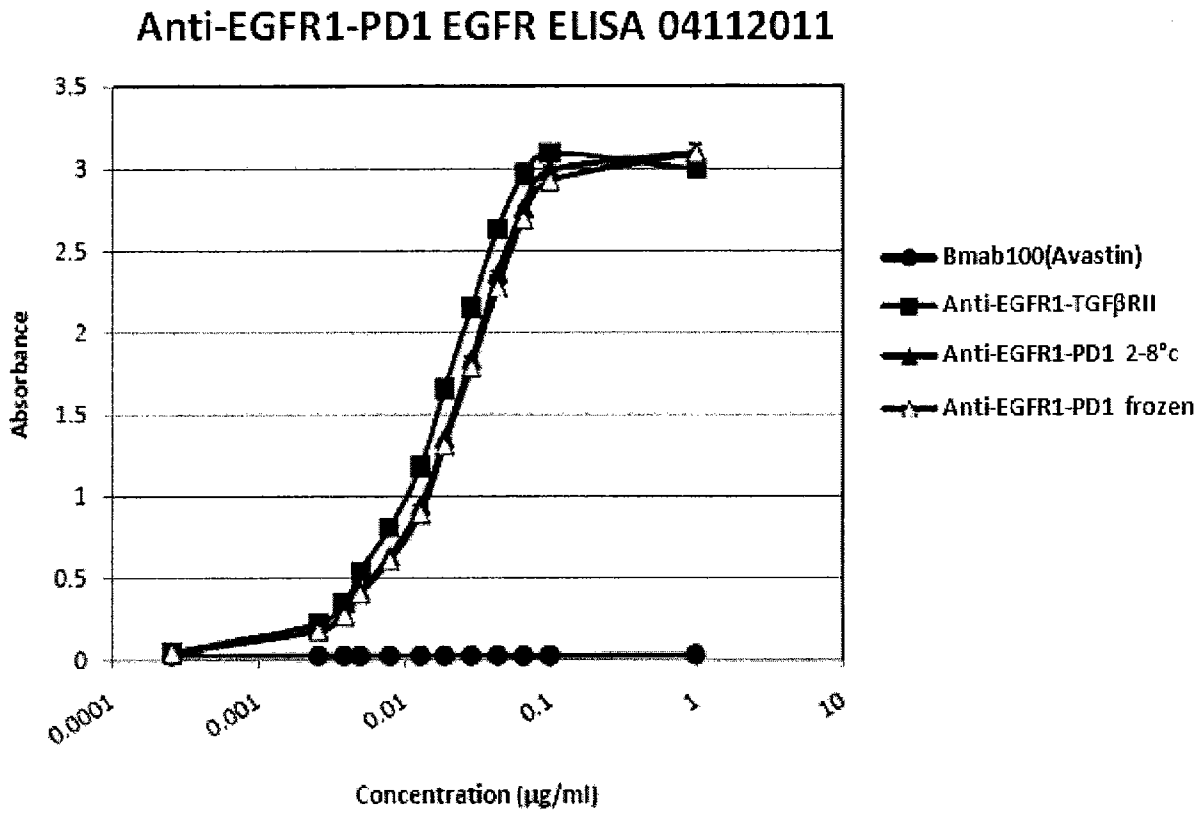

Anti-EGFR1-PD1 EGFR ELISA 04112011
Absorbance
3.5
3
2.5
2
1.5
1
0.5
0
0.0001
0.001
0.01
0.1
1
10
Concentration (µg/ml)
Bmab100(Avastin)
Anti-EGFR1-TGFβRII
Anti-EGFR1-PD1 2-8°c
Anti-EGFR1-PD1 frozen

Figure 32

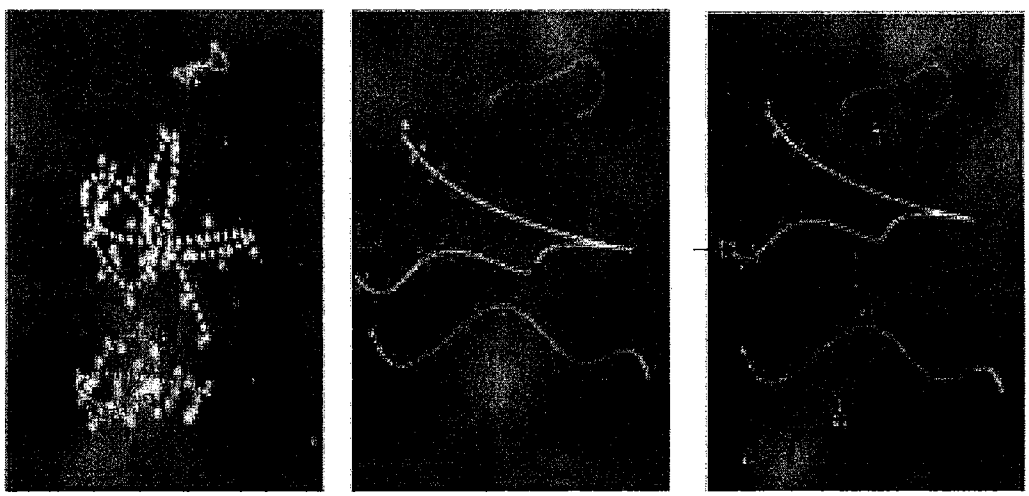

1
Measurement of whole protein
Molecular weight, presence of Glycosylation
2
Reduction alkylation → Heavy and light chain
and
peptide digestion→ primary Sequence
Identification and confirmation
3
MS/MS of protein and peptides
for proteome profiling ,
sequence and PTM confirmation

Figure 33

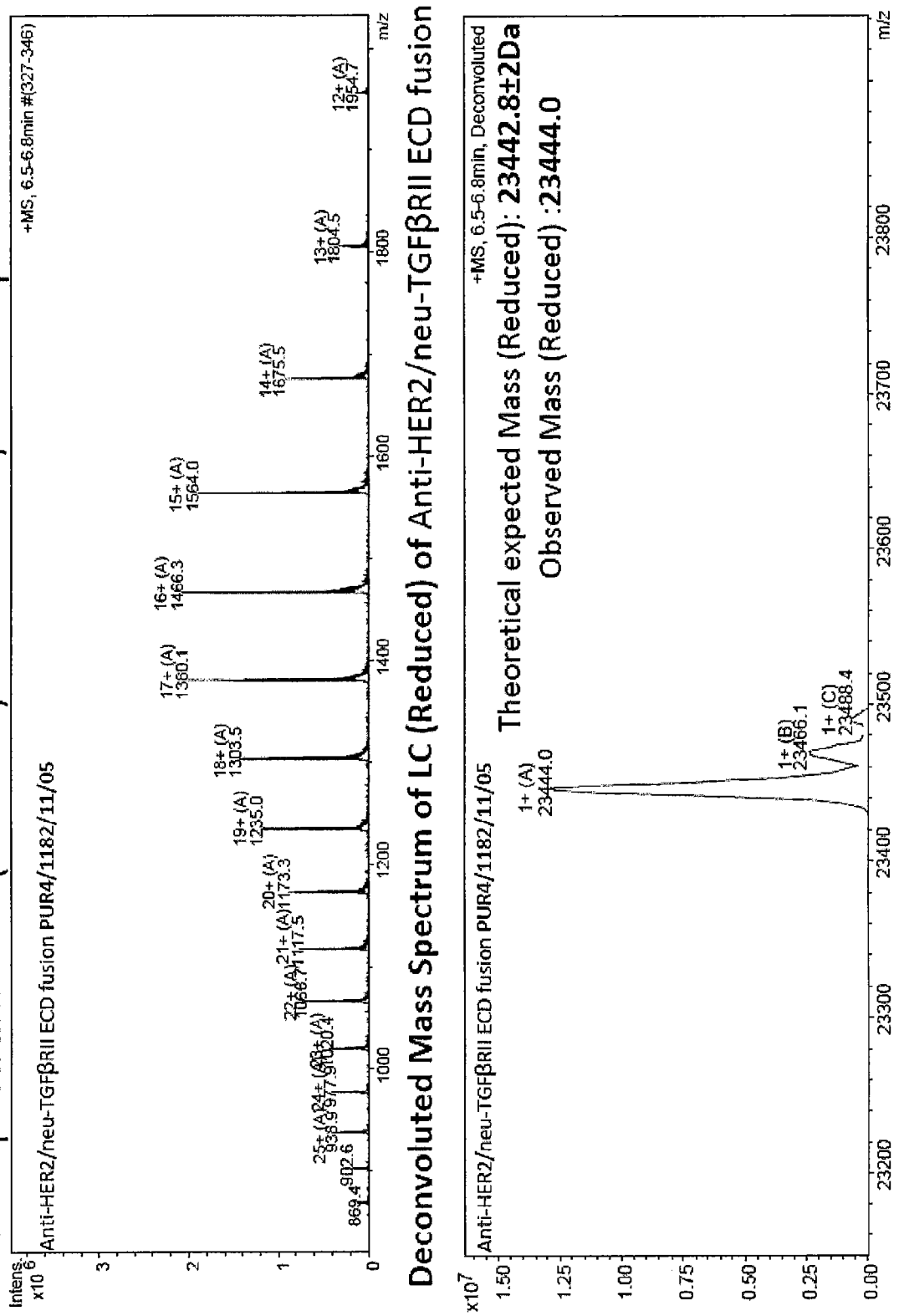

Mass Spectrum of LC (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion
Anti-HER2/neu-TGFβRII ECD fusion PUR4/1182/11/05
+MS, 6.5-6.8min #(327-346)
Intens.
x10 6
m/z
Deconvoluted Mass Spectrum of LC (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion
Anti-HER2/neu-TGFβRII ECD fusion PUR4/1182/11/05
+MS, 6.5-6.8min, Deconvoluted
Theoretical expected Mass (Reduced): 23442.8±2Da
Observed Mass (Reduced) :23444.0
x10 7
m/z

Figure 34

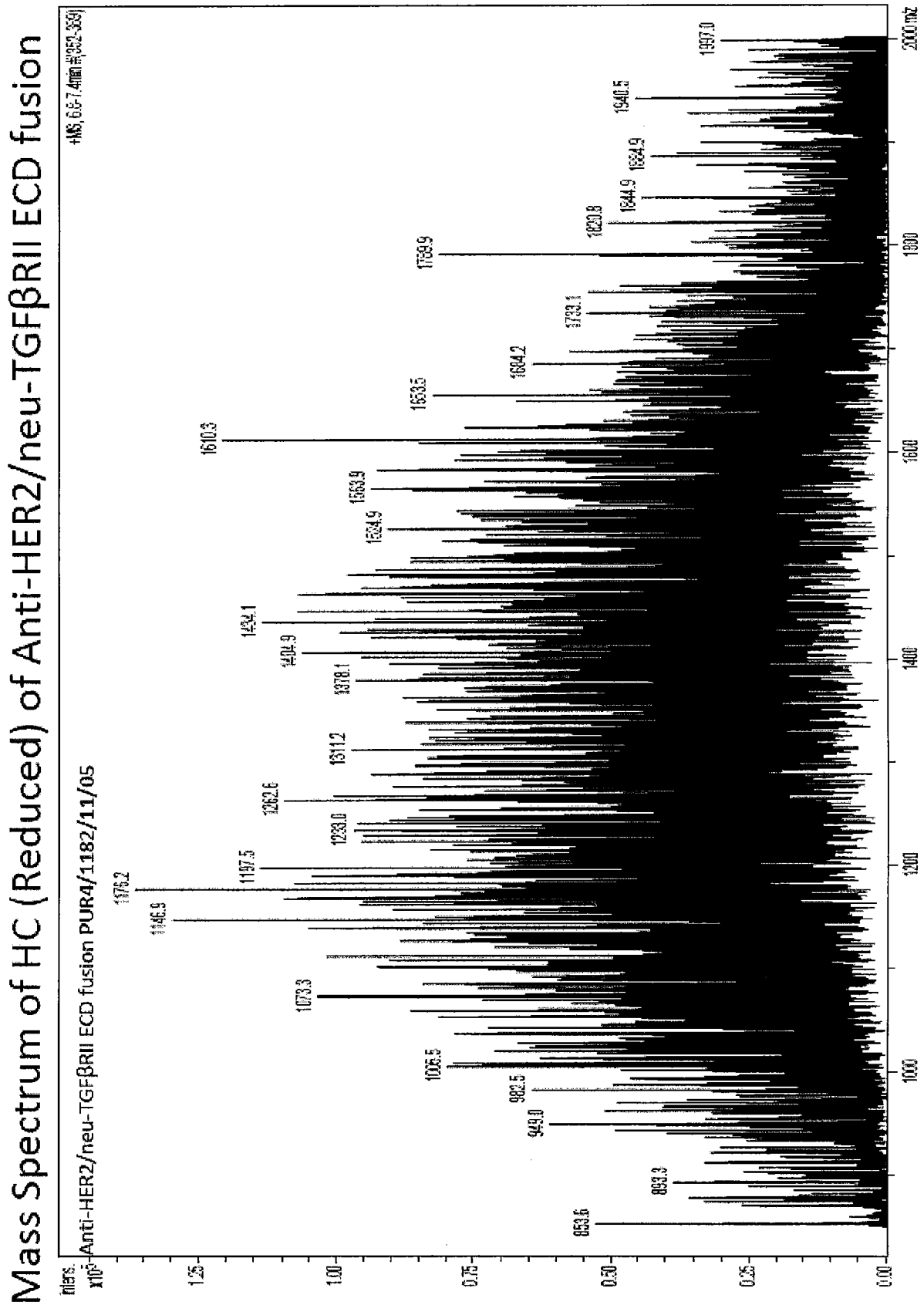

Mass Spectrum of HC (Reduced) of Anti-HER2/neu-TGFβRII ECD fusion
Anti-HER2/neu-TGFβRII ECD fusion PUR4/1182/11/05
+MS, 6.6-7.4min (352-359)
Counts
x10^5
1.25
1.00
0.75
0.50
0.25
0.00
1000
1200
1400
1600
1800
2000 m/z
853.6
893.3
949.0
982.5
1006.5
1078.3
1146.9
1176.2
1197.5
1233.0
1262.6
1311.2
1378.1
1404.9
1434.1
1524.9
1569.9
1610.3
1653.5
1684.2
1733.1
1759.9
1820.8
1844.9
1884.9
1940.5
1997.0

Figure 35

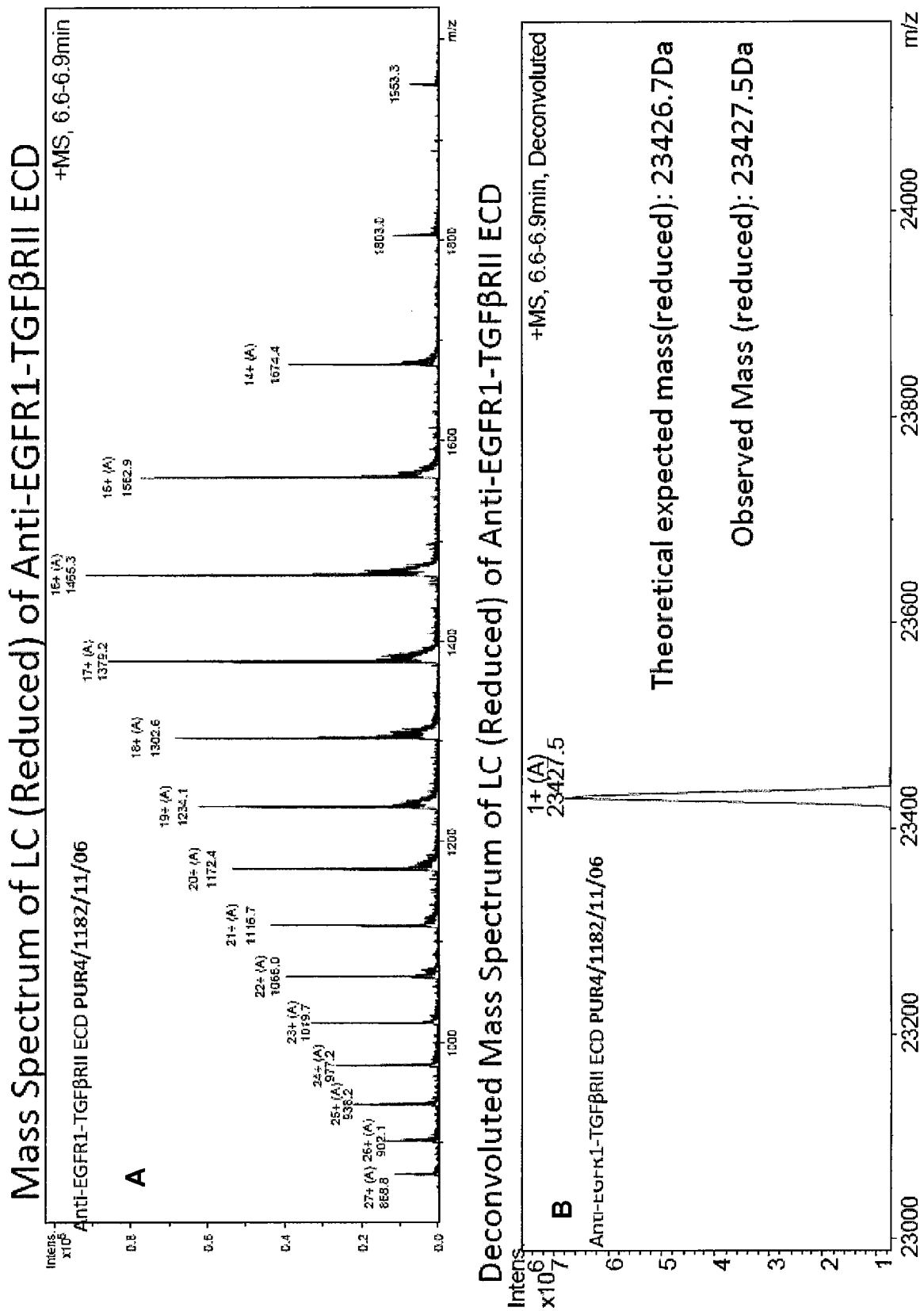

Mass Spectrum of LC (Reduced) of Anti-EGFR1-TGFβRII ECD
Anti-EGFR1-TGFβRII ECD PUR4/1182/11/06
+MS, 6.6-6.9min
A
Intens.
x10^6
m/z
Deconvoluted Mass Spectrum of LC (Reduced) of Anti-EGFR1-TGFβRII ECD
+MS, 6.6-6.9min, Deconvoluted
B
1+ (A)
23427.5
Theoretical expected mass(reduced): 23426.7Da
Observed Mass (reduced): 23427.5Da

Figure 36

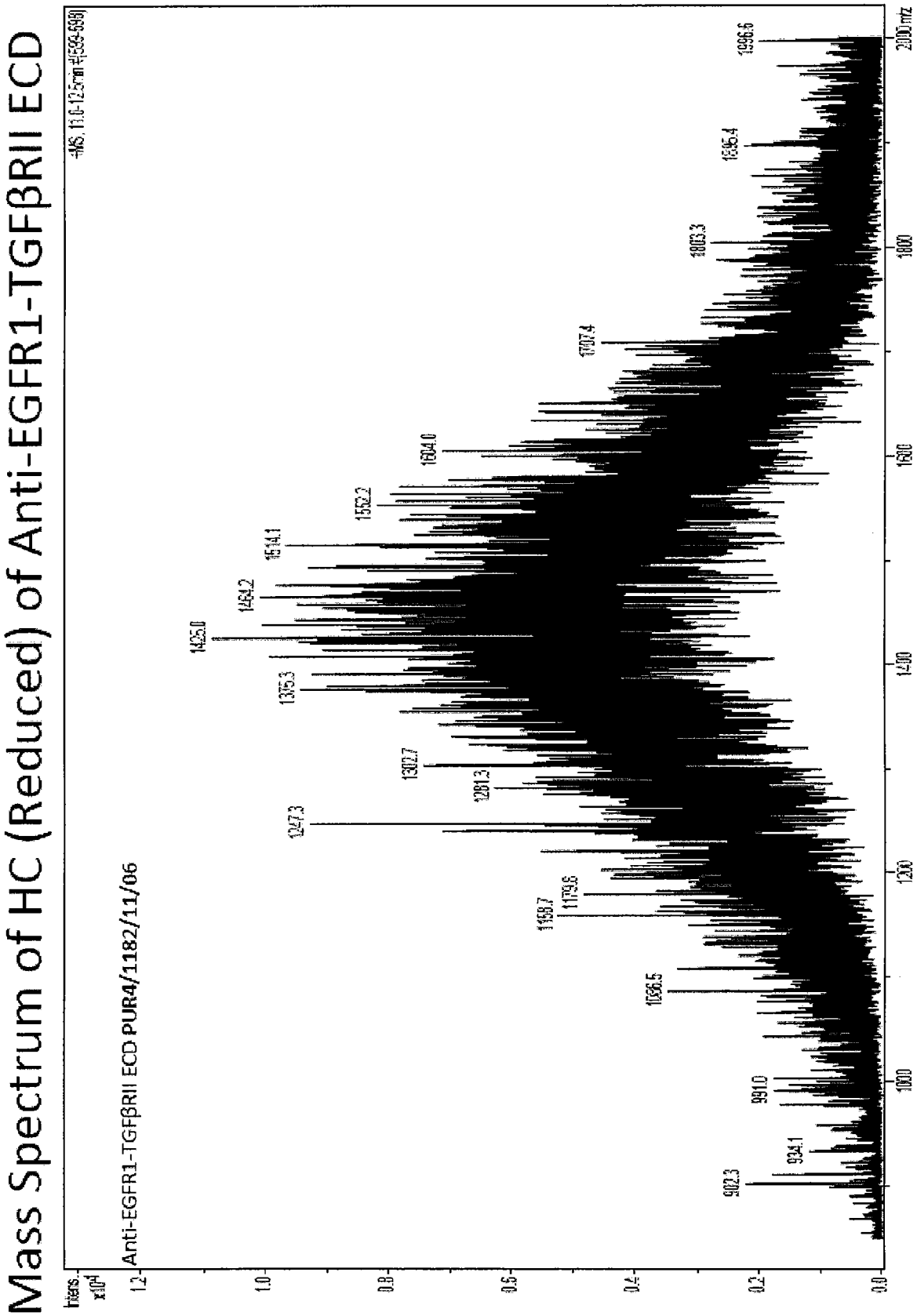

Mass Spectrum of HC (Reduced) of Anti-EGFR1-TGFβRII ECD
Anti-EGFR1-TGFβRII ECD PUR4/1182/11/06
Intens.
x10^4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
902.3
934.1
991.0
1096.5
1158.7
1179.6
1247.3
1281.3
1302.7
1375.3
1425.0
1464.2
1514.1
1562.2
1604.0
1707.4
1803.3
1845.4
1996.6
1000
1200
1400
1600
1800
2000 m/z

Figure 37

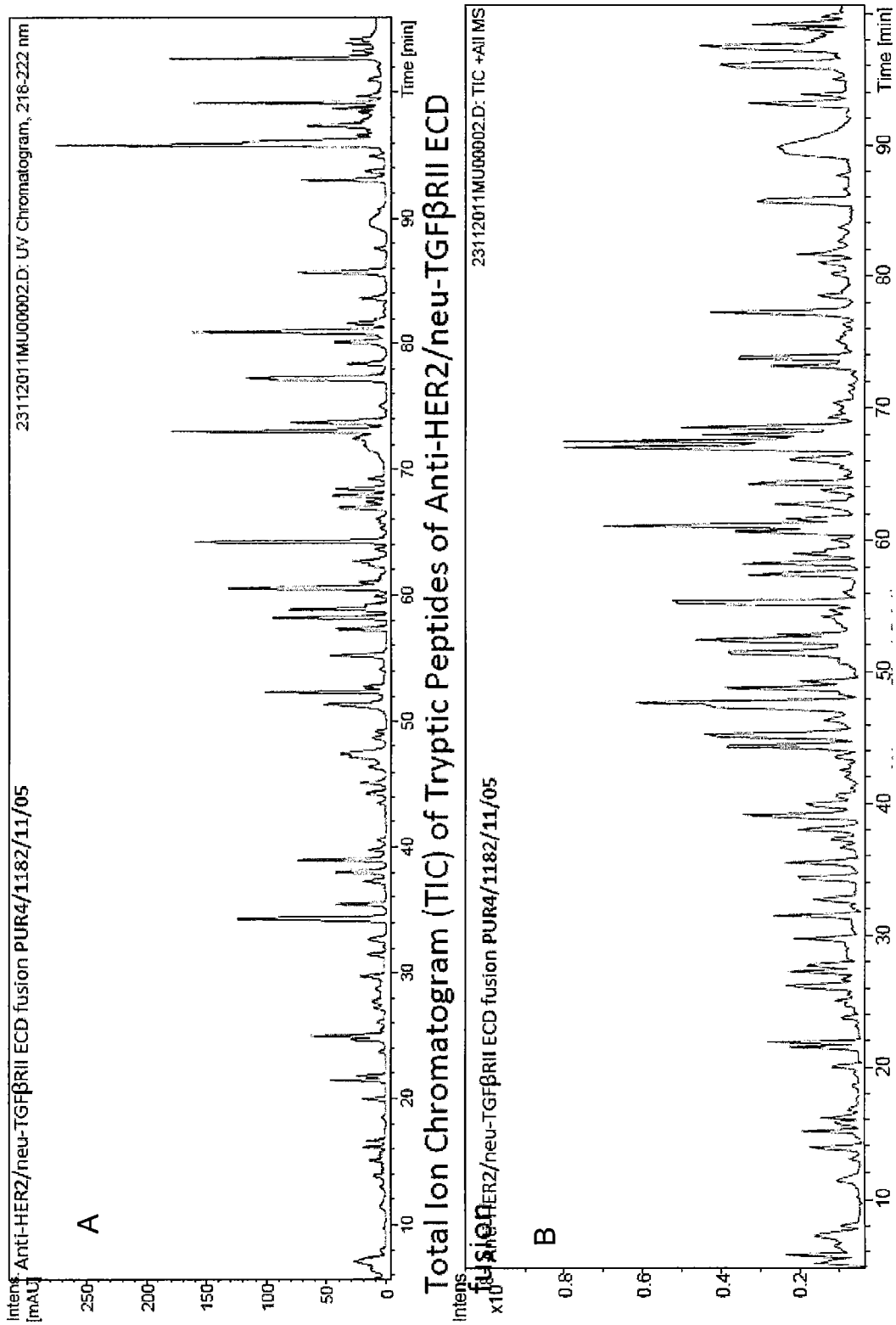

UV Chromatogram of Tryptic Peptides of Anti-HER2/neu-TGFβRII ECD fusion
A
Anti-HER2/neu-TGFβRII ECD fusion PUR4/1182/11/05
23112011MU000002.D: UV Chromatogram, 218-222 nm
Intens [mAU]
Time [min]
Total Ion Chromatogram (TIC) of Tryptic Peptides of Anti-HER2/neu-TGFβRII ECD
B
HER2/neu-TGFβRII ECD fusion PUR4/1182/11/05
23112011MU000002.D: TIC +All MS
Intens
x10
Time [min]

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)+	Observed Mass (M+H)+	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)	ASQDVNTAVAWYQQKPGK	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)	LLIYSASFLYSGVPSR	1772.9	1773.1	93.1	Yes
6	LC(62-66)	FSGSR	553.2	553.3	16.3	Yes
7	LC(67-103)	SGTDFTLTISSLQPEDFATYYCQQHYT	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)	SGTASVVCLLNNFYPR	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)	DSTYSLSSTLTLSK	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)+	Observed Mass (M+H)+	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)	DTYIHWVR	1089.5	1089.6, 545.3+2	64.2	YES
4	HC(39-43)	QAPGK	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 deteection range		
9	HC(68-76)	FTISADTSK	969.4	969.5, 485.3+2	45.3	YES
10	HC(77-87)	NTAYLQMNSLR	1310.6	1310.6, 655.8+2	61.4	YES
11	HC(88-98)	AEDTAVYYCSR	1334.6	1334.8, 667.9+2	38.9	YES
12	HC(99-124)	WGGDGFYAMDYWGQGTLVTVSSASTK	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)	DYFPEPVTVSWNSGALTS.....LGTQTYIC	6713.3	6, 1679.7+4,1343.8+5, 112(	102.6	NO
16	HC(214-216)	VDK	361.2	Out of deteection range		
17	HC(217-217)	K	147.1	Out of deteection 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)	THTCPPCPAPELLGGPSVFLFPPKPK	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 deteection range		
29	HC(326-329)	VSNK	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 deteection 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)	GFYPSDIAVEWESNGQPENNYK	2545.1	2545.1, 1273.1+2, 849.1+3	81.1	YES
38	HC(396-412)	TTPPVLDSDGSFFLYSK	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 deteection range		
41	HC(420-442)	WQQGNVFSCSVMHEALHNHYTQK	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)+	Observed Mass (M+H)+	RT(min)	MS/MS
1	LM(1-23)	GGGGSGGGGSGGGGSTIPPHVQK	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)	SCMSNCSITSICEKPQEVCVAVWR	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)	LPYHDFILEDAASPK	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)	KPGETFFMCSCSSDECNDNIIFSEEYNTSNPD	3794.8	Not Detected		

Figure 41

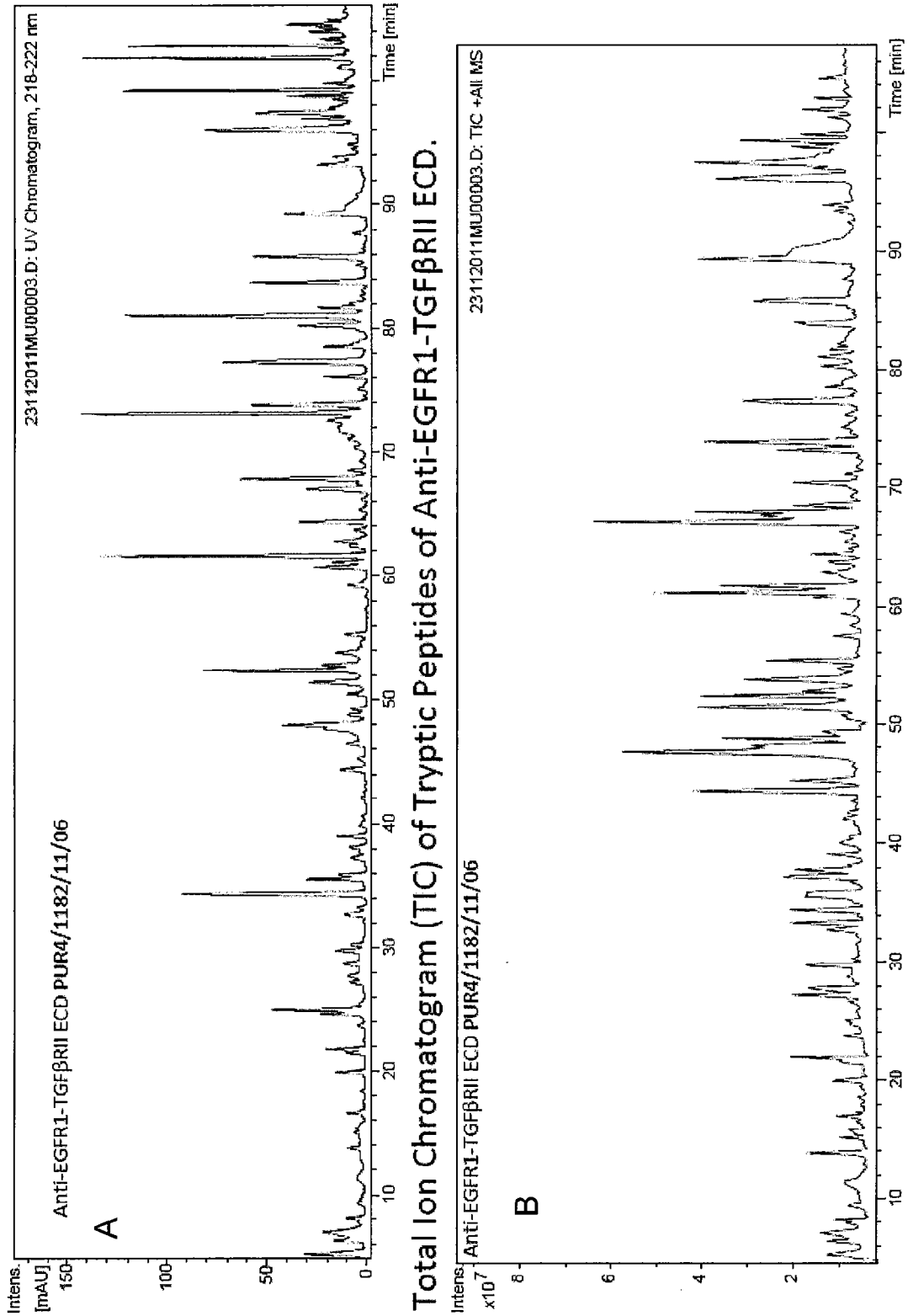

UV Chromatogram of Tryptic Peptide of Anti-EGFR1-TGFβRII ECD
23112011MU00003.D: UV Chromatogram, 218-222 nm
Anti-EGFR1-TGFβRII ECD PUR4/1182/11/06
A
Intens. [mAU]
Time [min]
Total Ion Chromatogram (TIC) of Tryptic Peptides of Anti-EGFR1-TGFβRII ECD.
23112011MU00003.D: TIC +All MS
Anti-EGFR1-TGFβRII ECD PUR4/1182/11/06
B
Intens. x10^7
Time [min]

Figure 42

List of expected/observed peptides of Light Chain(LC) of Anti-EGFR1-TGFβRII ECD.

S.No	Peptide	Sequence	Expected Mss(M+H)+	Observed Mass (M+H)+	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)	YASESISGIPSR	1266.6	1266.6	48	Yes
7	LC(62-103)	FSGSGSGTDFTLSINSVESED	4565.9	4565.9	93.1	NO
8	LC(104-107)	LELK	502.3	502.4	37.8	Yes
9	LC(108-108)	R	174.1	Out of detection range		
10	LC(109-126)	TVAAPSVFIFPPSDEQLK	1946	1946.2	89.5	Yes
11	LC(127-142)	SGTASVVCLLNNFYPR	1797.8	1797.2	97.1	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)	VDNALQSGNSQESVTEQDS	2135.9	2136.2	35.6	Yes
15	LC(170-183)	DSTYSLSSTLTLSK	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		
18	LC(191-207)	VYACEVTHQGLSSPVTK	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		

Figure 43

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

S.No	Peptide	Sequence	Expected Mass (M+H)+	Observed Mass (M+H)+	RT (Min)	MS/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)	GLEWLGVIWSGGNTDYNTPFTSR	2571.7	2571.4	101.8	yes
5	HC(67-71)	LSINK	574.3	574.5	33.2	YES
6	HC(72-75)	DNSK	463.2	Not Detected		
7	HC(76-96)	SQVFFMNSLQSNDTAIYYCAR	2515.1	Not Detected		
8	HC(97-122)	ALTYYDYEFAYWGQGTLVTVSAASTK	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)	DYFPEPVTVSWNSGALTSGVHTFPAVLQS'	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-TGFβRII ECD

S.No	Peptide	Sequence	Expected Mass (M+H)+	Observed Mass (M+H)+	RT (Min)	MS/MS
1	LM(1-23)	GGGGSGGGGSGGGGSTIPPHVQK	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)	SCMSNCSITSICEKPQEVCVAVWR	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)	LPYHDFILEDAASPK	1715.8	1715.9	77.1	YES
10	LM(114-117)	CIMK	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)	KPGETFFMCSCSSDECNDNIIFSEEYNTSNPD	3796.9	Not Detected		

Figure 45

Cantuzumab -TGFβRII fusion protein at LC constant region

Amino acid sequence of Cantuzumab heavy chain:

QVQLVQSGAEVKKPGETVKISCKASDYTFTYYGMNWVKQAPGQGLKWMG
WIDTTTGEPTYAQKFQGRIAFSLETSASTAYLQIKSLKSEDTATYFCARRGPYN
WYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Amino acid sequence of Cantuzumab light chain fusion protein:

DIVMTQSPLSVPVTPGEPVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIY
RMSNLVSGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGT
KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC
*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFPQL**
CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPD

Figure 46

Cixutumumab -TGFβRII fusion protein at LC constant region

Amino acid sequence of Cixutumumab heavy chain:

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGI
IPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAPLRFLE
WSTQDHYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Amino acid sequence of Cixutumumab light chain fusion protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYATWYQQKPGQAPILVIYGENK
RPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSGQHLVFGGGTKL
TVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAP
AECS*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKF**
PQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVW
RKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPG
ETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 47

Clivatuzumab -TGFβRII fusion protein at LC constant region

Amino acid sequence of Clivatuzumab heavy chain:

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYI
NPYNDGTQTNKKFKGKATLTRDTSINTAYMELSRLRSDDTAVYYCARGFGGS
YGFAYNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVNTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLNISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVNHEALHNHYTQKSLSLSPGK

Amino acid sequence of Clivatuzumab light chain fusion protein:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPGKAPKLWIYSTS
NLASGVPARFSGSGSGTDFTLTISSLQPEDSASYFCHQWNRYPYTFGGGTRLE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSPRKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFP**
QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWR
KNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGE
TFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 48

Pritumumab-TGFβRII fusion protein at LC constant region

Amino acid sequence of Pritumumab heavy chain:

EVQLLESGGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAI
TPSGGSTNYADSVKGRFTISRDNSQNTLYLQMNSLRVEDTAVYICGRVPYRST
WYPLYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Amino acid sequence of Pritumumab light chain fusion protein:

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASS
LHSKVPTQFSGSGSGTDFTLTISSLQPEDFATYYCLQYSTYPITFGGGTKVEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFPQL**
CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPD

Figure 49

Cantuzumab HC-4-1BB and LC-TGFβRII fusion protein

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

QVQLVQSGAEVKKPGETVKISCKASDYTFTYYGMNWVKQAPGQGLKWMG
WIDTTTGEPTYAQKFQGRIAFSLETSASTAYLQIKSLKSEDTATYFCARRGPYN
WYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGSACP*
WAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGM
FAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTK
ELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRS
AAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQR
LGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPS
PRSE

Amino acid sequence of Cantuzumab light chain fusion protein:

DIVMTQSPLSVPVTPGEPVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIY
RMSNLVSGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGT
KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC
*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFPQL**
CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPD

Figure 50

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

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

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGI
IPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAPLRFLE
WSTQDHYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGG*
GSGGGGSACPWAVSGARASPGSAASPRLREGPELSPDDPAG
LLDLRQGMFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTG
GLSYKEDTKELVVAKAGVYYVFFQLELRRVVAGEGSGSVSL
ALHLQPLRSAAGAAALALTVDLPPASSEARNSAFGFQGRL
LHLSAGQRLGVHLHTEARARHAWQLTQGATVLGLFRVT
PEIPAGLPSPRSE

Amino acid sequence of Cixutumumab light chain fusion protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYATWYQQKPGQAPILVIYGENK
RPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSGQHLVFGGGTKL
TVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAP
AECS*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKF**
PQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVW
RKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPG
ETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 51

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

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

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYI
NPYNDGTQTNKKFKGKATLTRDTSINTAYMELSRLRSDDTAVYYCARGFGGS
YGFAYNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVNTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLNISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVNHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGSAC*
PWAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQG
MFAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDT
KELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLR
SAAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQR
LGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPS
PRSE

Amino acid sequence of Clivatuzumab light chain fusion protein:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPGKAPKLWIYSTS
NLASGVPARFSGSGSGTDFTLTISSLQPEDSASYFCHQWNRYPYTFGGGTRLE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSPRKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFP**
QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWR
KNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGE
TFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 52

Pritumumab HC-4-1BB and LC-TGFβRII fusion protein

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

EVQLLESGGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAI
TPSGGSTNYADSVKGRFTISRDNSQNTLYLQMNSLRVEDTAVYICGRVPYRST
WYPLYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGSACP*
WAVSGARASPGSAASPRLREGPELSPDDPAGLLDLRQGM
FAQLVAQNVLLIDGPLSWYSDPGLAGVSLTGGLSYKEDTK
ELVVAKAGVYYVFFQLELRRVVAGEGSGSVSLALHLQPLRS
AAGAAALALTVDLPPASSEARNSAFGFQGRLLHLSAGQR
LGVHLHTEARARHAWQLTQGATVLGLFRVTPEIPAGLPS
PRSE

Amino acid sequence of Pritumumab light chain fusion protein:

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASS
LHSKVPTQFSGSGSGTDFTLTISSLQPEDFATYYCLQYSTYPITFGGGTKVEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFPQL**
CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPD

Figure 53

Cantuzumab - HC-PD1 and LC-TGFβRII fusion protein

Amino acid sequence of heavy chain-PD1 fusion protein:

QVQLVQSGAEVKKPGETVKISCKASDYTFTYYGMNWVKQAPGQGLKWMG
WIDTTTGEPTYAQKFQGRIAFSLETSASTAYLQIKSLKSEDTATYFCARRGPYN
WYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGSPGWF*
LDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRM
SPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRA
RRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPS
PRPAGQFQTLV

Amino acid sequence of Cantuzumab light chain fusion protein:

DIVMTQSPLSVPVTPGEPVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIY
RMSNLVSGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGT
KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC
*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFPQL**
CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPD

Figure 54

Cixutumumab HC-PD1 and LC-TGFβRII fusion protein

Amino acid sequence of heavy chain-PD1 fusion protein:

.

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGI
IPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAPLRFLE
WSTQDHYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGG*
GSGGGGSPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNT
SESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNG
RDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERR
AEVPTAHPSPSPRPAGQFQTLV

Amino acid sequence of Cixutumumab light chain fusion protein:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYATWYQQKPGQAPILVIYGENK
RPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSGQHLVFGGGTKL
TVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAP
AECS*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKF**
PQLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVW
RKNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPG
ETFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 55

Clivatuzumab HC-PD1 and LC-TGFβRII-fusion protein

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

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYI
NPYNDGTQTNKKFKGKATLTRDTSINTAYMELSRLRSDDTAVYYCARGFGGS
YGFAYNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVNTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLNISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVNHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGSPG*
WFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWY
RMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVV
RARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPS
PSPRPAGQFQTLV

Amino acid sequence of Clivatuzumab light chain fusion protein:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPGKAPKLWIYSTS
NLASGVPARFSGSGSGTDFTLTISSLQPEDSASYFCHQWNRYPYTFGGGTRLE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSPRKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFP**
QLCKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWR
KNDENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGE
TFFMCSCSSDECNDNIIFSEEYNTSNPD

Figure 56

Pritumumab HC-PD1 and LC-TGFβRII fusion protein

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

EVQLLESGGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAI
TPSGGSTNYADSVKGRFTISRDNSQNTLYLQMNSLRVEDTAVYICGRVPYRST
WYPLYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGSPGWF*
LDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNTSESFVLNWYRM
SPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRA
RRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPS
PRPAGQFQTLV

Amino acid sequence of Pritumumab light chain fusion protein:.

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASS
LHSKVPTQFSGSGSGTDFTLTISSLQPEDFATYYCLQYSTYPITFGGGTKVEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
*GGGGSGGGGSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFPQL**
CKFCDVRFSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKN
DENITLETVCHDPKLPYHDFILEDAASPKCIMKEKKKPGETF
FMCSCSSDECNDNIIFSEEYNTSNPD

Figure 57

Cantuzumab HC-TGFβRII-4-1BB fusion protein

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

QVQLVQSGAEVKKPGETVKISCKASDYTFTYYGMNWVKQAPGQGLKWMG
WIDTTTGEPTYAQKFQGRIAFSLETSASTAYLQIKSLKSEDTATYFCARRGPYN
WYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPP**
HVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS
CMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPY
HDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS
EEYNTSNPDepkscdk*ACPWAVSGARASPGSAASPRLREGP*
ELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDP
GLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVA
GEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARN
SAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGA
TVLGLFRVTPEIPAGLPSPRSE

Amino acid sequence of light chain

DIVMTQSPLSVPVTPGEPVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIY
RMSNLVSGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGT
KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC

Figure 58

Cixutumumab HC-TGFβRII-4-1BB fusion protein

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

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGI
IPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAPLRFLE
WSTQDHYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGG*
*GSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVR**
FSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLET
VCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSS
DECNDNIIFSEEYNTSNPD*epkscdk**ACPWAVSGARASPGSA***
ASPRLREGPELSPDDPAGLLDLRQGMFAQLVAQNVLLID
GPLSWYSDPGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFF
QLELRRVVAGEGSGSVSLALHLQPLRSAAGAAALALTVDL
PPASSEARNSAFGFQGRLLHLSAGQRLGVHLHTEARARH
AWQLTQGATVLGLFRVTPEIPAGLPSPRSE

Amino acid sequence of light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYATWYQQKPGQAPILVIYGENK
RPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSGQHLVFGGGTKL
TVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAP
AECS

Figure 59

Clivatuzumab HC-TGFβRII-4-1BB fusion protein

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

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYI
NPYNDGTQTNKKFKGKATLTRDTSINTAYMELSRLRSDDTAVYYCARGFGGS
YGFAYNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVNTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLNISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVNHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIP**
PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQK
SCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLP
YHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIF
SEEYNTSNPD*epkscdk**ACPWAVSGARASPGSAASPRLREG*
PELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSD
PGLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVV
AGEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEAR
NSAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQG
ATVLGLFRVTPEIPAGLPSPRSE

Amino acid sequence of light chain:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPGKAPKLWIYSTS
NLASGVPARFSGSGSGTDFTLTISSLQPEDSASYFCHQWNRYPYTFGGGTRLE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSPRKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC

Figure 60

Pritumumab HC-TGFβRII-4-1BB fusion protein

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

EVQLLESGGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAI
TPSGGSTNYADSVKGRFTISRDNSQNTLYLQMNSLRVEDTAVYICGRVPYRST
WYPLYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPP**
HVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS
CMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPY
HDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS
EEYNTSNPD*epkscdk*ACPWAVSGARASPGSAASPRLREGP
ELSPDDPAGLLDLRQGMFAQLVAQNVLLIDGPLSWYSDP
GLAGVSLTGGLSYKEDTKELVVAKAGVYYVFFQLELRRVVA
GEGSGSVSLALHLQPLRSAAGAAALALTVDLPPASSEARN
SAFGFQGRLLHLSAGQRLGVHLHTEARARHAWQLTQGA
TVLGLFRVTPEIPAGLPSPRSE

Amino acid sequence of light chain:

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASS
LHSKVPTQFSGSGSGTDFTLTISSLQPEDFATYYCLQYSTYPITFGGGTKVEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 61

Cantuzumab HC-TGFβRII-PD1 fusion protein

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

QVQLVQSGAEVKKPGETVKISCKASDYTFTYYGMNWVKQAPGQGLKWMG
WIDTTTGEPTYAQKFQGRIAFSLETSASTAYLQIKSLKSEDTATYFCARRGPYN
WYFDVWGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPP**
HVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS
CMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPY
HDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS
EEYNTSNPD*epkscdk**PGWFLDSPDRPWNPPTFSPALLVVTEGD*
NATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDC
RFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESL
RAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

Amino acid sequence of light chain:

DIVMTQSPLSVPVTPGEPVSISCRSSKSLLHSNGNTYLYWFLQRPGQSPQLLIY
RMSNLVSGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCLQHLEYPFTFGPGT
KLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQ
SGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC

Figure 62

Cixutumumab HC-TGFβRII-PD1 fusion protein

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

EVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGI
IPIFGTANYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCARAPLRFLE
WSTQDHYYYYYMDVWGKGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQ
TYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF
LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGG*
*GSGGGGS***TIPPHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVR**
FSTCDNQKSCMSNCSITSICEKPQEVCVAVWRKNDENITLET
VCHDPKLPYHDFILEDAASPKCIMKEKKKPGETFFMCSCSS
DECNDNIIFSEEYNTSNPD*epkscdk**PGWFLDSPDRPWNPPTFS*
PALLVVTEGDNATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPE
DRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISL
APKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

Amino acid sequence of light chain:

SSELTQDPAVSVALGQTVRITCQGDSLRSYYATWYQQKPGQAPILVIYGENK
RPSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCKSRDGSGQHLVFGGGTKL
TVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVK
AGVETTTPSKQSNNKYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAP
AECS

Figure 63

Clivatuzumab HC-TGFβRII-PD1 fusion protein

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

QVQLQQSGAEVKKFGASVKVSCEASGYTFPSYVLHWVKQAPGQGLEWIGYI
NPYNDGTQTNKKFKGKATLTRDTSINTAYMELSRLRSDDTAVYYCARGFGGS
YGFAYNGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV
TVSWNSGALTSGVNTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLNISRTPEVTC
VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVNHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIP**
PHVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQK
SCMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLP
YHDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIF
SEEYNTSNPD*epkscdk**PGWFLDSPDRPWNPPTFSPALLVVTEGD*
NATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDC
RFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESL
RAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

Amino acid sequence of light chain:

DIQLTQSPSSLSASVGDRVTMTCSASSSVSSSYLYWYQQKPGKAPKLWIYSTS
NLASGVPARFSGSGSGTDFTLTISSLQPEDSASYFCHQWNRYPYTFGGGTRLE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYEAKVQWKVDNALQSGNS
QESVTEQDSKDSTYSLSSTLTLSPRKADYEKHKVYACEVTHQGLSSPVTKSFNR
GEC

Figure 64

Pritumumab HC-TGFβRII-PD1 fusion protein

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

EVQLLESGGDLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSAI
TPSGGSTNYADSVKGRFTISRDNSQNTLYLQMNSLRVEDTAVYICGRVPYRST
WYPLYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEP
VTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP
SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT
CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVS
LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK*GGGGSGGGGSGGGGS***TIPP**
HVQKSVNNDMIVTDNNGAVKFPQLCKFCDVRFSTCDNQKS
CMSNCSITSICEKPQEVCVAVWRKNDENITLETVCHDPKLPY
HDFILEDAASPKCIMKEKKKPGETFFMCSCSSDECNDNIIFS
EEYNTSNPD*epkscdk**PGWFLDSPDRPWNPPTFSPALLVVTEGD*
NATFTCSFSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDC
RFRVTQLPNGRDFHMSVVRARRNDSGTYLCGAISLAPKAQIKESL
RAELRVTERRAEVPTAHPSPSPRPAGQFQTLV

Amino acid sequence of light chain:

DIQMTQSPSSLSASVGDRVTITCRASQDISNYLAWFQQKPGKAPKSLIYAASS
LHSKVPTQFSGSGSGTDFTLTISSLQPEDFATYYCLQYSTYPITFGGGTKVEIKR
TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 65

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/001155

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/495 C07K14/65 C07K14/705 C07K16/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/109789 A2 (UNIV JOHNS HOPKINS [US]; BEDI ATUL [US]; RAVI RAJANI [US]) 9 September 2011 (2011-09-09)	1-3,6-8, 15,16
Y	abstract paragraph [0003] - paragraph [0038] paragraph [0143] paragraph [0182]; claims 1-67; figures 2-4,6,13-17; example 2	4,5, 9-14, 17-32

X	WO 2009/114110 A1 (IMMUNGENE INC [US]) 17 September 2009 (2009-09-17) abstract page 4, paragraph 4 - page 6, paragraph 2 page 11, paragraph 3 - page 16, paragraph 3 page 18, paragraph 2 - page 19, paragraph 2; examples 1-3	1-3,6-8

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2013	01/10/2013

Name and mailing address of the ISA/	Authorized officer
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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International application No
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