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[Continued on next page]

(54) Title: BISPECIFIC HER2 AND CD3 BINDING MOLECULES

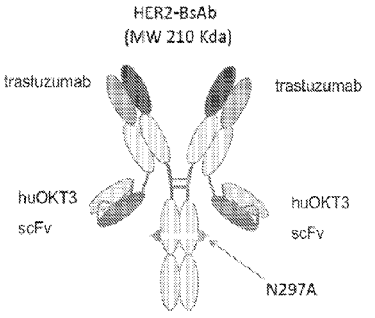

Fig. 1A

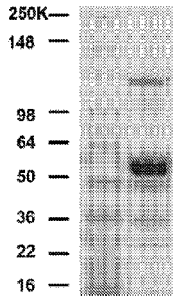

Fig. 1B

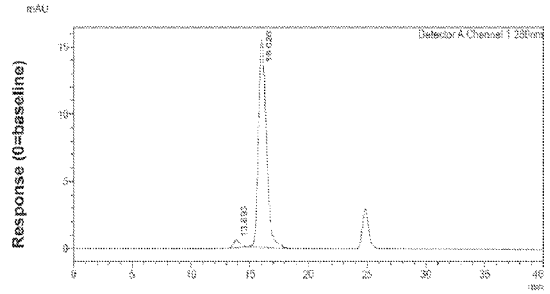

Fig. 1C

(57) Abstract: Provided herein are compositions, methods, and uses involving bispecific binding molecules that specifically bind to HER2, a receptor tyrosine kinase, and to CD3, a T cell receptor, and mediate T cell cytotoxicity for managing and treating disorders, such as cancer. Also provided herein are uses and methods for managing and treating HER2 -related cancers.

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BISPECIFIC HER2 AND CD3 BINDING MOLECULES

[0001] This application claims the benefit of U.S. Provisional Application No. 62/029,342, filed on July 25, 2014, which is incorporated by reference herein in its entirety.

[0002] This application incorporates by reference a Sequence Listing submitted with this application as text file entitled "Sequence_Listing_13542-006-228.txt" created on July 23, 2015 and having a size of 184 kbytes.

1. FIELD

[0003] Provided herein are compositions, methods, and uses involving bispecific binding molecules that specifically bind to HER2, a receptor tyrosine kinase, and to CD3, a T cell receptor, and mediate T cell cytotoxicity for managing and treating disorders, such as cancer.

2. BACKGROUND

[0004] HER2 is a receptor tyrosine kinase of the epidermal growth factor receptor family. Amplification or overexpression of HER2 has been demonstrated in the development and progression of cancers. Herceptin® (trastuzumab) is an anti-HER2 monoclonal antibody approved for treating HER2-positive metastatic breast cancer and HER2-positive gastric cancer (Trastuzumab [Highlights of Prescribing Information]. South San Francisco, CA: Genentech, Inc.; 2014). Ertumaxomab is a tri-specific HER2-CD3 antibody with intact Fc-receptor binding (*see*, for example, Kiewe et al. 2006, Clin Cancer Res, 12(10): 3085-3091). Ertumaxomab is a rat-mouse antibody; therefore, upon administration to humans, a human anti-mouse antibody response and a human anti-rat antibody response are expected. 2502A, the parental antibody of ertumaxomab, has low affinity for HER2 and low avidity (Diermeier-Daucher *et al.,* MAbs, 2012, 4(5): 614-622). There is a need for therapies capable of mediating T cell cytotoxicity in HER2-positive cancers.

3. SUMMARY

[0005] In certain embodiments, provided herein is a bispecific binding molecule comprising an aglycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion

polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, and wherein the first and second light chain fusion polypeptides are identical.

[0006] In certain embodiments of the bispecific binding molecule, the sequence of each heavy chain is any of SEQ ID NOs: 23 or 27. In certain embodiments of the bispecific binding molecule, the sequence of each light chain is SEQ ID NO: 25. In certain embodiments of the bispecific binding molecule, the sequence of the peptide linker is SEQ ID NO: 14. In certain embodiments of the bispecific binding molecule, the sequence of a V_H domain in the first scFv is any of SEQ ID NOs: 15 or 17. In certain embodiments of the bispecific binding molecule, the sequence of an intra-scFv peptide linker between a V_H domain and a V_L domain in the first scFv is of SEQ ID NO: 14. In certain embodiments of the bispecific binding molecule, the sequence of a V_L domain in the first scFv is of SEQ ID NO: 16. In certain embodiments of the bispecific binding molecule, the sequence of the scFv is SEQ ID NO: 19. In certain embodiments of the bispecific binding molecule, the sequence of the first light chain fusion polypeptide is SEQ ID NO: 29.

[0007] In certain embodiments of the bispecific binding molecule, the sequence of each heavy chain is any of SEQ ID NOs: 23, 27, 62 or 63. In certain embodiments of the bispecific binding molecule, the sequence of each light chain is SEQ ID NO: 25. In certain embodiments of the bispecific binding molecule, the sequence of the peptide linker is any of SEQ ID NOs: 14 or 35-41. In certain embodiments of the bispecific binding molecule, the sequence of a V_H domain in the first scFv is any of SEQ ID NOs: 15, 17 or 64. In certain embodiments of the bispecific binding molecule, the sequence of an intra-scFv peptide linker between a V_H domain and a V_L domain in the first scFv is any of SEQ ID NOs: 14 or 35-41. In certain embodiments of the bispecific binding molecule, the sequence of a V_L domain in the first scFv is any of SEQ ID NOs: 16 or 65. In certain embodiments of the bispecific binding molecule, the sequence of the scFv is any of SEQ ID NOs: 19 or 48-59. In certain embodiments of the bispecific binding molecule, the sequence of the first light chain fusion polypeptide is any of SEQ ID NOs: 29, 34, 42-47, or 60.

[0008] In certain embodiments of the bispecific binding molecule, the sequence of each heavy chain is SEQ ID NO: 27 and the sequence of each light chain is SEQ ID NO: 25. In

certain embodiments of the bispecific binding molecule, the sequence of the scFv is SEQ ID NO: 19. In certain embodiments of the bispecific binding molecule, the sequence of the heavy chain is SEQ ID NO: 27, the sequence of each light chain is SEQ ID NO: 25 and the sequence of the scFv is SEQ ID NO: 19. In certain embodiments of the bispecific binding molecule, the peptide linker is 5-30, 5-25, 5-15, 10-30, 10-20, 10-15, 15-30, or 15-25 amino acids in length. In certain embodiments, the sequence of the peptide linker is SEQ ID NO: 14.

[0009] In certain embodiments, the sequence of the first light chain fusion polypeptide is SEQ ID NO: 60. In certain embodiments, the sequence of the heavy chain is SEQ ID NO: 62 and the sequence of each light chain fusion polypeptide is SEQ ID NO: 60.

[0010] In certain embodiments, the sequence of the first light chain fusion polypeptide is SEQ ID NO: 47. In certain embodiments, the sequence of the heavy chain is SEQ ID NO: 27 and the sequence of each light chain fusion polypeptide is SEQ ID NO: 47.

[0011] In certain embodiments, the sequence of the first light chain fusion polypeptide is SEQ ID NO: 29. In certain embodiments, the sequence of the heavy chain is SEQ ID NO: 27 and the sequence of each light chain fusion polypeptide is SEQ ID NO: 29.

[0012] In certain embodiments of the bispecific binding molecule, the K_D is between 70 nM and 1 μM for CD3.

[0013] In certain embodiments of the bispecific binding molecule, the scFv of the bispecific binding molecule comprises one or more mutations to stabilize disulfide binding. In certain embodiments of the bispecific binding molecule, the stabilization of disulfide binding prevents aggregation of the bispecific binding molecule. In certain embodiments of the bispecific binding molecule, the stabilization of disulfide binding reduces aggregation of the bispecific binding molecule as compared to aggregation of the bispecific binding molecule without the stabilization of disulfide binding. In certain embodiments of the bispecific binding molecule, the one or more mutations to stabilize disulfide binding comprise a V_H G44C mutation and a V_L Q100C mutation (*e.g.*, as present in SEQ ID NOS: 54-59). In certain embodiments of the bispecific binding molecule, the one or more mutations to stabilize disulfide binding are the replacement of the amino acid residue at V_H44(according to the Kabat numbering system) with a cysteine and the replacement of the amino acid residue at V_L100 (according to the Kabat numbering system) with a cysteine so as to introduce a disulfide bond between V_H44 and V_L100 (*e.g.*, as present in SEQ ID NOS: 54-59).

[0014] In certain embodiments of the bispecific binding molecule, the bispecific binding molecule does not bind an Fc receptor in its soluble or cell-bound form. In certain embodiments of the bispecific binding molecule, the heavy chain has been mutated to destroy an N-linked glycosylation site. In certain embodiments of the bispecific binding molecule, the heavy chain has an amino acid substitution to replace an asparagine that is an N-linked glycosylation site, with an amino acid that does not function as a glycosylation site. In certain embodiments of the bispecific binding molecule, the heavy chain has been mutated to destroy a C1q binding site. In certain embodiments, the bispecific binding molecule does not activate complement.

[0015] In certain embodiments, provided herein is a bispecific binding molecule comprising an aglycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, wherein the first and second light chain fusion polypeptides are identical, and wherein (a) the sequence of each heavy chain is SEQ ID NO: 62; and (b) the sequence of each light chain fusion polypeptide is SEQ ID NO: 60.

[0016] In certain embodiments, provided herein is a bispecific binding molecule comprising an aglycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, wherein the first and second light chain fusion polypeptides are identical, and wherein (a) the sequence of each heavy chain is SEQ ID NO: 27; and (b) the sequence of each light chain fusion polypeptide is SEQ ID NO: 47.

[0017] In certain embodiments, provided herein is a bispecific binding molecule comprising an aglycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical heavy chains and two identical light chains, said light chains being a

first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, wherein the first and second light chain fusion polypeptides are identical, and wherein (a) the sequence of each heavy chain is SEQ ID NO: 27; and (b) the sequence of each light chain fusion polypeptide is SEQ ID NO: 29.

[0018] In certain embodiments, provided herein is a polynucleotide comprising nucleotide sequences encoding a light chain fusion polypeptide comprising an immunoglobulin light chain fused to a scFv, via a peptide linker, wherein the light chain binds to HER2 and wherein the scFv binds to CD3. In certain embodiments of the polynucleotide, the sequence of the light chain is SEQ ID NO: 25. In certain embodiments of the polynucleotide, the nucleotide sequence encoding the light chain is SEQ ID NO: 24. In certain embodiments of the polynucleotide, the sequence of the scFv is SEQ ID NO: 19. In certain embodiments of the polynucleotide, the nucleotide sequence encoding the scFv is SEQ ID NO: 18. In certain embodiments of the polynucleotide, the sequence of the light chain is SEQ ID NO: 25 and the sequence of the scFv is SEQ ID NO: 19. In certain embodiments of the polynucleotide, the nucleotide sequence encoding the light chain is SEQ ID NO: 24 and the nucleotide sequence encoding the scFv is SEQ ID NO: 18. In certain embodiments of the polynucleotide, the peptide linker is 5-30, 5-25, 5-15, 10-30, 10-20, 10-15, 15-30, or 15-25 amino acids in length. In certain embodiments of the polynucleotide, the sequence of the peptide linker is SEQ ID NO: 14. In certain embodiments of the polynucleotide, the nucleotide sequence encoding the peptide linker is SEQ ID NO: 13.

[0019] In certain embodiments, provided herein is a vector comprising a polynucleotide encoding nucleotide sequences encoding a light chain fusion polypeptide comprising an immunoglobulin light chain fused to a scFv, via a peptide linker, wherein the light chain binds to HER2 and wherein the scFv binds to CD3, operably linked to a promoter. In certain embodiments, provided herein is an *ex vivo* cell comprising the polynucleotide provided herein operably linked to a promoter. In certain embodiments, provided herein is an *ex vivo* cell comprising the vector.

[0020] In certain embodiments, provided herein is a vector comprising (i) a first polynucleotide comprising nucleotide sequences encoding a light chain fusion polypeptide

comprising an immunoglobulin light chain fused to a scFv, via a peptide linker, wherein the light chain binds to HER2 and wherein the scFv binds to CD3 operably linked to a first promoter, and (ii) a second polynucleotide encoding an immunoglobulin heavy chain that binds to HER2 operably linked to a second promoter. In certain embodiments, provided herein is an *ex vivo* cell comprising the vector.

[0021] In certain embodiments, provided herein is a method of producing a bispecific binding molecule comprising (a) culturing the cell comprising the vector comprising (i) a first polynucleotide comprising nucleotide sequences encoding a light chain fusion polypeptide comprising an immunoglobulin light chain fused to a scFv, via a peptide linker, wherein the light chain binds to HER2 and wherein the scFv binds to CD3 operably linked to a first promoter, and (ii) a second polynucleotide encoding an immunoglobulin heavy chain that binds to HER2 operably linked to a second promoter, to express the first and second polynucleotides such that a bispecific binding molecule comprising said light chain fusion polypeptide and said immunoglobulin heavy chain is expressed, and (b) recovering the bispecific binding molecule.

[0022] In certain embodiments, provided herein is a pharmaceutical composition comprising a therapeutically effective amount of (i) the first polynucleotide operably linked to the first promoter, and (ii) the second polynucleotide encoding an immunoglobulin heavy chain that binds to HER2 operably linked to the second promoter. In certain embodiments, provided herein is a pharmaceutical composition comprising a therapeutically effective amount of a vector comprising (i) the first polynucleotide operably linked to the first promoter, and (ii) the second polynucleotide encoding an immunoglobulin heavy chain that binds to HER2 operably linked to the second promoter. In certain embodiments, the vector is a viral vector.

[0023] In certain embodiments, provided herein is a mixture of polynucleotides comprising (i) a polynucleotide comprising nucleotide sequences encoding a light chain fusion polypeptide comprising an immunoglobulin light chain fused to a scFv, via a peptide linker, wherein the light chain binds to HER2 and wherein the scFv binds to CD3 operably linked to a first promoter, and (ii) a second polynucleotide encoding an immunoglobulin heavy chain that binds to HER2 operably linked to a second promoter. In certain embodiments of the mixture of polypeptides, the sequence of the heavy chain is SEQ ID NO: 27. In certain embodiments of the mixture of polypeptides, the nucleotide sequence encoding the heavy chain is SEQ ID NO: 26. In certain

embodiments, provided herein is an *ex vivo* cell comprising the mixture of polynucleotides provided herein.

[0024] In certain embodiments, provided herein is a method of producing a bispecific binding molecule, comprising (i) culturing the cell comprising the mixture of polynucleotides to express the first and second polynucleotides such that a bispecific binding molecule comprising said light chain fusion polypeptide and said immunoglobulin heavy chain is produced, and (ii) recovering the bispecific binding molecule.

[0025] In certain embodiments, provided herein is a method of producing a bispecific binding molecule, comprising (i) expressing the mixture of polynucleotides such that a bispecific binding molecule comprising said first light chain fusion polypeptide and said immunoglobulin heavy chain is produced, and (ii) recovering the bispecific binding molecule.

[0026] In certain embodiments, provided herein is a method of making a therapeutic T cell comprising binding a bispecific binding molecule described herein to a T cell. In certain embodiments, the T cell is a human T cell. In certain embodiments, the binding is noncovalently.

[0027] In certain embodiments, provided herein is a pharmaceutical composition comprising a therapeutically effective amount of the bispecific binding molecule and a pharmaceutically acceptable carrier.

[0028] In certain embodiments, provided herein is a pharmaceutical composition comprising a therapeutically effective amount of the bispecific binding molecule, a pharmaceutically acceptable carrier, and T cells. In certain embodiments, the T cells are bound to the bispecific binding molecule. In certain embodiments, the binding of the T cells to the bispecific binding molecule is noncovalently. In certain embodiments, the T cells are administered to a subject for treatment of a HER2-positive cancer in the subject. In certain embodiments, the T cells are autologous to the subject to whom they are administered. In certain embodiments, the T cells are allogeneic to the subject to whom they are administered. In certain embodiments, the T cells are human T cells.

[0029] In certain embodiments, provided herein is a method of treating a HER2-positive cancer in a subject in need thereof comprising administering a pharmaceutical composition provided herein. In certain embodiments, provided herein is a method of treating a HER2-positive cancer in a subject in need thereof comprising administering a therapeutically effective

amount of a bispecific binding molecule provided herein. In certain embodiments, the HER2-positive cancer is breast cancer, gastric cancer, an osteosarcoma, desmoplastic small round cell cancer, squamous cell carcinoma of head and neck cancer, ovarian cancer, prostate cancer, pancreatic cancer, glioblastoma multiforme, gastric junction adenocarcinoma, gastroesophageal junction adenocarcinoma, cervical cancer, salivary gland cancer, soft tissue sarcoma, leukemia, melanoma, Ewing's sarcoma, rhamdomyosarcoma, neuroblastoma, small cell lung cancer, or any other neoplastic tissue that expresses the HER2 receptor. In certain embodiments, the HER2-positive cancer is a primary tumor or a metastatic tumor, *e.g.*, a brain or peritoneal metastases.

[0030] In certain embodiments of the method of treating, the administering is intravenous. In certain embodiments of the method of treating, the administering is intraperitoneal, intrathecal, intraventricular, or intraparenchymal. In certain embodiments of the method of treating, the method further comprises administering to the subject doxorubicin, cyclophosphamide, paclitaxel, docetaxel, and/or carboplatin. In certain embodiments of the method of treating, the method further comprises administering to the subject radiotherapy. In certain embodiments of the method of treating, the administering is performed in combination with multi-modality anthracycline-based therapy. In certain embodiments of the method of treating, the administering is performed in combination with cytoreductive chemotherapy. In a specific embodiment, the administering is performed after treating the subject with cytoreductive chemotherapy. In certain embodiments of the method of treating, the bispecific binding molecule is not bound to a T cell. In certain embodiments of the method of treating, the bispecific binding molecule is bound to a T cell. In certain embodiments of the method of treating, the binding of the bispecific binding molecule to the T cell is non-covalently. In certain embodiments of the method of treating, the administering is performed in combination with T cell infusion. In a specific embodiment, the administering is performed after treating the patient with T cell infusion. In certain embodiments, the T cell infusion is performed with T cells that are autologous to the patient to whom the T cells are administered. In certain embodiments, the T cell infusion is performed with T cells that are allogeneic to the patient to whom the T cells are administered. In certain embodiments, the T cells can be bound to molecules identical to a bispecific binding molecule as described herein. In certain embodiments, the binding of the T cells to the molecules identical to a bispecific binding molecule is noncovalently. In certain embodiments, the T cells are human T cells.

[0031] In certain embodiments of the method of treating, the method further comprises administering to the subject an agent that increases cellular HER2 expression. In certain embodiments of the method of treating, the HER2-positive cancer is resistant to treatment with trastuzumab, cetuximab, lapatinib, erlotinib, or any other small molecule or antibody that targets the HER family of receptors. In certain embodiments of the method of treating, the subject is a human. In certain embodiments of the method of treating, the subject is a canine.

[0031a] In particular aspects provided herein is a bispecific binding molecule comprising a monoclonal antibody that is an immunoglobulin that binds to HER2, wherein said immunoglobulin is mutated in its Fc region to destroy a glycosylation site, said immunoglobulin comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, and wherein the first and second light chain fusion polypeptides are identical,

wherein the heavy chains comprise a V_H domain present in any of SEQ ID NOs: 23, 27, 62 or 63;

wherein the light chains comprise a V_L domain present in SEQ ID NO: 25;

wherein the first and second scFvs comprise a V_H domain having a sequence selected from the group consisting of SEQ ID NOs: 15, 17 and 64, and a V_L domain having a sequence selected from the group consisting of SEQ ID NOs: 16 and 65; and

wherein the first scFv is fused to the carboxyl end of the first light chain, and wherein the second scFv is fused to the carboxyl end of the second light chain.

[0031b] In particular aspects provided herein is a polynucleotide comprising nucleotide sequences encoding a light chain fusion polypeptide comprising an immunoglobulin light chain fused to a scFv, via a peptide linker, wherein the scFv is fused to the C-terminus of the light chain; wherein the light chain binds to HER2 and comprises a V_L domain present in SEQ ID NO: 25; and wherein the scFv binds to CD3 and comprises a V_H domain having a sequence selected from the group consisting of SEQ ID NOs: 15, 17 and 64, and a V_L domain having a sequence selected from the group consisting of SEQ ID NOs: 16 and 65; and

optionally wherein

(a) the sequence of the light chain is SEQ ID NO: 25, wherein optionally the nucleotide sequence encoding the light chain is SEQ ID NO: 24;

(b) the sequence of the scFv is SEQ ID NO: 52;

(c) the sequence of the scFv is SEQ ID NO: 19, wherein optionally the nucleotide sequence encoding the scFv is SEQ ID NO: 18;

(d) the sequence of the light chain is SEQ ID NO: 25, and the sequence of the scFv is SEQ ID NO: 52;

(e) the sequence of the light chain is SEQ ID NO: 25, and the sequence of the scFv is SEQ ID NO: 19, wherein optionally the nucleotide sequence encoding the light chain is SEQ ID NO: 24, and the nucleotide sequence encoding the scFv is SEQ ID NO: 18;

(f) the peptide linker is 5-30, 5-25, 5-15, 10-30, 10-20, 10-15, 15-30, or 15-25 amino acids in length, wherein optionally the sequence of the peptide linker is SEQ ID NO: 14, wherein optionally the nucleotide sequence encoding the peptide linker is SEQ ID NO: 13; or

(g) the sequence of the light chain fusion polypeptide is SEQ ID NO: 34 or SEQ ID NO: 29, wherein optionally the nucleotide sequence encoding the light chain fusion polypeptide of SEQ ID NO: 29 is SEQ ID NO: 28.

[0031c] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0031d] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0032] **Fig. 1A, Fig. 1B, Fig. 1C, Fig. 1D, and Fig. 1E** describe HER2-BsAb. Fig. 1A depicts a schematic of the HER2-BsAb. The arrow points to the N297A mutation introduced into the heavy chain to remove glycosylation. Fig. 1B depicts the purity of HER2-BsAb as demonstrated under reducing SDS-PAGE conditions. Fig. 1C depicts the purity of HER2-BsAb as demonstrated by SEC-HPLC. Fig. 1D demonstrates that the N297A mutation in the human IgG1-Fc inhibits binding to the CD16A Fc receptor. Fig. 1E demonstrates that the N297A mutation in the human IgG1-Fc inhibits binding to the CD32A Fc receptor.

[0033] **Fig. 2A and Fig. 2B** demonstrate that HER2-BsAb binds to a breast cancer cell line and to T cells. Fig. 2A depicts the staining of AU565 breast cancer cells with trastuzumab (left) or with HER2-BsAb (right). Fig. 2B depicts the staining of CD3+ T cells with huOKT3 (left) or with HER2-BsAb (right).

[0034] **Fig. 3** demonstrates that HER2-BsAb displays potent cytotoxic T lymphocyte activity in a 4 hour ^{51}Cr release assay. For a description of trastuzumab-mOKT3, *see*, Thakur *et al.*, 2010, Curr Opin Mol Ther, 12: 340.

[0035] **Fig. 4** compares the HER2 expression against HER2-BsAb T cell cytotoxicity in a panel of cancer cell lines.

[0036] **Fig. 5A and Fig. 5B** demonstrate that HER2-BsAb-redirected T cell cytotoxicity is antigen specific. Fig. 5A demonstrates that HER2-BsAb mediates T cell cytotoxicity against the HER2-positive cell line, UM SCC 47, but not the HER2-negative cell line HTB-132. Fig. 5B demonstrates that huOKT3 and trastuzumab can block the ability of HER2-BsAb to mediate T cell cytotoxicity.

[0037] **Fig. 6** demonstrates that HER2-BsAb detects low levels of HER2 by comparing the HER2-BsAb mediated T cell cytotoxicity to the HER2 threshold of detection by flow cytometry.

[0038] **Fig. 7A, Fig. 7B, and Fig. 7C** provide the specificity, affinity, and antiproliferative action of HER2-BsAb. Fig. 7A demonstrates that pre-incubation of the HER2-positive SKOV3 ovarian carcinoma cell line blocks binding of HER2-BsAb. Fig. 7B demonstrates that SKOV3 cells labeled with dilutions of trastuzumab or with HER2-BsAb display similar curves when mean fluorescence intensity (MFI) is plotted against antibody concentration. Fib. 7C demonstrates the antiproliferative action of HER2-BsAb compared against trastuzumab in the trastuzumab sensitive breast cancer cell line SKBR3.

[0039] **Fig. 8** demonstrates that HER2-BsAb is effective against squamous cell carcinoma of the head and neck (SCCHN) cell lines. A panel of SCCHN cells were analyzed for HER2-BsAb-mediated cytotoxicity and EC50 and compared to the expression level of HER2 in each cell line as determined by flow cytometry and by qRT-PCR.

[0040] **Fig. 9A, Fig. 9B, and Fig. 9C**. HER2-BsAb mediates T cell cytotoxicity against SCCHN resistant to other HER targeted therapies. Fig. 9A demonstrates that the SCCHN cell line PCI-30 expresses EGFR and HER2. Fig. 9B demonstrates that PCI-30 cells are resistant to HER-targeted therapies lapatinib, erlotinib, neratinib, trastuzumab, and cetuximab. Fig. 9C demonstrates that PCI-30 cells are sensitive to T cells in the presence of HER2-BsAb. Data represents the average of three different cytotoxicity assays.

[0041] **Fig. 10** demonstrates that HER2-BsAb is effective against osteosarcoma cell lines. A panel of osteosarcoma cell lines were analyzed for HER2-BsAb-mediated cytotoxicity and EC50 and compared to the expression level of HER2 in each cell line as determined by flow cytometry and by qRT-PCR

[0042] **Fig. 11A, Fig. 11B, and Fig. 11C** demonstrate that HER2-BsAb is effective against osteosarcoma cell lines resistant to other targeted therapies. Fig. 11A demonstrates that the osteosarcoma cell line U2OS expresses EGFR and HER2. Fig. 11B demonstrates that USOS cells are resistant to HER-targeted therapies lapatinib, erlotinib, neratinib, trastuzumab, and cetuximab. Fig. 11C demonstrates that USOS cells are sensitive to T cells in the presence of HER2-BsAb. Data represents the average of three different cytotoxicity assays.

[0043] **Fig. 12A, Fig. 12B, Fig. 12C and Fig. 12D** demonstrate that HER2-BsAb is effective against the HeLa cervical carcinoma cell line resistant to other targeted therapies. Fig. 12A demonstrates that HeLa cells express EGFR and HER2. Fig. 12B demonstrates that HeLa cells are resistant to HER-targeted therapies lapatinib, erlotinib, neratinib, trastuzumab, and

cetuximab. Fig. 12C demonstrates that HeLa cells are sensitive to T cells in the presence of HER2-BsAb. Data represents the average of three different cytotoxicity assays. Fig. 12D demonstrates that pre-treatment with lapatinib enhances HeLa sensitivity to HER2-BsAb.

[0044] **Fig. 13** demonstrates that HER2-BsAb reduces tumor growth *in vivo*. Fig. 13 demonstrates that HER2-BsAb protects against tumor progression in implanted MCF7 breast cancer cells mixed with PBMCs.

[0045] **Fig. 14** demonstrates that HER2-BsAb protects against tumor progression in implanted HCC1954 breast cancer mixed with peripheral blood mononuclear cells (PBMC) *in vivo*.

[0046] **Fig. 15** demonstrates that HER2-BsAb protects against a metastatic model of tumor progression induced by intravenous introduction of luciferase-tagged MCF7 cells *in vivo*.

[0047] **Fig. 16A, Fig. 16B, Fig. 16C, and Fig. 16D** demonstrate that HER2-BsAb blocks the metatstatic tumor growth of luciferase-tagged MCF7 cells *in vivo*. Fig. 16A represents mice without treatment. Fig. 16B represents mice treated with PBMC and HER2-C825. Fig. 16C represents mice treated with HER2-BsAb. Fig. 16D represents mice treated with PBMC and HER2-BsAb.

[0048] **Fig. 17A, Fig. 17B, and Fig. 17C** describe HER2-BsAb. Fig. 17A depicts a schematic of the HER2-BsAb. The arrow points to the N297A mutation introduced into the heavy chain to remove glycosylation. Fig. 17B depicts the purity of HER2-BsAb as demonstrated under reducing SDS-PAGE conditions. Fig. 17C depicts the purity of HER2-BsAb as demonstrated by size exclusion chromatography high performance liquid chromatography (SEC-HPLC).

[0049] **Fig. 18A, Fig. 18B, and Fig. 18C** demonstrate that HER2-BsAb has the same specificity, similar affinity, and antiproliferative effects as trastuzumab.

[0050] **Fig. 19A and Fig. 19B** demonstrate that HER2-BsAb redirected T cell cytotoxicity is HER2-specific and dependent on CD3.

[0051] **Fig. 20** depicts HER2 expression and half maximal effective concentration (EC50) in the presence of ATC and HER2-BsAb in 35 different cell lines from different tumor systems.

[0052] **Fig. 21A, Fig. 21B, Fig. 21C, Fig. 21D, Fig. 21E, Fig. 21F, Fig. 21G, Fig. 21H, and Fig. 21I** demonstrate that HER2-BsAb mediates cytotoxic responses against carcinoma cell lines resistant to other HER-targeted therapies.

[0053] **Fig. 22** demonstrates that the EC50 of HER2-BsAb correlates with the HER2 level of expression determined by flow-cytometry. pM=picomolar; MFI=mean fluorescence intensity.

[0054] **Fig. 23A, Fig. 23B, and Fig. 23C** demonstrates that HER2-BsAb mediates T cell cytotoxicity against PD-L1-positive HCC1954 targets in a manner that is relatively insensitive to PD-1 blockade by pembrolizumab, even with PD-1 expression on effector T cells.

[0055] **Fig. 24A and Fig. 24B** demonstrates that HER2-BsAb mediates T cell cytotoxicity against PD-L1-positive HEK-293 targets in a manner that is relatively insensitive to PD-1 expression on effector T cells. The cytotoxicity is an average of 6 experiments.

[0056] **Fig. 25A, Fig. 25B, Fig. 25C, and Fig. 25D** demonstrate that HER2-BsAb is effective against HER2-positive xenografts.

5. DETAILED DESCRIPTION

[0057] Provided herein are bispecific binding molecules that bind to both HER2 and CD3. Also provided herein are isolated nucleic acids (polynucleotides), such as complementary DNA (cDNA), encoding such bispecific binding molecules or fragments thereof. Further provided are vectors (*e.g.*, expression vectors) and cells (*e.g.*, *ex vivo* cells) comprising nucleic acids (polynucleotides) or vectors (*e.g.*, expression vectors) encoding such bispecific binding molecules or fragments thereof. Also provided herein are methods of making such bispecific binding molecules, cells, and vectors. Also provided herein are T cells bound to bispecific binding molecules provided herein. Also provided herein are methods of binding such bispecific binding molecules to T cells. In other embodiments, provided herein are methods and uses for treating HER2-positive cancers using the bispecific binding molecules, nucleic acids, vectors, and/or T cells described herein. Additionally, related compositions (*e.g.*, pharmaceutical compositions), kits, and diagnostic methods are also provided herein.

[0058] In certain embodiments, provided herein are bispecific binding molecules that specifically bind to HER2 and to CD3, and invoke T cell cytotoxicity for treating cancer. Without being bound by any theory, it is believed that the bispecific binding molecules described herein not only bind tumors to T cells, they also cross-link CD3 on T cells and initiate the activation cascade, and, this way, T cell receptor (TCR)-based cytotoxicity is redirected to desired tumor targets, bypassing major histocompatibility complex (MHC) restrictions.

5.1 BISPECIFIC BINDING MOLECULES

[0059] Provided herein are bispecific binding molecules that bind to HER2 and CD3. A binding molecule, which can be used within the methods provided herein, is a bispecific binding molecule comprising an aglycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, and wherein the first and second fusion polypeptides are identical.

[0060] HER2 is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases. In a specific embodiment, HER2 is human HER2. GenBank™ accession number NM_004448.3 (SEQ ID NO: 1) provides an exemplary human HER2 nucleic acid sequence. GenBank™ accession number NP_004439.2 (SEQ ID NO: 2) provides an exemplary human HER2 amino acid sequence. In another specific embodiment, HER2 is canine HER2. GenBank™ accession number NM_001003217.1 (SEQ ID NO: 3) provides an exemplary canine HER2 nucleic acid sequence. GenBank™ accession number NP_001003217.1 (SEQ ID NO: 4) provides an exemplary canine HER2 amino acid sequence.

[0061] CD3 is a T cell co-receptor comprised of a gamma chain, a delta chain, and two epsilon chains. In a specific embodiment, CD3 is a human CD3. GenBank™ accession number NM_000073.2 (SEQ ID NO: 5) provides an exemplary human CD3 gamma nucleic acid sequence. GenBank™ accession number NP_000064.1 (SEQ ID NO: 6) provides an exemplary human CD3 gamma amino acid sequence. GenBank™ accession number NM_000732.4 (SEQ ID NO: 7) provides an exemplary human CD3 delta nucleic acid sequence. GenBank™ accession number NP_000723.1 (SEQ ID NO: 8) provides an exemplary human CD3 delta amino acid sequence. GenBank™ accession number NM_000733.3 (SEQ ID NO: 9) provides an exemplary human CD3 epsilon nucleic acid sequence. GenBank™ accession number NP_000724.1 (SEQ ID NO: 10) provides an exemplary human CD3 epsilon amino acid sequence. In another specific embodiment, CD3 is a canine CD3. GenBank™ accession number NM_001003379.1 (SEQ ID NO: 11) provides an exemplary canine CD3 epsilon nucleic acid

sequence. GenBank™ accession number NP_001003379.1 (SEQ ID NO: 12) provides an exemplary canine CD3 epsilon amino acid sequence.

[0062] The immunoglobulin in the bispecific binding molecules of the invention can be, as non-limiting examples, a monoclonal antibody, a naked antibody, a chimeric antibody, a humanized antibody, or a human antibody. As used herein, the term "immunoglobulin" is used consistent with its well known meaning in the art, and comprises two heavy chains and two light chains. Methods for making antibodies are described in Section 5.3.

[0063] A chimeric antibody is a recombinant protein that contains the variable domains including the complementarity-determining regions (CDRs) of an antibody derived from one species, preferably a rodent antibody, while the constant domains of the antibody molecule is derived from those of a human antibody. For veterinary applications, the constant domains of the chimeric antibody may be derived from that of other species, such as, for example, horse, monkey, cow, pig, cat, or dog.

[0064] A humanized antibody is an antibody produced by recombinant DNA technology, in which some or all of the amino acids of a human immunoglobulin light or heavy chain that are not required for antigen binding (*e.g.*, the constant regions and the framework regions of the variable domains) are used to substitute for the corresponding amino acids from the light or heavy chain of the cognate, nonhuman antibody. By way of example, a humanized version of a murine antibody to a given antigen has on both of its heavy and light chains (1) constant regions of a human antibody; (2) framework regions from the variable domains of a human antibody; and (3) CDRs from the murine antibody. When necessary, one or more residues in the human framework regions can be changed to residues at the corresponding positions in the murine antibody so as to preserve the binding affinity of the humanized antibody to the antigen. This change is sometimes called "back mutation." Similarly, forward mutations may be made to revert back to murine sequence for a desired reason, *e.g.*, stability or affinity to antigen. Without being bound by any theory, humanized antibodies generally are less likely to elicit an immune response in humans as compared to chimeric human antibodies because the former contain considerably fewer non-human components.

[0065] The term "epitope" is art-recognized and is generally understood by those of skill in the art to refer to the region of an antigen that interacts with an antibody. An epitope of a protein

antigen can be linear or conformational, or can be formed by contiguous or noncontiguous amino acid sequences of the antigen.

[0066] A scFv is an art-recognized term. An scFv comprises a fusion protein of the variable regions of the heavy (V_H) and light (V_L) chains of an immunoglobulin, wherein the fusion protein retains the same antigen specificity as the whole immunoglobulin. The V_H is fused to the V_L via a peptide linker (such a peptide linker is sometimes referred to herein as an "intra-scFv peptide linker").

[0067] In certain embodiments of the invention, the scFv has a peptide linker that is between 5-30, 5-25, 5-15, 10-30, 10-20, 10-15, 15-30, or 15-25 amino acid residues in length. In certain embodiments, the scFv peptide linker displays one or more characteristics suitable for a peptide linker known to one of ordinary skill in the art. In certain embodiments, the scFv peptide linker comprises amino acids that allow for scFv peptide linker solubility, such as, for example, serine and threonine. In certain embodiments, the scFv peptide linker comprises amino acids that allow for scFv peptide linker flexibility, such as, for example, glycine. In certain embodiments, the scFv peptide linker connects the N-terminus of the V_H to the C-terminus of the V_L. In certain embodiments, the scFv peptide linker can connect the C-terminus of the V_H to the N-terminus of the V_L. In certain embodiments, the scFv peptide linker is a linker as described in Table 1, below (*e.g.*, any one of SEQ ID NOs: 14, or 35-41). In a preferred embodiment, the peptide linker is SEQ ID NO: 14.

[0068] In certain embodiments of the bispecific binding molecules of the invention, the scFv that binds to CD3 comprises the V_H and the V_L of a CD3-specific antibody known in the art, such as, for example, huOKT3 (*see*, for example, Adair *et al.,* 1994, Hum Antibodies Hybridomas 5 :41-47), YTH12.5 (*see*, for example Routledge *et al.,* 1991, Eur J Immunol, 21: 2717-2725), HUM291 (*see*, for example, Norman *et al.,* 2000, Clinical Transplantation, 70(12): 1707-1712), teplizumab (*see*, for example, Herold *et al.,* 2009, Clin Immunol, 132: 166-173), huCLB-T3/4 (*see*, for example, Labrijn *et al.,* 2013, Proceedings of the National Academy of Sciences, 110(13): 5145-5150), otelixizumab (*see*, for example, Keymeulen *et al.,* 2010, Diabetologia, 53: 614-623), blinatumomab (*see*, for example, Cheadle, 2006, Curr Opin Mol Ther, 8(1): 62-68), MT110 (*see*, for example, Silke and Gires, 2011, MAbs, 3(1): 31-37), catumaxomab (*see*, for example, Heiss and Murawa, 2010, Int J Cancer, 127(9): 2209-2221), 28F11 (*see*, for example, Canadian Patent Application CA 2569509 A1), 27H5 (*see*, for

example, Canadian Patent Application CA 2569509 A1), 23F10 (*see*, for example, Canadian Patent Application CA 2569509 A1), 15C3 (*see*, for example, Canadian Patent Application CA 2569509 A1), visilizumab (*see*, for example, Dean *et al.,* 2012, Swiss Med Wkly, 142: w13711), and Hum291 (*see*, for example, Dean *et al.,* 2012, Swiss Med Wkly, 142: w13711).

[0069] In certain embodiments, the scFv in a bispecific binding molecule of the invention binds to the same epitope as a CD3-specific antibody known in the art. In a specific embodiment, the scFv in a bispecific binding molecule of the invention binds to the same epitope as the CD3-specific antibody huOKT3. Binding to the same epitope can be determined by assays known to one skilled in the art, such as, for example, mutational analyses or crystallographic studies. In certain embodiments, the scFv competes for binding to CD3 with an antibody known in the art. In a specific embodiment, the scFv in a bispecific binding molecule of the invention competes for binding to CD3 with the CD3-specific antibody huOKT3. Competition for binding to CD3 can be determined by assays known to one skilled in the art, such as, for example, flow cytometry. *See*, for example, Section 6.1.2.4. In certain embodiments, the scFv comprises a V_H with at least 85%, 90%, 95%, 98%, or at least 99% similarity to the V_H of a CD3-specific antibody known in the art. In certain embodiments, the scFv comprises the V_H of a CD3-specific antibody known in the art, comprising between 1 and 5 conservative amino acid substitutions. In certain embodiments, the scFv comprises a V_L with at least 85%, 90%, 95%, 98%, or at least 99% similarity to the V_L of a CD3-specific antibody known in the art. In certain embodiments, the scFv comprises the V_L of a CD3-specific antibody known in the art, comprising between 1 and 5 conservative amino acid substitutions.

[0070] Conservative amino acid substitutions are amino acid substitutions that occur within a family of amino acids, wherein the amino acids are related in their side chains. Generally, genetically encoded amino acids are divided into families: (1) acidic, comprising aspartate and glutamate; (2) basic, comprising arginine, lysine, and histidine; (3) non-polar, comprising isoleucine, alanine, valine, proline, methionine, leucine, phenylalanine, tryptophan; and (4) uncharged polar, comprising cysteine, threonine, glutamine, glycine, asparagine, serine, and tyrosine. In addition, an aliphatic-hydroxy family comprises serine and threonine. In addition, an amide-containing family comprises asparagine and glutamine. In addition, an aliphatic family comprises alanine, valine, leucine and isoleucine. In addition, an aromatic family comprises phenylalanine, tryptophan, and tyrosine. Finally, a sulfur-containing side chain family comprises

cysteine and methionine. As an example, one skilled in the art would reasonably expect an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Preferred conservative amino acid substitution groups include: lysine-arginine, alanine-valine, phenylalanine-tyrosine, glutamic acid-aspartic acid, valine-leucine-isoleucine, cysteine-methionine, and asparagine-glutamine.

[0071] In a preferred embodiment, the scFv is derived from the huOKT3 antibody, and thus contains the V_H and V_L of huOKT3 monoclonal antibody (SEQ ID NOS: 15 and 16, respectively). *See*, for example, Van Wauwe *et al.,* 1991, nature, 349: 293-299. In specific embodiments of the bispecific binding molecule, the scFv is derived from the huOKT3 monoclonal antibody and has no more than 5 amino acid mutations relative to native huOKT3 V_H and V_L sequences. In certain embodiments of the bispecific binding molecule, the scFv is derived from the huOKT3 monoclonal antibody and comprises one or more mutations, relative to native huOKT3 V_H and V_L sequences, to stabilize disulfide binding. In certain embodiments of the bispecific binding molecule, the stabilization of disulfide binding prevents aggregation of the bispecific binding molecule. In certain embodiments of the bispecific binding molecule, the stabilization of disulfide binding reduces aggregation of the bispecific binding molecule as compared to aggregation of the bispecific binding molecule without the stabilization of disulfide binding. In certain embodiments of the bispecific binding molecule, the one or more mutations to stabilize disulfide binding comprise a V_H G44C mutation and a V_L Q100C mutation (*e.g.*, as present in SEQ ID NOS: 54-59). In certain embodiments of the bispecific binding molecule, the one or more mutations to stabilize disulfide binding are the replacement of the amino acid residue at V_H44(according to the Kabat numbering system) with a cysteine and the replacement of the amino acid residue at V_L100 (according to the Kabat numbering system) with a cysteine so as to introduce a disulfide bond between V_H44 and V_L100 (*e.g.*, as present in SEQ ID NOS: 54-59). In an especially preferred embodiment, the scFv comprises the V_H of huOKT3 comprising the amino acid substitution at numbered position 105, wherein the cysteine is substituted with a serine (SEQ ID NO: 17). In certain embodiments, the sequence of the V_H of the scFv is as described in Table 4, below (*e.g.*, any one of SEQ ID NOs: 15, 17, or 64). In certain

embodiments, the sequence of the V_L of the scFv is as described in Table 5, below (*e.g.*, any one of SEQ ID NOs: 16 or 65). In certain embodiments, the sequence of the scFv is as described in Table 6, below (*e.g.*, any one of SEQ ID NOs: 19 or 48-59). In a preferred embodiment, the sequence of the scFv is SEQ ID NO: 19. In a specific embodiment, the scFv comprises a variant of the V_H of huOKT3 that has no more than 5 amino acid mutations relative to the native sequence of huOKT3 V_H. In a specific embodiment, the scFv comprises a variant of the V_L of huOKT3 that has no more than 5 amino acid mutations relative to the native sequence of huOKT3 V_L.

[0072] The sequences of the variable regions of an anti-CD3 scFv may be modified by insertions, substitutions and deletions to the extent that the resulting scFv maintains the ability to bind to CD3, as determined by, for example, ELISA, flow cytometry, and BiaCore™. The ordinarily skilled artisan can ascertain the maintenance of this activity by performing the functional assays as described herein below, such as, for example, binding analyses and cytotoxicity analyses.

[0073] In certain embodiments, the peptide linker conjugating the immunoglobulin light chain and the scFv is between 5-30, 5-25, 5-15, 10-30, 10-20, 10-15, 15-30, or 15-25 amino acids in length. In certain embodiments, the peptide linker displays one or more characteristics suitable for a peptide linker known to one of ordinary skill in the art. In certain embodiments, the peptide linker comprises amino acids that allow for peptide linker solubility, such as, for example, serine and threonine. In certain embodiments, the peptide linker comprises amino acids that allow for peptide linker flexibility, such as, for example, glycine. In certain embodiments, the sequence of the peptide linker conjugating the immunoglobulin light chain and the scFv is as described in Table 1, below (*e.g.*, any one of SEQ ID NOs: 14 or 35-41). In preferred embodiments, the peptide linker is SEQ ID NO: 14.

[0074] In certain embodiments of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 comprises the heavy chain and/or the light chain of a HER2-specific antibody known in the art, such as, for example, trastuzumab (*see*, for example, Baselga et al. 1998, Cancer Res 58(13): 2825-2831), M-111 (*see*, for example, Higgins *et al.,* 2011, J Clin Oncol, 29(Suppl): Abstract TPS119), pertuzumab (*see*, for example, Franklin *et al.,* 2004, Cancer Cell, 5: 317-328), ertumaxomab (*see*, for example, Kiewe and Thiel, 2008, Expert Opin Investig Drugs, 17(10): 1553-1558), MDXH210 (*see*, for example, Schwaab *et al.,* 2001,

Journal of Immunotherapy, 24(1): 79-87), 2B1 (*see*, for example, Borghaei *et al.,* 2007, J Immunother, 30: 455-467), and MM-302 (*see*, for example, Wickham and Futch, 2012, Cancer Research, 72(24): Supplement 3). In certain embodiments of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 comprises the heavy chain of trastuzumab. In certain embodiments of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 comprises the sequence as set forth in SEQ ID NO: 23. In certain embodiments of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 comprises a variant of the heavy chain of trastuzumab (*see*, *e.g.*, Table 2, below). In a specific embodiment of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 comprises a variant of the light chain of trastuzumab that has no more than 5 amino acid mutations relative to the native sequence of trastuzumab. In certain embodiments of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 comprises the light chain of trastuzumab (SEQ ID NO: 25). In certain embodiments of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 comprises a variant of the light chain of trastuzumab. In a specific embodiment of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 comprises a variant of the light chain of trastuzumab that has no more than 5 amino acid mutations relative to the native sequence of trastuzumab.

[0075] In certain embodiments of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 binds to the same epitope as a HER2-specific antibody known in the art. In a specific embodiment, the immunoglobulin in a bispecific binding molecule of the invention binds to the same epitope as trastuzumab. Binding to the same epitope can be determined by assays known to one skilled in the art, such as, for example, mutational analyses or crystallographic studies. In certain embodiments, the immunoglobulin that binds to HER2 competes for binding to HER2 with an antibody known in the art. In a specific embodiment, the immunoglobulin in a bispecific binding molecule of the invention competes for binding to HER2 with trastuzumab. Competition for binding to HER2 can be determined by assays known to one skilled in the art, such as, for example, flow cytometry. *See*, for example, Section 6.1.2.4. In certain embodiments, the immunuglobulin comprises a V_H with at least 85%, 90%, 95%, 98%, or at least 99% similarity to the V_H of a HER2-specific antibody known in the art. In certain embodiments, the immunoglobulin comprises the V_H of a HER2-specific antibody

known in the art, comprising between 1 and 5 conservative amino acid substitutions. In certain embodiments, the immunoglobulin comprises a V_L with at least 85%, 90%, 95%, 98%, or at least 99% similarity to the V_L of a HER2-specific antibody known in the art. In certain embodiments, the immunoglobulin comprises the V_L of a HER2-specific antibody known in the art, comprising between 1 and 5 conservative amino acid substitutions. In certain embodiments, the immunoglobulin comprises a V_H of a heavy chain described in Table 2, below (*e.g.*, the V_H of any one of SEQ ID NOs: 23, 27, 62, or 63). In certain embodiments, the immunoglobulin comprises a V_L of a light chain described in Table 3, below (*e.g.*, the V_L of SEQ ID NO: 25).

[0076] The sequences of the variable regions of an anti-HER2 antibody may be modified by insertions, substitutions and deletions to the extent that the resulting antibody maintains the ability to bind to HER2, as determined by, for example, ELISA, flow cytometry, and BiaCore™. The ordinarily skilled artisan can ascertain the maintenance of this activity by performing the functional assays as described herein below, such as, for example, binding analyses and cytotoxicity analyses.

[0077] In certain embodiments of the bispecific binding molecules of the invention, the immunoglobulin that binds to HER2 is an IgG1 immunoglobulin.

[0078] Methods of producing human antibodies are known to one skilled in the art, such as, for example, phage display methods described above using antibody libraries derived from human immunoglobulin sequences. *See* also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/60433, WO 98/24893, WO 98/16664, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety. The techniques of Cole *et al.,* and Boerder *et al.,* are also available for the preparation of human monoclonal antibodies (Cole *et al.,* Monoclonal Antibodies and Cancer Therapy, Alan R. Riss, (1985); and Boerner *et al.,* J. Immunol., 147(1):86-95, (1991)).

[0079] In certain embodiments, human antibodies are produced using transgenic mice, which are incapable of expressing functional endogenous mouse immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be

rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, for example, all or a portion of a polypeptide provided herein. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, *see* Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, *see*, for example, PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,886,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, Calif.), Genpharm (San Jose, Calif.), and Medarex, Inc. (Princeton, N.J.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0080] Human monoclonal antibodies can also be made by immunizing mice transplanted with human peripheral blood leukocytes, splenocytes or bone marrows (*e.g.*, Trioma techniques of XTL). Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, for example, a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. *See*, for example, Jespers *et al.,* Bio/technology 12:899-903 (1988). Human antibodies may also be generated by in vitro activated B cells. *See* U.S. Pat. Nos. 5,567,610 and 5,229,275, which are incorporated in their entirety by reference.

[0081] Methods for making humanized antibodies are known to one skilled in the art. *See*, for example, Winter EP 0 239 400; Jones *et al.,* Nature 321:522-525 (1986); Riechmann *et al.,* Nature 332:323-327 (1988); Verhoeyen *et al.,* Science 239: 1534-1536 (1988); Queen *et al.,* Proc. Nat. Acad. ScL USA 86:10029 (1989); U.S. Pat. No. 6,180,370; and Orlandi *et al.,* Proc. Natl. Acad. Sd. USA 86:3833 (1989); the disclosures of all of which are incorporated by reference herein in their entireties. Generally, the transplantation of murine (or other non-human) CDRs onto a human antibody is achieved as follows. The cDNAs encoding heavy and light chain variable domains are isolated from a hybridoma. The DNA sequences of the variable domains, including the CDRs, are determined by sequencing. The DNAs, encoding the CDRs are inserted into the corresponding regions of a human antibody heavy or light chain variable domain coding sequences, attached to human constant region gene segments of a desired isotype (*e.g.*, gamma-l for CH and K for C_L), are gene synthesized. The humanized heavy and light chain genes are co-expressed in mammalian host cells (*e.g.*, CHO or NSO cells) to produce soluble humanized antibody. To facilitate large scale production of antibodies, it is often desirable select for high expressor using a DHFR gene or GS gene in the producer line. These producer cell lines are cultured in bioreactors, or hollow fiber culture system, or WAVE technology, to produce bulk cultures of soluble antibody, or to produce transgenic mammals (*e.g.*, goats, cows, or sheep) that express the antibody in milk (*see*, *e.g.*, U.S. Pat. No. 5,827,690).

[0082] Antibody fragments can be produced by enzymatic cleavage, synthetic or recombinant techniques, as known in the art and/or as described herein. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a combination gene encoding a $F(ab')_2$ heavy chain portion can be designed to include DNA sequences encoding the CH, domain and/or hinge region of the heavy chain. The various portions of antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques. In certain embodiments, elements of a human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.,* Nature Genet. 7:13 (1994),

Lonberg *et al.,* Nature 368:856 (1994), and Taylor *et al.,* Int. Immun. 6:579 (1994). A fully human antibody also can be constructed by genetic or chromosomal transfection methods, as well as phage display technology, all of which are known in the art. *See*, for example, McCafferty *et al.,* Nature 348:552-553 (1990) for the production of human antibodies and fragments thereof in vitro, from immunoglobulin variable domain gene repertoires from unimmunized donors. In this technique, antibody variable domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. In this way, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats, for their review, *see e.g.* Johnson and Chiswell, Current Opinion in Structural Biology 3:5564-571 (1993).

[0083] Antibody humanization can also be performed by, for example, synthesizing a combinatorial library comprising the six CDRs of a non-human target monoclonal antibody fused in frame to a pool of individual human frameworks. A human framework library that contains genes representative of all known heavy and light chain human germline genes can be utilized. The resulting combinatorial libraries can then be screened for binding to antigens of interest. This approach can allow for the selection of the most favorable combinations of fully human frameworks in terms of maintaining the binding activity to the parental antibody. Humanized antibodies can then be further optimized by a variety of techniques.

[0084] Antibody humanization can be used to evolve mouse or other non-human antibodies into "fully human" antibodies. The resulting antibody contains only human sequence and no mouse or non-human antibody sequence, while maintaining similar binding affinity and specificity as the starting antibody.

[0085] For full length antibody molecules, the immunoglobulin genes can be obtained from genomic DNA or mRNA of hybridoma cell lines. Antibody heavy and light chains are cloned in a mammalian vector system. Assembly is documented with double strand sequence analysis. The antibody construct can be expressed in other human or mammalian host cell lines. The construct can then be validated by transient transfection assays and Western blot analysis of the

expressed antibody of interest. Stable cell lines with the highest productivity can be isolated and screened using rapid assay methods.

[0086] In one approach, a hybridoma is produced by fusing a suitable immortal cell line (*e.g.*, a myeloma cell line such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U937, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMAIWA, NEURO 2A), or the like, or heteromylomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art. *See*, for example, the ATCC or LifeTech website, and the like, with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, avian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. *See*, for example, Ausubel, *supra*, and Colligan, Immunology, *supra*, chapter 2, entirely incorporated herein by reference. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Cells which produce antibodies with the desired specificity can be selected by a suitable assay (*e.g.*, ELISA).

[0087] In a preferred specific embodiment, the bispecific binding molecule comprises a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region, such that said molecule does not bind or has reduced binding to an Fc receptor (FcR), in soluble form or cell-bound form (including on immune-effector cells, such as, for example, NK cells, monocytes, and neutrophils). These FcRs include, but are not limited to, FcR1 (CD64), FcRII (CD32), and FcRIII (CD16). The affinity to FcR(n), the neonatal Fc receptor, is not affected, and thus maintained in the bispecific binding molecule. For example, if the immunoglobulin is an IgG, preferably, the IgG has reduced or no affinity for an Fc gamma receptor. In certain embodiments, one or more positions within the Fc region that makes a direct contact with Fc gamma receptor, such as, for example, amino acids 234-239

(hinge region), amino acids 265-269 (B/C loop), amino acids 297-299 (C'/E loop), and amino acids 327-332 (F/G) loop, are mutated such that the bispecific binding molecule has a decreased or no affinity for an Fc gamma receptor. *See*, for example, Sondermann *et al.,* 2000, Nature, 406: 267-273, which is incorporated herein by reference in its entirety. Preferably, for an IgG, the mutation N297A is made to destroy Fc receptor binding. In certain embodiments, affinity of the bispecific binding molecule or fragment thereof for an Fc gamma receptor is determined by, for example, BiaCore™ assay, as described, for example, in Okazaki *et al.,* 2004. J Mol Biol, 336(5):1239-49. *See* also, Section 6. In certain embodiments, the bispecific binding molecule comprising such a variant Fc region binds an Fc receptor on a FcR-bearing immune-effector cell with less than 25%, 20%, 15%, 10%, or 5% binding as compared to a reference Fc region. Without being bound by any particular theory, a bispecific binding molecule comprising such a variant Fc region will have a decreased ability to induce a cytokine storm. In preferred embodiments, the bispecific binding molecule comprising such a variant Fc region does not bind an Fc receptor in soluble form or as a cell-bound form.

[0088] In certain embodiments, the bispecific binding molecule comprises a variant Fc region, such as, for example, an Fc region with additions, deletions, and/or substitutions to one or more amino acids in the Fc region of an antibody provided herein in order to alter effector function, or enhance or diminish affinity of antibody to FcR. In a preferred embodiment, the affinity of the antibody to FcR is diminished. Reduction or elimination of effector function is desirable in certain cases, such as, for example, in the case of antibodies whose mechanism of action involves blocking or antagonism but not killing of the cells bearing a target antigen. In certain embodiments, the Fc variants provided herein may be combined with other Fc modifications, including but not limited to modifications that alter effector function. In certain embodiments, such modifications provide additive, synergistic, or novel properties in antibodies or Fc fusions. Preferably, the Fc variants provided herein enhance the phenotype of the modification with which they are combined.

[0089] In preferred embodiments, the bispecific binding molecule of the invention is aglycosylated. Preferably, this is achieved by mutating the anti-HER2 immunoglobulin portion of the bispecific binding molecule in its Fc receptor to destroy a glycosylation site, preferably an N-linked glycosylation site. In another specific embodiment, an immunoglobulin is mutated to destroy an N-linked glycosylation site. In certain preferred embodiments, the bispecific binding

molecule has been mutated to destroy an N-linked glycosylation site. In certain embodiments, the heavy chain of the bispecific binding molecule has an amino acid substitution to replace an asparagine that is an N-linked glycosylation site, with an amino acid that does not function as a glycosylation site. In a preferred embodiment, the method encompasses deleting the glycosylation site of the Fc region of a bispecific binding molecule, by modifying position 297 from asparagine to alanine (N297A). For example, in certain embodiments, the bispecific binding molecule comprises a heavy chain with the sequence of SEQ ID NO: 20. As used herein, "glycosylation sites" include any specific amino acid sequence in an antibody to which an oligosaccharide (i.e., carbohydrates containing two or more simple sugars linked together) will specifically and covalently attach. Oligosaccharide side chains are typically linked to the backbone of an antibody via either N- or O-linkages. N-linked glycosylation refers to the attachment of an oligosaccharide moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of an oligosaccharide moiety to a hydroxyamino acid, *e.g.*, serine, threonine. Methods for modifying the glycosylation content of antibodies are well known in the art, *see*, for example, U.S. Pat. No. 6,218,149; EP 0 359 096 B1; U.S. Publication No. US 2002/0028486; WO 03/035835; U.S. Publication No. 2003/0115614; U.S. Pat. No. 6,218,149; U.S. Pat. No. 6,472,511; all of which are incorporated herein by reference in their entirety. In another embodiment, aglycosylation of the bispecific binding molecules of the invention can be achieved by recombinantly producing the bispecific binding molecule in a cell or expression system incapable of glycosylation, such as, for example, bacteria. In another embodiment, aglycosylation of the bispecific binding molecules of the invention can be achieved by enzymatically removing the carbohydrate moieties of the glycosylation site.

[0090] In preferred embodiments, the bispecific binding molecule of the invention does not bind or has reduced binding affinity (relative to a reference or wild type immunoglobulin) to the complement component C1q. Preferably, this is achieved by mutating the anti-HER2 immunoglobulin portion of the bispecific binding molecule to destroy a C1q binding site. In certain preferred embodiments, the method encompasses deleting the C1q binding site of the Fc region of an antibody, by modifying position 322 from lysine to alanine (K322A). For example, in certain embodiments, the bispecific binding molecule comprises a heavy chain with the sequence of SEQ ID NO: 21. In certain embodiments, affinity of the bispecific binding molecule or fragment thereof for the complement component C1q is determined by, for example,

BiaCore™ assay, as described, for example, in Okazaki *et al.,* 2004. J Mol Biol, 336(5):1239-49. *See* also, Section 6. In certain embodiments, the bispecific binding comprising an anti-HER2-immunoglobulin comprising a destroyed C1q binding site binds the complement component C1q with less than 25%, 20%, 15%, 10%, or 5% binding compared to a reference or wild type immunoglobulin. In certain embodiments, the bispecific binding molecule does not activate complement.

[0091] In preferred embodiments, the bispecific binding molecule of the invention comprises an immunoglobulin, wherein the immunoglobulin (i) comprises at least one amino acid modification relative to a wild-type Fc region, such that said molecule does not bind or has reduced binding to an Fc receptor in soluble form or as cell-bound form; (ii) comprises one or more mutations in the Fc region to destroy an N-linked glycosylation site; and (iii) does not or has reduced binding to the complement component C1q. For example, in certain embodiments, the bispecific binding molecule comprises an IgG comprising a first mutation, N297A, in the Fc region to (i) abolish or reduce binding to an Fc receptor in soluble form or as cell-bound form; and (ii) destroy an N-linked glycosylation site in the Fc region; and a second mutation, K322A, in the Fc region to (iii) abolish or reduce binding to the complement component C1q. *See*, for example, SEQ ID NO: 27.

[0092] In a preferred embodiment, the immunoglobulin that binds to HER2 comprises the variable regions of trastuzumab (*see, e.g.,* Tables 2 and 3), and preferably a human IgG1 constant region. In a preferred embodiment, the immunoglobulin that binds to HER2 comprises the variable regions of trastuzumab wherein the sequence of the heavy chain is SEQ ID NO: 27 and wherein the sequence of the light chain is SEQ ID NO: 25. In a preferred embodiment, the immunoglobulin that binds to HER2 is a variant of trastuzumab, wherein the heavy chain does not bind or has reduced binding to an Fc receptor in soluble form or as cell-bound form. In a preferred embodiment, the heavy chain that does not bind an Fc receptor in soluble form or as a cell-bound form comprises a mutation in the Fc region to destroy an N-linked glycosylation site. In a preferred embodiment, the heavy chain has an amino acid substitution to replace an asparagine that is an N-linked glycosylation site, with an amino acid that does not function as a glycosylation site. In a preferred embodiment, the mutation to destroy an N-linked glycosylation site is N297A in the Fc region (SEQ ID NO: 20). In a preferred embodiment, the immunoglobulin that binds to HER2 comprises the variable regions of trastuzumab, wherein the

sequence of the heavy chain comprises a mutation in the Fc region to destroy a C1q binding site. In a preferred embodiment, the immunoglobulin does not activate complement. In a preferred embodiment, the mutation to destroy a C1q binding site is K322A in the Fc region (SEQ ID NO: 21). In an especially preferred embodiment, the immunoglobulin that binds to HER2 comprises the variable regions of trastuzumab, wherein the immunglobulin heavy chain comprises a mutation in the Fc region to destroy an N-linked glycosylation site and a mutation in the Fc region to destroy a C1q binding site (*see*, for example, SEQ ID NO: 27). In an especially preferred embodiment, the immunoglobulin that binds to HER2 comprises the variable regions of trastuzumab wherein the sequence of the heavy chain of the immunoglobulin has been mutated in the Fc region and is SEQ ID NO: 27 and wherein the sequence of the light chain is SEQ ID NO: 25. In an especially preferred embodiment, the sequence of the light chain fusion polypeptide is SEQ ID NO: 29. In certain embodiments, the heavy chain comprises the constant region of trastuzumab. In certain embodiments, the heavy chain comprises the constant region of a heavy chain described in Table 2, below (*e.g.*, the constant region of any one of SEQ ID NOs: 23, 27, 62, or 63). In certain embodiments, the sequence of the heavy chain is as described in Table 2, below (*e.g.*, any one of SEQ ID NOs: 23, 27, 62, or 63). In certain embodiments, the light chain comprises the constant region of a light chain described in Table 3, below (*e.g.*, the constant region of SEQ ID NO: 25). In certain embodiments, the sequence of the light chain is as described in Table 3, below (*e.g.*, SEQ ID NO: 25).

[0093] In certain embodiments, the bispecific binding molecule has a trastuzumab-derived sequence that contains one or more of the modifications in the trastuzumab immunoglobulin, and has a huOKT3-derived sequence that contains one or more of the modifications in the huOKT3 V_H and V_L sequences, as described in Table 8, below. Bispecific binding molecules having other immunoglobulin or scFv sequences can contain analogous mutations at corresponding positions in these other immunoglobulin or scFv sequences. In certain embodiments, the bispecific binding molecule is (a) derived from trastuzumab and huOKT3; and (b) contains one or more of the modifications as described in Table 8, below. In certain embodiments, the sequence of the peptide linker conjugating the immunoglobulin light chain and the scFv is as described in Table 1, below (*e.g.*, any one of SEQ ID NOs: 14 or 35-41). In certain embodiments, the sequence of the heavy chain is as described in Table 2, below (*e.g.*, any one of SEQ ID NOs: 23, 27, 62, or 63). In certain embodiments, the sequence of the light chain is as described in Table 3, below

(*e.g.*, SEQ ID NO: 25). In certain embodiments, the sequence of the V_H of the scFv is as described in Table 4, below (*e.g.*, any one of SEQ ID NOs: 15, 17, or 64). In certain embodiments, the sequence of the V_L of the scFv is as described in Table 5, below (*e.g.*, any one of SEQ ID NOs: 16 or 65). In certain embodiments, the sequence of the scFv peptide linker is as described in Table 1, below (*e.g.*, any one of SEQ ID NOs: 14 or 35-41). In certain embodiments, the sequence of the scFv is as described in Table 6, below (*e.g.*, any one of SEQ ID NOs: 19 48-59, or 66). In certain embodiments, the sequence of the light chain fusion polypeptide is as described in Table 7, below (*e.g.*, any one of SEQ ID NOs: 29, 34, 42-47, or 60).

[0094] In certain embodiments, the bispecific binding molecule comprises a glycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, wherein the first and second light chain fusion polypeptides are identical, wherein the sequence of each heavy chain is SEQ ID NO: 62, and wherein the sequence of each light chain fusion polypeptide is SEQ ID NO: 60.

[0095] In certain embodiments, the bispecific binding molecule comprises a glycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, wherein the first and second light chain fusion polypeptides are identical, wherein the sequence of each heavy chain is SEQ ID NO: 27, and wherein the sequence of each light chain fusion polypeptide is SEQ ID NO: 47.

[0096] In certain embodiments, the bispecific binding molecule comprises a glycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical

heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, wherein the first and second light chain fusion polypeptides are identical, wherein the sequence of each heavy chain is SEQ ID NO: 27, and wherein the sequence of each light chain fusion polypeptide is SEQ ID NO: 29.

[0097] In certain embodiments, the bispecific binding molecule has low immunogenicity. Low or acceptable immunogenicity and/or high affinity, as well as other suitable properties, can contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as raising significant HAHA, HACA or HAMA responses in less than about 75%, or preferably less than about 50% of the patients treated and/or raising low titres in the patient treated (Elliott *et al.,* Lancet 344:1125-1127 (1994), entirely incorporated herein by reference).

[0098] The bispecific binding molecules provided herein can bind HER2 and CD3 with a wide range of affinities. The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method. *See*, for example, Berzofsky, *et al.,* "Antibody-Antigen Interactions," In Fundamental Immunology, Paul, W. E., Ed., Raven Press: New York, N.Y. (1984); Kuby, Janis Immunology, W.H. Freeman and Company: New York, N.Y. (1992); and methods described herein. The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (*e.g.*, salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters are preferably made with standardized solutions of antibody and antigen, and a standardized buffer, such as the buffer described herein. The affinity, K_D is a ratio of k_{on}/k_{off}. Generally, a K_D in the micromolar range is considered low affinity. Generally, a K_D in the picomolar range is considered high affinity. In another specific embodiment, the bispecific binding molecule has high affinity for HER2 and low affinity for CD3. In another specific embodiment, the bispecific binding molecule has high affinity for HER2 and average affinity for CD3. In a specific embodiment, the bispecific binding molecule has a K_D of between 70 nM and 1 μM for CD3. In a specific embodiment, the bispecific binding molecule has a K_D of between 70 nM and 500 nM for CD3. In a specific embodiment, the bispecific binding molecule has a K_D of between 500 nM and 1 μM for CD3.

[0099] In certain embodiments, the bispecific binding molecule binds to one or more HER2-positive carcinoma cell lines, as determined by assays known to one skilled in the art, such as, for example, ELISA, BiaCore™, and flow cytometry. In certain embodiments, the carcinoma cell line is a breast carcinoma cell line, such as, for example, MDA-MB-361, MDA-MB-468, AU565, SKBR3, HTB27, HTB26, HCC1954, and/or MCF7. In certain embodiments, the carcinoma cell line is an ovarian carcinoma cell line, such as, for example, OVCAR3 and/or SKOV3. In certain embodiments, the carcinoma cell line is a melanoma cell line, such as, for example, HT144, SKMEL28, M14, and/or HTB63. In certain embodiments, the carcinoma cell line is an osteosarcoma cell line, such as, for example, RG160, RG164, CRL1427, and/or U2OS. In certain embodiments, the carcinoma cell line is a Ewings sarcoma cell line, such as, for example, SKEAW and/or SKES-1. In certain embodiments, the carcinoma cell line is a rhabdomyosarcoma cell line, such as, for example, HTB82. In certain embodiments, the carcinoma cell line is a neuroblastoma cell line, such as, for example, NMB7, SKNBE(2)C, IMR32, SKNBE(2)S, SKNBE(1)N, and/or NB5. In certain embodiments, the carcinoma cell line is a squamous cell carcinoma head and neck (SCCHN) cell lines, such as, for example, 15B, 93-VU-147T, PCI-30, UD-SCC2, PCI-15B, SCC90, and/or UMSCC47. In certain embodiments, the carcinoma cell line is a cervical cancer cell line, such as, for example, HeLa. In certain embodiments, the carcinoma cell line is a small cell lung cancer cell line, such as, for example, NCI-H524, NCI-H69, and/or NCI-H345. In certain embodiments, the bispecific binding molecule binds to the HER2-positive carcinoma cell line with an EC50 in the picomolar range. *See*, for example, Section 6.1.3.4 and Section 6.1.3.6.

[00100] In certain embodiments, the bispecific binding molecule binds to CD3+ T cells, as determined by assays known to one skilled in the art, such as, for example, ELISA, BiaCore™, and flow cytometry. In certain preferred embodiments, the bispecific binding molecule binds to CD3+ T cells with greater than 15-fold less binding than huOKT3 binding to CD3+ T cells. *See*, for example, Section 6.1.3.1. In certain embodiments, the CD3+ T cells are human T cells.

[00101] In certain embodiments, the bispecific binding molecule the bispecific binding molecule mediates T cell cytotoxicity against HER2-positive cells, as determined by assays known to one skilled in the art, such as, for example, cytotoxicity assays. In preferred embodiments, the bispecific binding molecule mediates T cell cytotoxicity against HER2-positive cell lines with an EC50 in the picomolar range. In certain embodiments, the HER2-

positive cells are breast carcinoma cell lines, such as, for example, MDA-MB-361, MDA-MB-468, AU565, SKBR3, HTB27, HTB26, and/or MCF7. In certain embodiments, the HER2-positive cells are ovarian carcinoma cell lines, such as, for example, OVCAR3 and/or SKOV3. In certain embodiments, the HER2-positive cells are melanoma cell lines, such as, for example, HT144, SKMEL28, M14, and/or HTB63. In certain embodiments, the HER2-positive cells are osteosarcoma cell lines, such as, for example, RG160, RG164, CRL1427, and/or U2OS. In certain embodiments, the HER2-positive cells are Ewings sarcoma cell lines, such as, for example, SKEAW and/or SKES-1. In certain embodiments, the HER2-positive cells are rhabdomyosarcoma cell lines, such as, for example, HTB82. In certain embodiments, the HER2-positive cells are neuroblastoma cell lines, such as, for example, NMB7, SKNBE(2)C, IMR32, SKNBE(2)S, SKNBE(1)N, and/or NB5. In certain embodiments, the HER2-positive cells are squamous cell carcinoma head and neck (SCCHN) cell lines, such as, for example, 15B, 93-VU-147T, PCI-30, UD-SCC2, PCI-15B, SCC90, and/or UMSCC47. In certain embodiments, the HER2-positive cells are a cervical cancer cell line, such as, for example, HeLa. In certain embodiments, the HER2-positive cells are a small cell lung cancer cell line, such as, for example, NCI-H524, NCI-H69, and/or NCI-H345. *See*, for example, Section 6.1.3.4 and Section 6.1.3.6.

[00102] In certain embodiments, preincubation of HER2-positive cells with huOKT3 blocks the ability of the bispecific binding molecule to induce T cell cytotoxicity. In certain embodiments, preincubation of HER2-positive cells with trastuzumab blocks the ability of the bispecific binding molecule to induce T cell cytotoxicity. *See*, for example, Section 6.1.3.3.

[00103] In certain embodiments, the bispecific binding molecule mediates T cell cytotoxicity against HER2-positive cells, wherein the level of HER2-expression in said cells is below the threshold of detection by flow cytometry performed with the bispecific binding molecule. *See*, for example, Section 6.1.3.4.

[00104] In certain embodiments, the bispecific binding molecule mediates T cell cytotoxicity against HER2-positive cells resistant to other HER-targeted therapies, such as, for example, trastuzumab, cetuximab, lapatinib, erlotinib, neratinib, or any other small molecule or antibody that targets the HER family of receptors. In a specific embodiment, the tumor that is resistant to HER-targeted therapies, such as, for example, trastuzumab, cetuximab, lapatinib, erlotinib, neratinib, or any other small molecule or antibody that targets the HER family of receptors is

responsive to treatment with a bispecific binding molecule to the invention. *See*, for example, Section 6.1.3.7, Section 6.1.3.8, Section 6.1.3.9, and Section 6.1.3.10.

[00105] In certain embodiments, the bispecific binding molecule reduces HER2-positive tumor progression, metastasis, and/or tumor size. *See*, for example, Section 6.1.3.11.

[00106] In certain embodiments, the bispecific binding molecule is bound to a T cell. In certain embodiments, the binding of the bispecific binding molecule to a T cell is noncovalently. In certain embodiments, the T cell is administered to a subject. In certain embodiments, the T cell is autologous to the subject to whom the T cell is to be administered. In certain embodiments, the T cell is allogeneic to the subject to whom the T cell is to be administered. In certain embodiments, the T cell is a human T cell.

[00107] In certain embodiments, the bispecific binding molecule is not bound to a T cell.

[00108] In certain embodiments, the bispecific binding molecule is conjugated to an organic moiety, a detectable marker, and/or isotope as described in Section 5.2.

[00109] In certain embodiments, the bispecific binding molecule or fragment thereof is produced as described in Section 5.3. In certain embodiments, the bispecific binding molecule or fragment thereof is encoded by a polynucleotide as described in Section 5.3.1. In certain embodiments, the bispecific binding molecule or fragment thereof is encoded by a vector (*e.g.*, expression vector) as described in Section 5.3.2. In certain embodiments, the bispecific binding molecule or fragment thereof is produced from a cell as described in Section 5.3.2.

[00110] In certain embodiments, the bispecific binding molecule is a component of a composition (*e.g.*, pharmaceutical composition) and/or as part of a kit as described in Section 5.5.

[00111] In certain embodiments, the bispecific binding molecule is used according to the methods provided in Section 5.6. In certain embodiments, the bispecific binding molecule is used as a diagnostic tool according to the methods provided in Section 5.6.2. In certain embodiments, the bispecific binding molecule is used as a therapeutic according to the methods provided in Section 5.6.1. In certain embodiments, the bispecific binding molecule is administered to a subject, such as a subject described in Section 5.7, for use according to the methods provided in Section 5.6. In certain embodiments, the bispecific binding molecule is administered to a subject as part of a combination therapy as described in Section 5.9, for use according to the methods provided in Section 5.6.

[00112] Table. 1. Linker Sequence

DESCRIPTION	SEQUENCE (SEQ ID NO:)
$(G_4S)_3$	GGGGSGGGGSGGGGS (SEQ ID NO: 14)
$TS(G_4S)_3$ Linker	TSGGGGSGGGGSGGGGS (SEQ ID NO: 35)
G_4S Linker	GGGGS (SEQ ID NO: 36)
$(G_4S)_2$ Linker	GGGGSGGGGS (SEQ ID NO: 37)
$(G_4S)_3$ Linker	GGGGSGGGGSGGGGS (SEQ ID NO: 38)
$(G_4S)_4$ Linker	GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 39)
$(G_4S)_5$ Linker	GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 40)
$(G_4S)_6$ Linker	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 41)

[00113] Table 2. Heavy Chain Sequence. The non-italicized, non-underlined sequence represents the V_H domain. The italicized sequence represents the constant region. The underlined, italicized, and bold sequences represent the mutations described in the "DESCRIPTION" column.

DESCRIPTION	SEQUENCE (SEQ ID NO:)
Trastuzumab V_H domain with human IgG1 constant region	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKG LEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS*ASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO: 23)
Trastuzumab V_H domain with human IgG1 constant region; N297A; K322A	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKG LEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS*ASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQY**A**STYRVVSVLTVLHQDWLNGKEY KC**A**VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO: 27)
Trastuzumab V_H domain with human IgG1 constant region; N297A	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKG LEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS*ASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQY**A**STYRVVSVLTVLHQDWLNGKEY*

	KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 62)
Trastuzumab V_H domain with human IgG1 constant region; K322A	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKG LEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS*ASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KC**A**VSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK* (SEQ ID NO:63)

[00114] Table 3. Light Chain Sequence. The non-italicized sequence represents the V_L domain. The italicized sequence represents the constant region.

DESCRIPTION	SEQUENCE (SEQ ID NO:)
Trastuzumab light chain	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGECTS* (SEQ ID NO: 25)

[00115] Table 4. scFv V_H Sequence. The underlined, italicized, and bold sequences represent the mutations described in the "DESCRIPTION" column.

DESCRIPTION	SEQUENCE (SEQ ID NO:)
huOKT3 V_H	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHYCLDYWGQGTPVTVSS (SEQ ID NO: 15)
huOKT3 V_H; C105S	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS (SEQ ID NO: 17)
huOKT3 V_H; C105S + V_H-G44C	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK ***C***LEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS (SEQ ID NO: 64)

[00116] Table 5. scFv V_L Sequence. The underlined, italicized, and bold sequence represent the mutations described in the "DESCRIPTION" column.

DESCRIPTION	SEQUENCE (SEQ ID NO:)
huOKT3 V_L	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKR WIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWS SNPFTFGQGTKLQITR (SEQ ID NO: 16)
huOKT3 V_L; V_L-	DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKR

Q100C	WIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWS SNPFTFG***C***GTKLQITR (SEQ ID NO:65)

[00117] Table 6. scFv Sequence. The uppercase, non-italicized, non-bold, non-underlined sequence represents the V_H domain. The uppercase, italicized sequence represents the V_L domain. The uppercase, underlined, italicized, and bold sequences represent the mutations described in the "DESCRIPTION" column. The lowercase bold sequences represent the intra-scFv linker.

DESCRIPTION	SEQUENCE (SEQ ID NO:)
huOKT3 scFv C105S; 15 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggs***DI QMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDT SKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGQ GTKLQITR* (SEQ ID NO: 19)
huOKT3 scFv C105S; 5 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggs***DIQMTQSPSS LSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPS RFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGQGTKLQITR* (SEQ ID NO: 48)
huOKT3 scFv C105S; 10 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggs***DIQMT QSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLA SGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGQGTKL QITR* (SEQ ID NO: 49)
huOKT3 scFv C105S; 20 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsgg ggs***DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRW IYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFT FGQGTKLQITR* (SEQ ID NO: 50)
huOKT3 scFv C105S; 25 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsgg ggsggggs***DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKA PKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWS SNPFTFGQGTKLQITR* (SEQ ID NO: 51)
huOKT3 scFv C105S; 30 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsgg ggsggggsggggs***DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTP GKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQ*

	QWSSNPFTFGQGTKLQITR (SEQ ID NO: 52)
huOKT3 scFv C105S; V_L-Q100C; V_H-G44C; 5 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK ***C***LEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggs***DIQMTQSPSS LSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVPS RFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFG***C***GTKLQITR (SEQ ID NO: 53)
huOKT3 scFv C105S; V_L-Q100C; V_H-G44C; 10 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK ***C***LEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggs***DIQMT QSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLA SGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFG***C***GTKL QITR* (SEQ ID NO: 54)
huOKT3 scFv C105S; V_L-Q100C; V_H-G44C; 15 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK ***C***LEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggs***DI QMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDT SKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFG***C*** *GTKLQITR* (SEQ ID NO: 55)
huOKT3 scFv C105S; V_L-Q100C; V_H-G44C; 20 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK ***C***LEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsgg ggs***DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRW IYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFT FG***C***GTKLQITR* (SEQ ID NO: 56)
huOKT3 scFv C105S; V_L-Q100C; V_H-G44C; 25 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK ***C***LEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsgg ggsggggs***DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKA PKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWS SNPFTFG***C***GTKLQITR* (SEQ ID NO: 57)
huOKT3 scFv C105S; V_L-Q100C; V_H-G44C; 30 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK ***C***LEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsgg ggsggggsggggs***DIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTP GKAPKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQ QWSSNPFTFG***C***GTKLQITR* (SEQ ID NO: 58)
huOKT3; 15 amino acid intra-scFv linker	QVQLVQSGGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK GLEWIGYINPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRP EDTGVYFCARYYDDHYCLDYWGQGTPVTVSS**ggggsggggsggggs***DI QMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDT SKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGQ GTKLQITR* (SEQ ID NO: 59)

[00118] Table 7. Light Chain Fusion Polypeptide Sequence. The uppercase, non-italicized, non-bold, non-underlined sequence represents the V_L domain of the trastuzumab light

chain. The uppercase, italicized sequence represents the constant region of the trastuzumab light chain. The lowercase, non-italicized, non-bold, non-underlined sequence represents the linker conjugating the light chain to the scFv. The uppercase, underlined sequence represents the V_H domain of the scFv. The uppercase, bold sequence represents the V_L domain of the scFv. The uppercase, underlined, italicized, and bold sequences represent the mutations described in the "DESCRIPTION" column. The lowercase bold sequences represent the intra-scFv linker.

DESCRIPTION	**SEQUENCE (SEQ ID NO:)**
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the scFv; 15 amino acid intra-scFv peptide linker	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsDIQMTQSP SSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTS KLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPF TFGQGTKLQITR** (SEQ ID NO: 29)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the scFv; 5 amino acid intra-scFv peptide linker	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsDIQMTQSPSSLSASVG DRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVP SRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGQGTK LQITR** (SEQ ID NO: 30)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the scFv; 10 amino acid intra-scFv peptide linker	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsDIQMTQSPSSLS ASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLA SGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFG QGTKLQITR** (SEQ ID NO: 31)
Trastuzumab light chain; C105S; 17 amino acid linker	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF*

conjugating the light chain to the scFv; 20 amino acid intra-scFv peptide linker	*YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsggggsDIQMT QSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIY DTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSS NPFTFGQGTKLQITR** (SEQ ID NO: 32)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the scFv; 25 amino acid intra-scFv peptide linker	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsggggsggggsDI QMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPK RWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQ QWSSNPFTFGQGTKLQITR** (SEQ ID NO: 33)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the scFv; 30 amino acid intra-scFv peptide linker	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsggggsggggsgg ggsDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGK APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATY YCQQWSSNPFTFGQGTKLQITR** (SEQ ID NO: 34)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the huOKT3 scFv; 5 amino acid intra-scFv peptide linker; V_L-Q100C; V_H-G44C	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK***C***LEWIGYI NPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsDIQMTQSPSSLSASVG DRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLASGVP SRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFGCGTK LQITR** (SEQ ID NO: 42)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the huOKT3	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS

scFv; 10 amino acid intra-scFv peptide linker; V_L-Q100C; V_H-G44C	GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK***C***LEWIGYI NPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggs**DIQMTQSPSSLS ASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTSKLA SGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPFTFG CGTKLQITR** (SEQ ID NO: 43)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the huOKT3 scFv; 15 amino acid intra-scFv peptide linker; V_L-Q100C; V_H-G44C	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK***C***LEWIGYI NPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsDIQMTQSP SSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTS KLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPF TFGCGTKLQITR** (SEQ ID NO: 44)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the huOKT3 scFv; 20 amino acid intra-scFv peptide linker; V_L-Q100C; V_H-G44C	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK***C***LEWIGYI NPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsggggsDIQMT QSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIY DTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSS NPFTFGCGTKLQITR** (SEQ ID NO: 45)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the huOKT3 scFv; 25 amino acid intra-scFv peptide linker; V_L-Q100C; V_H-G44C	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK***C***LEWIGYI NPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsggggsggggsDI QMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPK RWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQ QWSSNPFTFGQCGTKLQITR** (SEQ ID NO: 46)
Trastuzumab light chain; C105S; 17 amino acid linker conjugating the light chain to the huOKT3 scFv; 30 amino acid intra-scFv peptide	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGK***C***LEWIGYI NPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF

linker; V_L-Q100C; V_H-G44C	CARYYDDHY***S***LDYWGQGTPVTVSS**ggggsggggsggggsggggsggggsgg ggsDIQMTQSPSSLSASVGDRVTITCSASSSVSYMNWYQQTPGK APKRWIYDTSKLASGVPSRFSGSGSGTDYTFTISSLQPEDIATY YCQQWSSNPFTFGCGTKLQITR** (SEQ ID NO: 47)
Trastuzumab light chain; 17 amino acid linker conjugating the light chain to the scFv; 15 amino acid intra-scFv peptide linker	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAP KLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQH YTTPPTFGQGTKVEIKR*TVAAPSVFIFPPSDEQLKSGTASVVCLLNNF YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE KHKVYACEVTHQGLSSPVTKSFNRGEC*tsggggsggggsggggsQVQLVQS GGGVVQPGRSLRLSCKASGYTFTRYTMHWVRQAPGKGLEWIGY INPSRGYTNYNQKFKDRFTISRDNSKNTAFLQMDSLRPEDTGVYF CARYYDDHYCLDYWGQGTPVTVSS**ggggsggggsggggs**DIQMTQSP SSLSASVGDRVTITCSASSSVSYMNWYQQTPGKAPKRWIYDTS KLASGVPSRFSGSGSGTDYTFTISSLQPEDIATYYCQQWSSNPF TFGQGTKLQITR** (SEQ ID NO: 60)

[00119] Table 8. Modifications to bispecific binding molecules

LOCATION OF MODIFICATION	DESCRIPTION
Heavy chain	Mutation to reduce binding to the Fc receptor (as an example, N297A mutation)
	Mutation to destroy a glycosylation site (as an example, N297A mutation)
	Mutation to reduce C1q binding (as an example, K322A mutation)
Linker conjugating the light chain to the huOKT3 scFv	Increase or decrease the length of the linker
huOKT3 scFv V_H	Mutation to increase stabilization and/or reduce aggregation (as an example, introduce disulfide binding between V_H40 and V_L100 (according to Kabat numbering), as an example, V_H G44C and V_L Q100C)
	Reduce aggregation (as an example, C105S mutation)
huOKT3 scFv V_L	Mutation to increase stabilization and/or reduce aggregation (as an example,, introduce disulfide binding between V_H40 and V_L100 (according to Kabat numbering), as an example, V_H G44C and V_L Q100C)
huOKT3 intra-scFv linker	Increase or decrease the length of the linker

5.2 BISPECIFIC BINDING MOLECULE CONJUGATES

[00120] In preferred embodiments, a bispecific binding molecule provided herein is not conjugated to any other molecule, such as an organic moiety, a detectable label, or an isotope. In

alternative embodiments, a bispecific binding molecule provided herein is conjugated to one or more organic moieties. In alternative embodiments, a bispecific binding molecule provided herein is conjugated to one or more detectable labels. In alternative embodiments, a bispecific binding molecule provided herein is conjugated to one or more isotopes.

5.2.1 DETECTABLE LABELS AND ISOTOPES

[00121] In certain embodiments, a bispecific binding molecule provided herein is conjugated to one or more detectable labels or isotopes, *e.g.*, for imaging purposes. In certain embodiments, a bispecific binding molecule is detectably labeled by covalent or non-covalent attachment of a chromogenic, enzymatic, radioisotopic, isotopic, fluorescent, toxic, chemiluminescent, nuclear magnetic resonance contrast agent or other label.

[00122] Non-limiting examples of suitable chromogenic labels include diaminobenzidine and 4-hydroxyazo-benzene-2-carboxylic acid.

[00123] Non-limiting examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast-alcohol dehydrogenase, alpha-glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

[00124] Non-limiting examples of suitable radioisotopic labels include ^{3}H, ^{111}In, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{57}To, ^{58}Co, ^{59}Fe, ^{75}Se, ^{152}Eu, ^{90}Y, ^{67}Cu, ^{217}Ci, ^{211}At, ^{212}Pb, ^{47}Sc, ^{223}Ra, ^{223}Ra, ^{89}Zr, ^{177}Lu, and ^{109}Pd. In certain embodiments, ^{111}In is a preferred isotope for *in vivo* imaging as it avoids the problem of dehalogenation of ^{125}I or ^{131}I-labeled bispecific binding molecules in the liver. In addition, ^{111}In has a more favorable gamma emission energy for imaging (Perkins *et al.,* Eur. J. Nucl. Med. 70:296-301 (1985); Carasquillo et ah, J. Nucl. Med. 25:281-287 (1987)). For example, ^{111}In coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA has shown little uptake in non-tumorous tissues, particularly the liver, and therefore enhances specificity of tumor localization (Esteban *et al.,* J. Nucl. Med. 28:861-870 (1987)).

[00125] Non-limiting examples of suitable non-radioactive isotopic labels include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Tr, and ^{56}Fe.

[00126] Non-limiting examples of suitable fluorescent labels include a ^{152}Eu label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a

phycocyanin label, an allophycocyanin label, a Green Fluorescent Protein (GFP) label, an o-phthaldehyde label, and a fluorescamine label.

[00127] Non-limiting examples of chemiluminescent labels include a luminol label, an isoluminol label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label.

[00128] Non-limiting examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

[00129] Techniques known to one of ordinary skill in the art for binding the above-described labels to a bispecific binding molecule provided herein are described in, for example, Kennedy et at., Clin. CMm. Acta 70:1-31 (1976), and Schurs *et al.,* Clin. CMm. Acta 81:1-40 (1977). Coupling techniques mentioned in the latter are the glutaraldehyde method, the periodate method, the dimaleimide method, the m-maleimidobenzyl-N-hydroxy-succinimide ester method, all of which methods are incorporated by reference herein.

[00130] In certain embodiments, the bispecific binding molecule is conjugated to a diagnostic agent. A diagnostic agent is an agent useful in diagnosing or detecting a disease by locating the cells containing the antigen. Useful diagnostic agents include, but are not limited to, radioisotopes, dyes (such as with the biotin-streptavidin complex), contrast agents, fluorescent compounds or molecules and enhancing agents (*e.g.*, paramagnetic ions) for magnetic resonance imaging (MRI). U.S. Pat. No. 6,331,175 describes MRI technique and the preparation of antibodies conjugated to a MRI enhancing agent and is incorporated in its entirety by reference. Preferably, the diagnostic agents are selected from the group consisting of radioisotopes, enhancing agents for use in magnetic resonance imaging, and fluorescent compounds. In order to load an antibody component with radioactive metals or paramagnetic ions, it may be necessary to react it with a reagent having a long tail to which are attached a multiplicity of chelating groups for binding the ions. Such a tail can be a polymer such as a polylysine, polysaccharide, or other derivatized or derivatizable chain having pendant groups to which can be bound chelating groups such as, for example, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and like groups known to be useful for this purpose. Chelates are coupled to the antibodies using standard chemistries. The chelate is normally linked to the antibody by a group which enables formation of a bond to the molecule with minimal loss of

immunoreactivity and minimal aggregation and/or internal cross-linking other, more unusual, methods and reagents for conjugating chelates to antibodies are disclosed in U.S. Pat. No. 4,824,659 to Hawthorne, entitled "Antibody Conjugates," issued Apr. 25, 1989, the disclosure of which is incorporated herein in its entirety by reference. Particularly useful metal-chelate combinations include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, used with diagnostic isotopes for radio-imaging. The same chelates, when complexed with non-radioactive metals, such as manganese, iron and gadolinium are useful for MRI, when used along bispecific binding molecules provided herein. Macrocyclic chelates such as NOTA, DOTA, and TETA are of use with a variety of metals and radiometals, most particularly with radionuclides of gallium, yttrium and copper, respectively. Such metal-chelate complexes can be made very stable by tailoring the ring size to the metal of interest. Other ring-type chelates such as macrocyclic polyethers, which are of interest for stably binding nuclides, such as ^{223}Ra for RAIT are encompassed herein.

5.2.2 ORGANIC CONJUGATES

[00131] In certain embodiments, the bispecific binding molecules provided herein comprise one or more organic moieties that are covalently bonded, directly or indirectly, to the bispecific binding molecule. Such modification can produce an antibody or antigen-binding fragment with improved pharmacokinetic properties (*e.g.*, increased *in vivo* serum half-life). The organic moiety can be a hydrophilic polymeric group, fatty acid group, or fatty acid ester group. As used herein, the term "fatty acid" encompasses mono-carboxylic acids and di-carboxylic acids. As used herein, a "hydrophilic polymeric group" refers to an organic polymer that is more soluble in water than in octane, *e.g.*, polylysine. Hydrophilic polymers suitable for modifying a bispecific binding molecule provided herein can be linear or branched and include, for example, polyalkane glycols (*e.g.*, polyethylene glycol, (PEG), monomethoxy-polyethylene glycol, and polypropylene glycol), carbohydrates (*e.g.*, dextran, cellulose, oligosaccharides, and polysaccharides), polymers of hydrophilic amino acids (*e.g.*, polylysine, polyarginine, and polyaspartate), polyalkane oxides (*e.g.*, polyethylene oxide and polypropylene oxide) and polyvinyl pyrolidone. In certain embodiments, the hydrophilic polymer that modifies a bispecific binding molecule provided herein has a molecular weight of about 800 to about 150,000 Daltons as a separate molecular entity. For example PEG_{5000} and $PEG_{20,000}$, wherein the subscript is the average molecular weight of the polymer in Daltons, can be used. The hydrophilic polymeric group can be substituted with

one to about six alkyl, fatty acid or fatty acid ester groups. Hydrophilic polymers that are substituted with a fatty acid or fatty acid ester group can be prepared by employing suitable methods. For example, a polymer comprising an amine group can be coupled to a carboxylate of the fatty acid or fatty acid ester, and an activated carboxylate (*e.g.*, activated with N,N-carbonyl diimidazole) on a fatty acid or fatty acid ester can be coupled to a hydroxyl group on a polymer.

[00132] Fatty acids and fatty acid esters suitable for modifying bispecific binding molecules provided herein can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying bispecific binding molecules provided herein include, for example, n-dodecanoate, n-tetradecanoate, n-octadecanoate, n-eicosanoate, n-docosanoate, n-triacontanoate, n-tetracontanoate, cis-delta-9-octadecanoate, all cis-delta-5,8,11,14-eicosatetraenoate, octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic acid, and the like. Suitable fatty acid esters include mono-esters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably one to about six, carbon atoms.

[00133] The bispecific binding molecule conjugates provided herein can be prepared using suitable methods, such as by reaction with one or more modifying agents. As used herein, an "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as, for example, tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acrylolyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (*see*, for example, Hernanson, G. T., Bioconjugate Techniques, Academic Press: San Diego, Calif. (1996)). An activating group can be bonded directly to the organic group (*e.g.*, hydrophilic polymer, fatty acid, fatty acid ester), or through a linker moiety, for example a divalent C_1-C_{12} group, wherein one or more carbon atoms can be replaced by a heteroatom such as oxygen, nitrogen or sulfur. Suitable linker moieties include, for example, tetraethylene glycol, $(CH_2)_3$, and NH. Modifying agents that

comprise a linker moiety can be produced, for example, by reacting a mono-Boc-alkyldiamine (*e.g.*, mono-Boc-ethylenediamine or mono-Boc-diaminohexane) with a fatty acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) to form an amide bond between the free amine and the fatty acid carboxylate. The Boc protecting group can be removed from the product by treatment with trifluoroacetic acid (TFA) to expose a primary amine that can be coupled to another carboxylate as described, or can be reacted with maleic anhydride and the resulting product cyclized to produce an activated maleimido derivative of the fatty acid. (*See*, for example, Thompson, *et al.,* WO 92/16221 the entire teachings of which are incorporated herein by reference.)

[00134] As used herein, a "modifying agent" refers to a suitable organic group (*e.g.*, hydrophilic polymer, a fatty acid, and a fatty acid ester) that comprises an activating group. For example, the organic moieties can be bonded to the bispecific binding molecule in a non-site specific manner by employing an amine-reactive modifying agent, for example, an N-hydroxysuccinimide ester of PEG. Modified bispecific binding molecules can also be prepared by reducing disulfide bonds (*e.g.*, intra-chain disulfide bonds) of bispecific binding molecule. The reduced bispecific binding molecule can then be reacted with a thiol-reactive modifying agent to produce the modified bispecific binding molecule provided herein. Modified bispecific binding molecules comprising an organic moiety that is bonded to specific sites of a bispecific binding molecule provided herein can be prepared using suitable methods, such as reverse proteolysis (Fisch *et al.,* Bioconjugate Chem., 3:147-153 (1992); Werlen *et al.,* Bioconjugate Chem., 5:411-417 (1994); Kumaran *et al.,* Protein Sci. 6(10):2233-2241 (1997); Itoh *et al.,* Bioorg. Chem., 24(1): 59-68 (1996); Capellas *et al.,* Biotechnol. Bioeng., 56(4):456-463 (1997)), and the methods described in Hermanson, G. T., Bioconjugate Techniques, Academic Press: San Diego, Calif. (1996).

5.3 BISPECIFIC BINDING MOLECULE PRODUCTION

[00135] Provided herein are methods for producing bispecific binding molecules as described in Section 5.1 and Section 5.2. In certain embodiments, provided herein are methods for producing a bispecific binding molecule comprising an aglycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFV), via a peptide linker,

to create a first fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, and wherein the first and second fusion polypeptides are identical.

[00136] Methods to produce bispecific binding molecules described herein are known to one of ordinary skill in the art, for example, by chemical synthesis, by purification from biological sources, or by recombinant expression techniques, including, for example, from mammalian cell or transgenic preparations. The methods described herein employs, unless otherwise indicated, conventional techniques in molecular biology, microbiology, genetic analysis, recombinant DNA, organic chemistry, biochemistry, PCR, oligonucleotide synthesis and modification, nucleic acid hybridization, and related fields within the skill of the art. These techniques are described, for example, in the references cited herein and are fully explained in the literature. *See*, for example, Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press; Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press; Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY; Ausubel *et al.,* Current Protocols in Molecular Biology, John Wiley & Sons (1987 and annual updates); Current Protocols in Immunology, John Wiley & Sons (1987 and annual updates) Gait (ed.) (1984) Oligonucleotide Synthesis: A Practical Approach, IRL Press; Eckstein (ed.) (1991) Oligonucleotides and Analogues: A Practical Approach, IRL Press; Birren et al. (eds.) (1999) Genome Analysis: A Laboratory Manual, Cold Spring Harbor Laboratory Press.

[00137] A variety of methods exist in the art for the production of bispecific binding molecules. For example, the bispecific binding molecule may be made by recombinant DNA methods, such as those described in U.S. Pat. No. 4,816,567. The one or more DNAs encoding a bispecific binding molecule provided herein can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies, or such chains from human, humanized, or other sources). Once isolated, the DNA may be placed into expression vectors, which are then transformed into host cells such as NS0 cells, Simian COS cells, Chinese hamster ovary (CHO) cells, yeast cells, algae cells, eggs, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of the bispecific binding

molecules in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains of a desired species in place of the homologous human sequences (U.S. Pat. No. 4,816,567; Morrison *et al.,* supra) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of a bispecific binding molecule provided herein. In certain embodiments, the DNA is as described in Section 5.3.1.

[00138] Bispecific binding molecules provided herein can also be prepared using at least one bispecific binding molecule-encoding polynucleotide to provide transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce such antibodies in their milk. Such animals can be provided using known methods. *See*, for example, but not limited to, U.S. Pat. Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616, 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

[00139] In certain embodiments, bispecific binding molecules provided herein can additionally be prepared using at least one bispecific binding molecule-encoding polynucleotide provided herein to provide transgenic plants and cultured plant cells (for example, but not limited to tobacco and maize) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom. As a non-limiting example, transgenic tobacco leaves expressing recombinant proteins have been successfully used to provide large amounts of recombinant proteins, for example, using an inducible promoter. *See*, for example , Cramer *et al.,* Curr. Top. Microbol. Immunol. 240:95-118 (1999) and references cited therein. Also, transgenic maize have been used to express mammalian proteins at commercial production levels, with biological activities equivalent to those produced in other recombinant systems or purified from natural sources. *See*, for example, Hood *et al.,* Adv. Exp. Med. Biol. 464:127-147 (1999) and references cited therein. Antibodies have also been produced in large amounts from transgenic plant seeds including antibody fragments, such as scFvs, including tobacco seeds and potato tubers. *See*, for example, Conrad *et al.,* Plant Mol. Biol. 38:101-109 (1998) and references cited therein. Thus, bispecific binding molecules can also be produced using transgenic plants, according to known methods. *See* also, for example, Fischer *et al.,* Biotechnol. Appl. Biochem. 30:99-108 (October, 1999), Ma *et al.,* Trends Biotechnol. 13:522-7 (1995); Ma *et al.,* Plant Physiol. 109:341-6 (1995); Whitelam *et al.,* Biochem Soc. Trans. 22:940-944 (1994);

and references cited therein. Each of the above references is entirely incorporated herein by reference.

[00140] In certain embodiments, bispecific binding molecules provided herein can be prepared using at least one bispecific binding molecule-encoding polynucleotide provided herein to provide bacteria that produce such bispecific binding molecules. As a non-limiting example, *E. coli* expressing recombinant proteins has been successfully used to provide large amounts of recombinant proteins. *See*, for example, Verma *et al.,* 1998, 216(1-2): 165-181 and references cited therein.

[00141] *See*, also, Section 6.1.2.1 for a detailed example for the design and production of a bispecific binding molecule described herein.

[00142] In certain embodiments, the bispecific binding molecules can be recovered and purified from recombinant cell cultures by well-known methods including, but not limited to, protein A purification, protein G purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography ("HPLC") can also be employed for purification. *See*, for example, Colligan, Current Protocols in Immunology, or Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y., (1997-2001), *e.g.*, chapters 1, 4, 6, 8, 9, and 10, each entirely incorporated herein by reference.

[00143] In certain embodiments, the bispecific binding molecules provided herein include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a eukaryotic host, including, for example, yeast, higher plant, insect and mammalian cells. In preferred embodiments, the bispecific binding molecule is generated in a host such that the bispecific binding molecule is aglycosylated. In another preferred embodiment, the bispecific binding molecule is generated in a bacterial cell such that the bispecific binding molecule is aglycosylated. Such methods are described in many standard laboratory manuals, such as Sambrook, supra, Sections 17.37-17.42; Ausubel, supra, Chapters 10, 12, 13, 16, 18 and 20, Colligan, Protein Science, supra, Chapters 12-14, all entirely incorporated herein by reference.

[00144] Purified antibodies can be characterized by, for example, ELISA, ELISPOT, flow cytometry, immunocytology, Biacore™ analysis, Sapidyne KinExA™ kinetic exclusion assay,

SDS-PAGE and Western blot, or by HPLC analysis as well as by a number of other functional assays disclosed herein.

5.3.1 POLYNUCLEOTIDES

[00145] In certain embodiments, provided herein are polynucleotides comprising a nucleotide sequence encoding a bispecific binding molecule described herein or a fragment thereof (*e.g.*, a heavy chain and/or a light chain fusion polypeptide) that immunospecifically binds to HER2 and CD3, as described in Section 5.1 and Section 5.2. Also provided herein are vectors comprising such polynucleotides. *See*, Section 5.3.2. Also provided herein are polynucleotides encoding antigens of the bispecific binding molecules provided herein. Also provided herein are polynucleotides that hybridize under stringent or lower stringency hybridization conditions to polynucleotides that encode a bispecific binding molecule or fragment thereof provided herein.

[00146] The language "purified" includes preparations of polynucleotide or nucleic acid molecule having less than about 15%, 10%, 5%, 2%, 1%, 0.5%, or 0.1% (in particular less than about 10%) of other material, *e.g.*, cellular material, culture medium, other nucleic acid molecules, chemical precursors and/or other chemicals. In a specific embodiment, a nucleic acid molecule(s) encoding a bispecific binding molecule described herein is isolated or purified.

[00147] Nucleic acid molecules provided herein can be in the form of RNA, such as mRNA, hnRNA, tRNA or any other form, or in the form of DNA, including, but not limited to, cDNA and genomic DNA obtained by cloning or produced synthetically, or any combinations thereof. The DNA can be triple-stranded, double-stranded or single-stranded, or any combination thereof. Any portion of at least one strand of the DNA or RNA can be the coding strand, also known as the sense strand, or it can be the non-coding strand, also referred to as the anti-sense strand.

[00148] In certain embodiments, provided herein is a polynucleotide comprising nucleotide sequences encoding a bispecific binding molecule or fragment thereof as described in Section 5.1 and Section 5.2, wherein the bispecific binding molecule comprises an aglycosylated monoclonal antibody that is an immunoglobulin that binds to HER2, comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first scFv, via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second

scFv (i) are identical, and (ii) bind to CD3, and wherein the first and second light chain fusion polypeptides are identical.

[00149] For a detailed example for the generation of a bispecific binding molecule as described herein, *see*, Section 6.1.2.1 for a detailed example for the design and production of a bispecific binding molecule described herein.

[00150] In certain embodiments, provided herein is a polynucleotide comprising nucleotide sequences encoding a light chain fusion polypeptide comprising a light chain fused to a scFv, via a peptide linker, wherein the light chain binds to HER2 and wherein the scFv binds to CD3. In certain embodiments, the light chain is the light chain of a HER2-specific antibody known in the art, such as, for example, trastuzumab, M-111, pertuzumab, ertumaxomab, MDXH210, 2B1, and MM-302. In certain embodiments, the scFv comprises the V_H and V_L of an anti-CD3 antibody known in the art, such as, for example, huOKT3, YTH12.5, HUM291, teplizumab, huCLB-T3/4, otelixizumab, blinatumomab, MT110, catumaxomab, 28F11, 27H5, 23F10, 15C3, visilizumab, and Hum291. In a preferred embodiment, the anti-CD3 antibody is huOKT3. In an especially preferred embodiment, the scFv comprises the VH of huOKT3, further comprising the amino acid substitution at numbered position 105, wherein the cysteine is substituted with a serine. *See*, for example, Kipriyanov et al. 1997, Protein Eng. 445-453. In certain embodiments, the scFv is derived from the huOKT3 monoclonal antibody and comprises one or more mutations, relative to the native huOKT3 V_H and V_L, to stabilize disulfide binding. In certain embodiments, the stabilization of disulfide binding prevents aggregation of the bispecific binding molecule. In certain embodiments, the stabilization of disulfide binding reduces aggregation of the bispecific binding molecule as compared to aggregation of the bispecific binding molecule without the stabilization of disulfide binding. In certain embodiments of the bispecific binding molecule, the one or more mutations to stabilize disulfide binding comprise a V_H G44C mutation and a V_L Q100C mutation (*e.g.*, as present in SEQ ID NOS: 54-59). In certain embodiments of the bispecific binding molecule, the one or more mutations to stabilize disulfide binding are the replacement of the amino acid residue at V_H44(according to the Kabat numbering system) with a cysteine and the replacement of the amino acid residue at V_L100 (according to the Kabat numbering system) with a cysteine so as to introduce a disulfide bond between V_H44 and V_L100 (*e.g.*, as present in SEQ ID NOS: 54-59). In certain embodiments, the peptide linker is between 5-30, 5-25, 5-15, 10-30, 10-20, 10-15, 15-30, or 15-25 amino acid residues in length. In certain

embodiments, the sequence of the peptide linker is as described in Table 1, above (*e.g.*, any one of SEQ ID NOs: 14 or 35-41). In a particularly preferred embodiment, the sequence of the peptide linker is SEQ ID NO: 14. In certain embodiments, the sequence to the scFv comprises one or more modifications as described in Table 8, above.

[00151] In particular aspects, provided herein are polynucleotides comprising nucleotide sequences encoding bispecific binding molecules or fragments thereof, which specifically bind to HER2 and CD3, and comprise an amino acid sequence as described herein, as well as antibodies which compete with such bispecific binding molecules for binding to HER2 and/or CD3, or which binds to the same epitope as that of such antibodies.

[00152] In a preferred embodiment, the sequence of the light chain is SEQ ID NO: 25. In a preferred embodiment, the nucleotide sequence encoding the light chain is SEQ ID NO: 24. In a preferred embodiment, the sequence of the scFv SEQ ID NO: 19. In a preferred embodiment, the nucleotide sequence encoding the scFv SEQ ID NO: 18. In a preferred embodiment, the sequence of the light chain is SEQ ID NO: 25 and the sequence of the scFv is SEQ ID NO: 19. In a preferred embodiment, the nucleotide sequence encoding the light chain is SEQ ID NO: 24 and the nucleotide sequence encoding the scFv is SEQ ID NO: 18. In a preferred embodiment, the sequence of the light chain fusion polypeptide is SEQ ID NO: 29. In a preferred embodiment, the nucleotide sequence encoding the light chain fusion polypeptide is SEQ ID NO: 28.

[00153] In certain embodiments, the bispecific binding molecule has a trastuzumab-derived sequence that contains one or more of the modifications in the trastuzumab immunoglobulin, and has a huOKT3-derived sequence that contains one or more of the modifications in the huOKT3 V_H and V_L sequences, as described in Table 8, below. Bispecific binding molecules having other immunoglobulin or scFv sequences can contain analogous mutations at corresponding positions in these other immunoglobulin or scFv sequences. In certain embodiments, the bispecific binding molecule is (a) derived from trastuzumab and huOKT3; and (b) contains one or more of the modifications as described in Table 8, above. In certain embodiments, the sequence of the peptide linker conjugating the immunoglobulin light chain and the scFv is as described in Table 1, above (*e.g.*, any one of SEQ ID NOs: 14 or 35-41). In certain embodiments, the sequence of the heavy chain is as described in Table 2, above (*e.g.*, any one of SEQ ID NOs: 23, 27, 62, or 63). In certain embodiments, the sequence of the light chain is as described in Table 3, above

(*e.g.*, SEQ ID NO: 25). In certain embodiments, the sequence of the V_H of the scFv is as described in Table 4, above (*e.g.*, any one of SEQ ID NOs: 15, 17, or 64). In certain embodiments, the sequence of the V_L of the scFv is as described in Table 5, above (*e.g.*, any one of SEQ ID NOs: 16 or 65). In certain embodiments, the sequence of the scFv peptide linker is as described in Table 1, above (*e.g.*, any one of SEQ ID NOs: 14 or 35-41). In certain embodiments, the sequence of the scFv is as described in Table 6, above (*e.g.*, any one of SEQ ID NOs: 19 or 48-59). In certain embodiments, the sequence of the light chain fusion polypeptide is as described in Table 7, above (*e.g.*, any one of SEQ ID NOs: 29, 34, 42-47, or 60).

[00154] In certain embodiments, provided herein is a polynucleotide comprising nucleotide sequences encoding the heavy chain of a HER2-specific antibody described in Section 5.2. In certain embodiments, the heavy chain is the heavy chain a HER2-specific antibody known in the art, such as, for example, trastuzumab, M-111, pertuzumab, ertumaxomab, MDXH210, 2B1, and MM-302. In a preferred embodiment, the antibody comprises the V_H of trastuzumab, wherein the sequence of the heavy chain is SEQ ID NO: 27. In a preferred embodiment, the antibody comprises the V_H of trastuzumab, wherein the nucleotide sequence encoding the heavy chain is SEQ ID NO: 26. In a preferred embodiment, the sequence of the heavy chain is comprises the V_H of trastuzumab and comprises the amino acid substitution N297A in the Fc region (SEQ ID NO: 26). In a preferred embodiment, the nucleotide sequence encoding the heavy chain comprises the nucleotide sequence encoding the trastuzumab V_H and comprises the amino acid substitution N297A in the Fc region (SEQ ID NO: 26). In preferred embodiment, the sequence of the heavy chain comprises the sequence of the trastuzumab V_H and comprises the amino acid substitution K322A in the Fc region (SEQ ID NO: 27). In a preferred embodiment, the nucleotide sequence encoding the heavy chain comprises the nucleotide sequence encoding the trastuzumab V_H and comprises the amino acid substitution K322A in the Fc region (SEQ ID NO: 26). In an especially preferred embodiment, the sequence of the heavy chain comprises the sequence of the trastuzumab V_H and comprises the amino acid substitutions N297A and K322A in the Fc region (SEQ ID NO: 27). In an especially preferred embodiment, the nucleotide sequence encoding the heavy chain comprises the nucleotide sequence encoding the trastuzumab V_H and comprises the amino acid substitutions N297A and K322A in the Fc region (SEQ ID NO: 26).

[00155] The polynucleotides provided herein can be obtained by any method known in the art. For example, if the nucleotide sequence encoding a bispecific binding molecule or fragment thereof described herein is known, a polynucleotide encoding the bispecific binding molecule or fragment thereof can be may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.,* BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[00156] Alternatively, a polynucleotide encoding a bispecific binding molecule or fragment thereof may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular bispecific binding molecule or fragment thereof is not available, but the sequence of the bispecific binding molecule or fragment thereof is known, a nucleic acid encoding the bispecific binding molecule or fragment thereof may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody provided herein) by PCR amplification using synthetic primers that hybridize to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, for example, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art. *See*, for example, Section 5.3.2.

[00157] In certain embodiments, the amino acid sequence of the antibody of the bispecific binding molecule is known in the art. In such embodiments, a polypeptide encoding such an antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (*see*, for example, the techniques described in Sambrook *et al.,* 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. and Ausubel *et al.,* eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., which are both incorporated by reference herein in their entireties), to generate bispecific binding molecules having a different amino acid sequence, for example, to create amino acid substitutions, deletions, and/or insertions. For example, such manipulations can be performed to

render the encoded amino acid aglycosylated, or to destroy the antibody's ability to bind to C1q, Fc receptor, or to activate the complement system.

[00158] Isolated nucleic acid molecules provided herein can include nucleic acid molecules comprising an open reading frame (ORF), optionally with one or more introns, for example, but not limited to, at least one specified portion of at least one complementarity determining region (CDR), as CDR1, CDR2 and/or CDR3 of at least one heavy chain or light chain; nucleic acid molecules comprising the coding sequence for an anti-HER2 antibody or variable region, an anti-CD3 scFv, or a single chain fusion polypeptide; and nucleic acid molecules which comprise a nucleotide sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode at least one bispecific binding molecule as described herein and/or as known in the art.

[00159] Also provided herein are isolated nucleic acids that hybridize under selective hybridization conditions to a polynucleotide disclosed herein. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising such polynucleotides. For example, polynucleotides provided herein can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated, or otherwise complementary to, a cDNA from a human or mammalian nucleic acid library.

[00160] The nucleic acids can conveniently comprise sequences in addition to a polynucleotide provided herein. For example, a multi-cloning site comprising one or more endonuclease restriction sites can be inserted into the nucleic acid to aid in isolation of the polynucleotide. In addition, translatable sequences can be inserted to aid in the isolation of the translated polynucleotide provided herein. For example, a hexa-histidine marker sequence provides a convenient means to purify the polypeptides provided herein. The nucleic acid provided herein—excluding the coding sequence—is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide provided herein.

[00161] Additional sequences can also be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. (*See*, *e.g.*, Ausubel, supra; or Sambrook, supra).

[00162] In a specific embodiment, using routine recombinant DNA techniques, one or more of the CDRs of an antibody described herein may be inserted within framework regions. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (*see*, *e.g.*, Chothia *et al.,* J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds HER2. One or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are provided herein and within the skill of the art.

[00163] In certain embodiments, the isolated or purified nucleic acid molecule, or fragment thereof, upon linkage with another nucleic acid molecule, can encode a fusion protein. The generation of fusion proteins is within the ordinary skill in the art and can involve the use of restriction enzyme or recombinational cloning techniques (*see*, for example, Gateway.TM.. (Invitrogen)). *See*, also, U.S. Pat. No. 5,314,995.

[00164] In certain embodiments, a polynucleotide provided herein is in the form of a vector (*e.g.*, expression vector) as described in Section 5.3.2.

5.3.2 CELLS AND VECTORS

[00165] In certain embodiments, provided herein are cells (*e.g.*, *ex vivo* cells) expressing (*e.g.*, recombinantly) bispecific binding molecules as described herein. Also provided herein are vectors (*e.g.*, expression vectors) comprising nucleotide sequences (*see*, for example, Section 5.3.1) encoding a bispecific binding molecule or fragment thereof described herein for recombinant expression in host cells, preferably in mammalian cells. Also provided herein are cells (*e.g.*, *ex vivo* cells) comprising such vectors or nucleotide sequences for recombinantly expressing a bispecific binding molecule described here. Also provided herein are methods for producing a bispecific binding molecule described herein, comprising expressing such bispecific binding molecule from a cell (*e.g.*, *ex vivo* cell). In a preferred embodiment, the cell is an *ex vivo* cell.

[00166] A vector (*e.g.,* expression vector) is a DNA molecule comprising a gene that is expressed in a cell (*e.g.*, *ex vivo* cell). Typically, gene expression is placed under the control of certain regulatory elements, including constitutive or inducible promoters, tissue-specific regulatory elements and enhancers. Such a gene is said to be "operably linked to" the regulatory elements, *e.g.*, a promoter. A recombinant host may be any prokaryotic or eukaryotic cell that contains either a cloning vector or expression vector. This term also includes those prokaryotic or eukaryotic cells, as well as a transgenic animal, that have been genetically engineered to contain the cloned gene(s) in the chromosome or genome of the host cell or cells of the host cells (*e.g.*, *ex vivo* cells).

[00167] In a preferred embodiment, the promoter is the CMV promoter.

[00168] In certain embodiments, provided herein is a vector comprising one or more polynucleotide as described in Section 5.3.1.

[00169] In certain embodiments, a polynucleotide as described in Section 5.3.1 can be cloned into a suitable vector and can be used to transform or transfect any suitable host. Vectors and methods to construct such vectors are known to one of ordinary skill in the art and are described in general technical references (*see*, in general, "Recombinant DNA Part D," Methods in Enzymology, Vol. 153, Wu and Grossman, eds., Academic Press (1987)). In certain embodiments, the vector comprises regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host (*e.g.*, bacterium, fungus, plant, insect, or mammal) into which the vector is to be introduced, as appropriate and taking into consideration whether the vector is DNA or RNA. In certain embodiments, the vector comprises regulatory sequences that are specific to the genus of the host. In certain embodiments, the vector comprises regulatory sequences that are specific to the species of the host.

[00170] In certain embodiments, the vector comprises one or more marker genes, which allow for selection of transformed or transfected hosts. Non-limiting examples of marker genes include biocide resistance, *e.g.*, resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. In a preferred embodiment, the vector comprises ampicillin and hygromycin selectable markers.

[00171] In certain embodiments, an expression vector can comprise a native or normative promoter operably linked to a polynucleotide as described in Section 5.3.1. The selection of

promoters, for example, strong, weak, inducible, tissue-specific and developmental-specific, is within the skill in the art. Similarly, the combining of a nucleic acid molecule, or fragment thereof, as described above with a promoter is also within the skill in the art.

[00172] Non-limiting examples of suitable vectors include those designed for propagation and expansion or for expression or both. For example, a cloning vector can be selected from the group consisting of the pUC series, the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.). Bacteriophage vectors, such as lamda-GT10, lamda-GT11, lamda-ZapII (Stratagene), lamda-EMBL4, and lamda-NM1149, can also be used. Non-limiting examples of plant expression vectors include pBI110, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Non-limiting examples of animal expression vectors include pEUK-C1, pMAM and pMAMneo (Clontech). The TOPO cloning system (Invitrogen, Carlsbad, Calif.) can also be used in accordance with the manufacturer's recommendations.

[00173] In certain embodiments, the vector is a mammalian vector. In certain embodiments, the mammalian vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the bispecific binding molecule coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. In certain embodiments, the mammalian vector contains additional elements, such as, for example, enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. In certain embodiments, highly efficient transcription can be achieved with, for example, the early and late promoters from SV40, the long terminal repeats (LTRS) from retroviruses, for example, RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (*e.g.*, the human actin promoter). Non-limiting examples of mammalian expression vectors include, vectors such as pIRESlneo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clonetech Labs, Palo Alto, Calif.), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Non-limiting example of mammalian host cells that can be used in combination with such mammalian vectors include human Hela 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

[00174] In certain embodiments, the vector is a viral vector, for example, retroviral vectors, parvovirus-based vectors, *e.g.*, adeno-associated virus (AAV)-based vectors, AAV-adenoviral chimeric vectors, and adenovirus-based vectors, and lentiviral vectors, such as Herpes simplex (HSV)-based vectors. In certain embodiments, the viral vector is manipulated to render the virus replication deficient. In certain embodiments, the viral vector is manipulated to eliminate toxicity to the host. These viral vectors can be prepared using standard recombinant DNA techniques described in, for example, Sambrook *et al.,* Molecular Cloning, a Laboratory Manual, 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989); and Ausubel *et al.,* Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994).

[00175] In certain embodiments, a vector or polynucleotide described herein can be transferred to a cell (*e.g.*, an *ex vivo* cell) by conventional techniques and the resulting cell can be cultured by conventional techniques to produce a bispecific binding molecule described herein. Accordingly, provided herein are cells comprising a polynucleotide encoding a bispecific binding molecule or fragment thereof, a heavy or light chain thereof, or a light chain fusion polypeptide thereof, operably linked to a promoter for expression of such sequences in the host cell. In certain embodiments, a vector encoding the heavy chain operably linked to a promoter and a vector encoding the light chain fusion polypeptide operably linked to a promoter can be co-expressed in the cell for expression of the entire bispecific binding molecule, as described below. In certain embodiments, a cell comprises a vector comprising a polynucleotide encoding both the heavy chain and the light chain fusion polypeptide of a bispecific binding molecule described herein operably linked to a promoter. In certain embodiments, a cell comprises two different vectors, a first vector comprising a polynucleotide encoding a heavy chain operably linked to a promoter, and a second vector comprising a polynucleotide encoding a light chain fusion polypeptide operably linked to a promoter. In certain embodiments, a first cell comprises a first vector comprising a polynucleotide encoding a heavy chain of a bispecific binding molecule described herein, and a second cell comprises a second vector comprising a polynucleotide encoding a light chain fusion polypeptide of a bispecific binding molecule described herein. In certain embodiments, provided herein is a mixture of cells comprising such first cell and such second cell. In a preferred embodiment, the cell expresses the vector or vectors such that the oligonucleotide is both transcribed and translated efficiently by the cell.

[00176] In embodiment, the cell expresses the vector, such that the oligonucleotide, or fragment thereof, is both transcribed and translated efficiently by the cell.

[00177] In certain embodiments, the cell is present in a host, which can be an animal, such as a mammal. Examples of cells include, but are not limited to, a human cell, a human cell line, E. coli (*e.g.*, E. coli TB-1, TG-2, DH5a, XL-Blue MRF' (Stratagene), SA2821 and Y1090), B. subtilis, P. aerugenosa, S. cerevisiae, N. crassa, insect cells (*e.g.*, Sf9, Ea4) and others set forth herein below. In a preferred embodiment, the cell is a CHO cell. In an especially preferred embodiment, the cell is a CHO-S cell.

[00178] In certain embodiments, a polynucleotide described herein can be expressed in a stable cell line that comprises the polynucleotide integrated into a chromosome by introducing the polynucleotide into the cell. In certain embodiments, the polynucleotide is introduced into the cell by, for example, electroporation. In certain embodiments, the polynucleotide is introduced into the cell by, for example, transfection of a vector comprising the polynucleotide into the cell. In certain embodiments, the vector is co-transfected with a selectable marker such as DHFR, GPT, neomycin, or hygromycin to allow for the identification and isolation of the transfected cells. In certain embodiments, the transfected polynucleotide can also be amplified to express large amounts of the encoded bispecific binding molecule. For example, the DHFR (dihydrofolate reductase) marker can be utilized to develop cell lines that carry several hundred or even several thousand copies of the polynucleotide of interest. Another example of a selection marker is the enzyme glutamine synthase (GS) (Murphy, *et al.,* Biochem. J. 227:277-279 (1991); Bebbington, *et al.,* Bio/Technology 10:169-175 (1992)). Using these markers, the cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of bispecific binding molecules.

[00179] In a preferred embodiment, the vector comprises (i) a first polynucleotide sequence encoding a light chain fusion polypeptide comprising an immunoglobulin light chain fused to a scFv, via a peptide linker, wherein the light chain binds to HER2 and wherein the scFv binds to CD3, operably linked to a first promoter and (ii) a second polynucleotide encoding an immunoglobulin heavy chain that binds to HER2 operably linked to a second promoter. In certain embodiments, the vector is a viral vector.

5.4 T CELLS BOUND TO BISPECIFIC BINDING MOLECULES

[00180] Without being bound by any theory, it is believed that when the bispecific binding molecules provided herein are bound to T cells, by, for example, procedures such as those described herein, an anti-CD3 scFv of the bispecific binding molecule binds to CD3 on the surface of the T cell. Without being bound by any theory, it is believed that binding of the bispecific binding molecule to the T cell (*i.e.*, binding of an anti-CD3 scFv to CD3 expressed on the T cell) activates the T cell, and consequently, allows for the T cell receptor-based cytotoxicity to be redirected to desired tumor targets, bypassing MHC restrictions.

[00181] Thus, the invention also provides T cells which are bound to a bispecific binding molecule of the invention (*e.g.*, as described in Section 5.1 and Section 5.2). In specific embodiments, the T cells are bound to the bispecific binding molecule noncovalently. In specific embodiments, the T cells are are autologous to a subject to whom the T cells are to be administered. In specific embodiments, the T cells are allogeneic to a subject to whom the T cells are to be administered. In specific embodiments, the T cells are human T cells.

[00182] In specific embodiments, the T cells which are bound to bispecific binding molecules of the invention are used in accordance with the methods described in Section 5.6. In specific embodiments, the T cells which are bound to bispecific binding molecules of the invention are used as part of a combination therapy as described in Section 5.9.

5.5 PHARMACEUTICAL COMPOSITIONS AND KITS

[00183] In certain embodiments, provided herein are compositions (*e.g.*, pharmaceutical compositions) and kits comprising a pharmaceutically effective amount of one or more bispecific binding molecule as described in Section 5.1 or Section 5.2. Compositions may be used in the preparation of individual, single unit dosage forms. Compositions provided herein can be formulated for parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intra-Ommaya, intraocular, intravitreous, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, intrathecal, intraventricular in the brain, intraparenchymal in the brain, or

transdermal administration. In a preferred embodiment, the composition is formulated for parenteral administration. In an especially preferred embodiment, the composition is formulated for intravenous administration. In a preferred embodiment, the composition is formulated for intraperitoneal administration. In a specific embodiment, the composition is formulated for intraperitoneal administration to treat peritoneal metastases. In a preferred embodiment, the composition is formulated for intrathecal administration. In a specific embodiment, the composition is formulated for intrathecal administration to treat brain metastases. *See*, for example, Kramer *et al.,* 2010, 97: 409-418. In a preferred embodiment, the composition is formulated for intraventricular administration in the brain. In a specific embodiment, the composition is formulated for intraventricular administration to treat brain metastases. *See*, for example, Kramer *et al.,* 2010, 97: 409-418. In a preferred embodiment, the composition is formulated for intraparenchymal administration in the brain. In a specific embodiment, the composition is formulated for intraparenchymal administration to treat a brain tumor or brain tumor metastases. *See*, for example, Luther *et al.,* 2014, Neuro Oncol, 16: 800-806, and Clinical Trial ID NO NCT01502917.

[00184] In a specific embodiment, the composition is formulated for intraperitoneal administration for peritoneal metastases.

[00185] In certain embodiments, provided herein is a composition comprising one or more polynucleotide comprising nucleotide sequences encoding a bispecific binding molecule as described herein. In certain embodiments, provided herein is a composition comprising a cell, wherein the cell comprises one or more polynucleotide comprising nucleotide sequences encoding a bispecific binding molecule as described herein. In certain embodiments, provided herein is a composition comprising a vector, wherein the vector comprises one or more polynucleotide comprising nucleotide sequences encoding a bispecific binding molecule as described herein. In certain embodiments, provided herein is a composition comprising a cell, wherein the cell comprises a vector, wherein the vector comprises one or more polynucleotide comprising nucleotide sequences encoding a bispecific binding molecule as described herein.

[00186] In certain embodiments, a composition described herein is a stable or preserved formulation. In certain embodiments, the stable formulation comprises a phosphate buffer with saline or a chosen salt. In certain embodiments, a composition described is a multi-use preserved formulation, suitable for pharmaceutical or veterinary use. In certain embodiments, a

composition described herein comprises a preservative. Preservatives are known to one of ordinary skill in the art. Non-limiting examples of preservatives include phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride (*e.g.*, hexahydrate), alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, and sodium dehydroacetate and thimerosal, or mixtures thereof in an aqueous diluent. Any suitable concentration or mixture can be used as known in the art, such as 0.001-5%, or any range or value therein, such as, but not limited to 0.001, 0.003, 0.005, 0.009, 0.01, 0.02, 0.03, 0.05, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.3, 4.5, 4.6, 4.7, 4.8, 4.9, or any range or value therein. Non-limiting examples include, no preservative, 0.1-2% m-cresol (*e.g.*, 0.2, 0.3. 0.4, 0.5, 0.9, 1.0%), 0.1-3% benzyl alcohol (*e.g.*, 0.5, 0.9, 1.1, 1.5, 1.9, 2.0, 2.5%), 0.001-0.5% thimerosal (*e.g.*, 0.005, 0.01), 0.001-2.0% phenol (*e.g.*, 0.05, 0.25, 0.28, 0.5, 0.9, 1.0%), 0.0005-1.0% alkylparaben(s) (*e.g.*, 0.00075, 0.0009, 0.001, 0.002, 0.005, 0.0075, 0.009, 0.01, 0.02, 0.05, 0.075, 0.09, 0.1, 0.2, 0.3, 0.5, 0.75, 0.9, 1.0%), and the like.

[00187] It can be sometimes desirable to deliver the compositions provided herein to a subject over prolonged periods of time, for example, for periods of one week to one year or more from a single administration. Various slow release, depot or implant dosage forms can be utilized. For example, a dosage form can contain a pharmaceutically acceptable non-toxic salt of the compounds that has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from *e.g.*, N,N'-dibenzyl-ethylenediamine or ethylenediamine; or (c) combinations of (a) and (b) *e.g.*, a zinc tannate salt. Additionally, a composition provided herein, preferably, a relatively insoluble salt such as those just described, can be formulated in a gel, for example, an aluminum monostearate gel with, *e.g.*, sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the compound or salt dispersed for encapsulated in a slow degrading, non-toxic, non-antigenic polymer such as a

polylactic acid/polyglycolic acid polymer, for example, as described in U.S. Pat. No. 3,773,919. The compounds or, preferably, relatively insoluble salts such as those described above can also be formulated in cholesterol matrix silastic pellets, particularly for use in animals. Additional slow release, depot or implant compositons, *e.g.*, gas or liquid liposomes are known in the literature (U.S. Pat. No. 5,770,222 and "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., N.Y., 1978).

[00188] The range of at least one bispecific binding molecule composition provided herein includes amounts yielding upon reconstitution, if in a wet/dry system, concentrations from about 1.0 microgram/ml to about 1000 mg/ml, although lower and higher concentrations are operable and are dependent on the intended delivery vehicle, *e.g.*, solution formulations will differ from transdermal patch, pulmonary, transmucosal, or osmotic or micro pump methods.

[00189] In certain embodiments, compositions provided herein comprise at least one of any suitable auxiliary, such as, but not limited to, diluent, binder, stabilizer, buffers, salts, lipophilic solvents, preservative, adjuvant or the like. In certain embodiments, pharmaceutically acceptable auxiliaries are preferred. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but not limited to, Gennaro, Ed., Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing Co. (Easton, Pa.) 1990. Pharmaceutically acceptable carriers can be routinely selected that are suitable for the mode of administration, solubility and/or stability of the bispecific binding molecule as described herein.

[00190] In certain embodiments, compositions provided herein contain one or more pharmaceutical excipient and/or additive. Non-limiting examples of pharmaceutical excipients and additives are proteins, peptides, amino acids, lipids, and carbohydrates (*e.g.*, sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Non-limiting examples of protein excipients include serum albumin such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Non-limiting examples of amino acid/antibody components, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. In certain embodiments, the amino acid is glycine. Non-limiting examples of carbohydrate

excipients include monosaccharides such as fructose, maltose, galactose, glucose, D-mannose, sorbose, and the like; disaccharides, such as lactose, sucrose, trehalose, cellobiose, and the like; polysaccharides, such as raffinose, melezitose, maltodextrins, dextrans, starches, and the like; and alditols, such as mannitol, xylitol, maltitol, lactitol, xylitol sorbitol (glucitol), myoinositol and the like. In certain embodiments, the carbohydrate excipient is mannitol, trehalose, or raffinose.

[00191] In certain embodiments, a composition provided herein includes one or more buffer or a pH adjusting agent; typically, the buffer is a salt prepared from an organic acid or base. Non-limiting examples of buffers include organic acid salts such as salts of citric acid, ascorbic acid, gluconic acid, carbonic acid, tartaric acid, succinic acid, acetic acid, or phthalic acid; Tris, tromethamine hydrochloride, or phosphate buffers. In certain embodiments, the buffer is an organic acid salts such as citrate. Other excipients, *e.g.*, isotonicity agents, buffers, antioxidants, preservative enhancers, can be optionally and preferably added to the diluent. An isotonicity agent, such as glycerin, is commonly used at known concentrations. A physiologically tolerated buffer is preferably added to provide improved pH control. The compositions can cover a wide range of pHs, such as from about pH 4 to about pH 10, and preferred ranges from about pH 5 to about pH 9, and a most preferred range of about 6.0 to about 8.0. Preferably, the compositions provided herein have pH between about 6.8 and about 7.8. Preferred buffers include phosphate buffers, most preferably sodium phosphate, particularly phosphate buffered saline (PBS).

[00192] In certain embodiments, a composition provided herein includes one or more polymeric excipient/additive such as, for example, polyvinylpyrrolidones, ficolls (a polymeric sugar), dextrates (*e.g.*, cyclodextrins, such as 2-hydroxypropyl-.beta.-cyclodextrin), polyethylene glycols, flavoring agents, antimicrobial agents, sweeteners, antioxidants, antistatic agents, surfactants (*e.g.*, polysorbates such as "TWEEN 20" and "TWEEN 80"), lipids (*e.g.*, phospholipids, fatty acids), steroids (*e.g.*, cholesterol), and/or chelating agents (*e.g.*, EDTA).

[00193] Other additives, such as a pharmaceutically acceptable solubilizers like Tween 20 (polyoxyethylene (20) sorbitan monolaurate), Tween 40 (polyoxyethylene (20) sorbitan monopalmitate), Tween 80 (polyoxyethylene (20) sorbitan monooleate), Pluronic F68 (polyoxyethylene polyoxypropylene block copolymers), and PEG (polyethylene glycol) or non-ionic surfactants such as polysorbate 20 or 80 or poloxamer 184 or 188, Pluronic.RTM. polyls, other block co-polymers, and chelators such as EDTA and EGTA can optionally be added to the

compositions to reduce aggregation. These additives are particularly useful if a pump or plastic container is used to administer the composition. The presence of pharmaceutically acceptable surfactant mitigates the propensity for the protein to aggregate.

[00194] Additional pharmaceutical excipients and/or additives suitable for use in a composition provided herein are known to one of skill in the art and are referenced in, for example, "Remington: The Science & Practice of Pharmacy", 19.sup.th ed., Williams & Williams, (1995), and in the "Physician's Desk Reference", 52nd ed., Medical Economics, Montvale, N.J. (1998), which are entirely incorporated herein by reference. In certain preferred embodiments, the carrier or excipient materials are carbohydrates (*e.g.*, saccharides and alditols) and buffers (*e.g.*, citrate) or polymeric agents.

[00195] Preferably, the aqueous diluent optionally further comprises a pharmaceutically acceptable preservative. Preferred preservatives include those selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof. The concentration of preservative used in the composition is a concentration sufficient to yield an anti-microbial effect. Such concentrations are dependent on the preservative selected and are readily determined by the skilled artisan.

[00196] The compositions provided herein can be prepared by a process which comprises mixing at least one bispecific binding molecule as described herein and a preservative selected from the group consisting of phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, alkylparaben, (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal or mixtures thereof in an aqueous diluent. Mixing the at least one bispecific binding molecule and preservative in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable composition, for example, a measured amount of at least one bispecific binding molecule in buffered solution is combined with the desired preservative in a buffered solution in quantities sufficient to provide the bispecific binding molecule and preservative at the desired concentrations. The compositions provided herein can be prepared by a process that comprises mixing at least one bispecific binding molecule as described herein and a selected buffer, preferably a phosphate buffer containing saline or a chosen salt. Mixing the at least one

bispecific binding molecule and buffer in an aqueous diluent is carried out using conventional dissolution and mixing procedures. To prepare a suitable composition, for example, a measured amount of at least one bispecific binding molecule in water or buffer is combined with the desired buffering agent in water in quantities sufficient to provide the protein and buffer at the desired concentrations. Variations of these processes would be recognized by one of ordinary skill in the art. For example, the order the components are added, whether additional additives are used, the temperature and pH at which the composition is prepared, are all factors that can be optimized for the concentration and means of administration used.

[00197] In specific embodiments involving combination therapy with infusion of T cells, provided herein is a pharmaceutical composition comprising (a) a bispecific binding molecule described herein (*see, e.g.,* Section 5.1 or 5.2); (b) T cells; and/or (c) a pharmaceutically effective carrier. In specific embodiments, the T cells are autologous to the subject to whom the T cells are administered. In certain embodiments, the T cells are allogeneic to the subject to whom the T cells are administered. In specific embodiments, the T cells are bound to the bispecific binding molecule. In specific embodiments, the binding of the T cells to the bispecific binding molecule is noncovalently. In specific embodiments, the T cells are human T cells. Methods that can be used to bind bispecific binding molecules to T cells are known in the art. *See, e.g.*, Lum *et al.,* 2013, Biol Blood Marrow Transplant, 19:925-33, Janeway *et al.,* Immunobiology: The Immune System in Health and Disease, 5th edition, New York: Garland Science; Vaishampayan *et al.,* 2015, Prostate Cancer, 2015:285193, and Stromnes *et al.,* 2014, Immunol Rev. 257(1):145-164. *See, also*, Vaishampayan *et al.,* 2015, Prostate Cancer, 2015:285193, which describes the following exemplary, non-limiting method for binding bispecific binding molecules to T cells:

> Peripheral blood mononuclear cells (PBMCs) are collected to obtain lymphocytes for activated T cell expansion from 1 or 2 leukopheresis. PBMCs are activated with, for example, 20 ng/mL of OKT3 and expanded in 100 IU/mL of IL-2 to generate 40-320 billion activated T cells during a maximum of 14 days of culture under cGMP conditions as described in Ueda *et al.*, 1993, Transplantation, 56(2):351-356 and Uberti *et al.*, 1994, Clinical Immunology and Immunopathology, 70(3):234-240. Cells are grown in breathable flasks (FEP Bag Type 750-Cl, American Fluoroseal Corporation, Gaithersburg, MD) in RPMI 1640 medium (Lonza) supplemented with 2% pooled heat inactivated human

serum. Activated T cells are split approximately every 2-3 days based on cell counts. After 14 days, activated T cells are cultured with 50 ng of a bispecific binding molecule described herein per 10^6 activated T cells. The mixture is then washed and cryopreserved.

[00198] In certain embodiments, a pharmaceutical composition described herein is to be used in accordance with the methods provided herein (*see*, *e.g.*, Section 5.6).

5.5.1 PARENTERAL FORMULATIONS

[00199] In certain embodiments, a composition provided herein is formulated for parenteral injectable administration. As used herein, the term "parenteral" includes intravenous, intravascular, intramuscular, intradermal, subcutaneous, and intraocular. For parenteral administration, the composition can be formulated as a solution, suspension, emulsion or lyophilized powder in association, or separately provided, with a pharmaceutically acceptable parenteral vehicle. Non-limiting examples of such vehicles are water, saline, Ringer's solution, dextrose solution, glycerol, ethanol, and 1-10% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils can also be used. The vehicle or lyophilized powder can contain additives that maintain isotonicity (*e.g.*, sodium chloride, mannitol) and chemical stability (*e.g.*, buffers and preservatives). The formulation is sterilized by known or suitable techniques.

[00200] Suitable pharmaceutical carriers are described in the most recent edition of Remington's Pharmaceutical Sciences, A. Osol, a standard reference text in this field.

[00201] Formulations for parenteral administration can contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Aqueous or oily suspensions for injection can be prepared by using an appropriate emulsifier or humidifier and a suspending agent, according to known methods. Agents for injection can be a non-toxic, non-orally administrable diluting agent such as aqueous solution or a sterile injectable solution or suspension in a solvent. As the usable vehicle or solvent, water, Ringer's solution, isotonic saline, etc. are allowed; as an ordinary solvent, or suspending solvent, sterile involatile oil can be used. For these purposes, any kind of involatile oil and fatty acid can be used, including natural or synthetic or semisynthetic fatty oils or fatty acids; natural or synthetic or semisynthetic mono- or di- or tri-glycerides. Parental administration is known in the art and includes, but is not limited to, conventional means of

injections, a gas pressured needle-less injection device as described in U.S. Pat. No. 5,851,198, and a laser perforator device as described in U.S. Pat. No. 5,839,446 entirely incorporated herein by reference.

5.5.2 PULMONARY FORMULATIONS

[00202] In certain embodiments, a composition comprising a bispecific binding molecule described herein is formulated for pulmonary administration. For pulmonary administration, the composition is delivered in a particle size effective for reaching the lower airways of the lung or sinuses. Compositions for pulmonary administration can be delivered by any of a variety of inhalation or nasal devices known in the art for administration of a therapeutic agent by inhalation. These devices capable of depositing aerosolized formulations in the sinus cavity or alveoli of a patient include metered dose inhalers, nebulizers, dry powder generators, sprayers, and the like. Other devices suitable for directing the pulmonary or nasal administration of bispecific binding molecules described herein are also known in the art. All such devices use formulations suitable for the administration for the dispensing of a bispecific binding molecule described herein in an aerosol. Such aerosols can be comprised of either solutions (both aqueous and non aqueous) or solid particles. Metered dose inhalers like the Ventolin® metered dose inhaler, typically use a propellent gas and require actuation during inspiration (*See*, *e.g.*, WO 94/16970, WO 98/35888). Dry powder inhalers like Turbuhaler™ (Astra), Rotahaler®. (Glaxo), Diskus® (Glaxo), devices marketed by Inhale Therapeutics, to name a few, use breath-actuation of a mixed powder (U.S. Pat. No. 4,668,218 Astra, EP 237507 Astra, WO 97/25086 Glaxo, WO 94/08552 Dura, U.S. Pat. No. 5,458,135 Inhale, WO 94/06498 Fisons, entirely incorporated herein by reference). Nebulizers like the Ultravent® nebulizer (Mallinckrodt), and the Acorn II® nebulizer (Marquest Medical Products) (U.S. Pat. No. 5,404,871 Aradigm, WO 97/22376), the above references entirely incorporated herein by reference, produce aerosols from solutions, while metered dose inhalers, dry powder inhalers, etc. generate small particle aerosols. Such examples of commercially available inhalation devices are non-limiting examples are not intended to be limiting in scope.

[00203] In certain embodiments, a spray comprising a bispecific binding molecule as described herein can be produced by forcing a suspension or solution of at least one bispecific binding molecule as described herein through a nozzle under pressure. The nozzle size and configuration, the applied pressure, and the liquid feed rate can be chosen to achieve the desired

output and particle size. An electrospray can be produced, for example, by an electric field in connection with a capillary or nozzle feed. Advantageously, particles of a composition comprising at least one bispecific binding molecule described herein delivered by a sprayer have a particle size less than about 10 um, preferably in the range of about 1 um to about 5 um, and most preferably about 2 um to about 3 um.

[00204] Formulations of a composition comprising at least one bispecific binding molecule described herein suitable for use with a sprayer typically include the at least one bispecific binding molecule in an aqueous solution at a concentration of about 0.1 mg to about 100 mg per ml of solution or mg/gm, or any range or value therein, *e.g.*, but not limited to, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/ml or mg/gm. The formulation can include agents such as an excipient, a buffer, an isotonicity agent, a preservative, a surfactant, and, preferably, zinc. The formulation can also include an excipient or agent for stabilization of the bispecific binding molecule composition, such as a buffer, a reducing agent, a bulk protein, or a carbohydrate. Bulk proteins useful in formulating such a composition include albumin, protamine, or the like. Typical carbohydrates useful in formulating antibody composition proteins include sucrose, mannitol, lactose, trehalose, glucose, or the like. The composition can also include a surfactant, which can reduce or prevent surface-induced aggregation of the composition caused by atomization of the solution in forming an aerosol. Various conventional surfactants can be employed, such as polyoxyethylene fatty acid esters and alcohols, and polyoxy ethylene sorbitol fatty acid esters. Amounts will generally range between 0.001 and 14% by weight of the formulation. Preferred surfactants are polyoxyethylene sorbitan monooleate, polysorbate 80, polysorbate 20, or the like.

[00205] In certain embodiments, the composition is administered via a nebulizer, such as jet nebulizer or an ultrasonic nebulizer. Typically, in a jet nebulizer, a compressed air source is used to create a high-velocity air jet through an orifice. As the gas expands beyond the nozzle, a low-pressure region is created, which draws a solution of antibody composition protein through a capillary tube connected to a liquid reservoir. The liquid stream from the capillary tube is sheared into unstable filaments and droplets as it exits the tube, creating the aerosol. A range of configurations, flow rates, and baffle types can be employed to achieve the desired performance characteristics from a given jet nebulizer. In an ultrasonic nebulizer, high-frequency electrical

energy is used to create vibrational, mechanical energy, typically employing a piezoelectric transducer. This energy is transmitted to the formulation of antibody composition protein either directly or through a coupling fluid, creating an aerosol including the antibody composition protein. Advantageously, particles of antibody composition protein delivered by a nebulizer have a particle size less than about 10 um, preferably in the range of about 1 um to about 5 um, and most preferably about 2 um to about 3 um.

[00206] In certain embodiments, the composition is administered via a metered dose inhaler (MDI), wherein a propellant, at least one bispecific binding molecule described herein, and any excipients or other additives are contained in a canister as a mixture including a liquefied compressed gas. Actuation of the metering valve releases die mixture as an aerosol, preferably containing particles in the size range of less than about 10 um, preferably about 1 um to about 5 um, and most preferably about 2 um to about 3 um. The desired aerosol particle size can be obtained by employing a formulation of antibody composition protein produced by various methods known to those of skill in the art, including jet-milling, spray drying, critical point condensation, or the like. Preferred metered dose inhalers include those manufactured by 3M or Glaxo and employing a hydrofluorocarbon propellant.

[00207] Formulations of a bispecific binding molecule described herein for use with a metered-dose inhaler device will generally include a finely divided powder containing at least one Anti-IL-6 antibody as a suspension in a non-aqueous medium, for example, suspended in a propellant with the aid of a surfactant. The propellant can be any conventional material employed for this purpose, such as chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol and 1,1,1,2-tetrafluoroethane, HFA-134a (hydrofluoroalkane-134a), HFA-227 (hydrofluoroalkane-227), or the like. Preferably the propellant is a hydrofluorocarbon. The surfactant can be chosen to stabilize the at least one bispecific binding molecule as a suspension in the propellant, to protect the active agent against chemical degradation, and the like. Suitable surfactants include sorbitan trioleate, soya lecithin, oleic acid, or the like. In some cases solution aerosols are preferred using solvents such as ethanol. Additional agents known in the art for formulation of a protein can also be included in the formulation.

5.5.3 ORAL FORMULATIONS

[00208] In certain embodiments, a composition provided herein is formulated for oral administration. In certain embodiments, for oral administration, compositions and methods of administering at least one bispecific binding molecule described herein rely on the co-administration of adjuvants such as, for example, resorcinols and nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecylpolyethylene ether, to artificially increase the permeability of the intestinal walls, as well as the co-administration of enzymatic inhibitors such as, for example, pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) and trasylol, to inhibit enzymatic degradation. The active constituent compound of the solid-type dosage form for oral administration can be mixed with at least one additive, including sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, arginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, and glyceride. These dosage forms can also contain other type(s) of additives, such as, for example, inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, alpha.-tocopherol, antioxidant such as cysteine, disintegrator, binder, thickener, buffering agent, sweetening agent, flavoring agent, perfuming agent, etc.

[00209] In certain embodiments, tablets and pills for oral administration can be further processed into enteric-coated preparations. In certain embodiments, liquid preparations for oral administration include, for example, emulsion, syrup, elixir, suspension and solution preparations allowable for medical use. These preparations can contain inactive diluting agents ordinarily used in said field, for example, water. Liposome preparations can be utilized for oral administration preparations, for example, as described for insulin and heparin (U.S. Pat. No. 4,239,754). Additionally, microspheres of artificial polymers of mixed amino acids (proteinoids) can be utilized to in oral administration of pharmaceuticals, for example, as described in U.S. Pat. No. 4,925,673. Furthermore, carrier compounds, such as those described in U.S. Pat. No. 5,879,681 and U.S. Pat. No. 5,871,753, are used in oral administration of biologically active agents.

5.5.4 MUCOSAL FORMULATIONS

[00210] In certain embodiments, a composition provided herein is formulated for absorption through mucosal surfaces. In certain embodiments, for absorption through mucosal surfaces, compositions and methods of administering at least one bispecific binding molecule described

herein include an emulsion comprising a plurality of submicron particles, a mucoadhesive macromolecule, a bioactive peptide, and an aqueous continuous phase, which promotes absorption through mucosal surfaces by achieving mucoadhesion of the emulsion particles (U.S. Pat. No. 5,514,670). Mucous surfaces suitable for application of the emulsions provided herein can include, for example, corneal, conjunctival, buccal, sublingual, nasal, vaginal, pulmonary, stomachic, intestinal, and rectal routes of administration. Formulations for vaginal or rectal administration, for example, suppositories, can contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for intranasal administration can be solid and contain as excipients, for example, lactose or can be aqueous or oily solutions of nasal drops. For buccal administration excipients include, for example, sugars, calcium stearate, magnesium stearate, pregelinatined starch, and the like (U.S. Pat. No. 5,849,695).

5.5.5 TRANSDERMAL FORMULATIONS

[00211] In certain embodiments, a composition provided herein is formulated for transdermal administration. In certain embodiments, for transdermal administration, the composition comprises at least one bispecific binding molecule described herein encapsulated in a delivery device such as, for example, a liposome or polymeric nanoparticles, microparticle, microcapsule, or microspheres (referred to collectively as microparticles unless otherwise stated). A number of suitable devices are known for transdermal administration, including microparticles made of synthetic polymers such as polyhydroxy acids such as polylactic acid, polyglycolic acid and copolymers thereof, polyorthoesters, polyanhydrides, and polyphosphazenes, and natural polymers such as collagen, polyamino acids, albumin and other proteins, alginate and other polysaccharides, and combinations thereof (U.S. Pat. No. 5,814,599).

5.5.6 KITS

[00212] Provided herein are kits comprising one or more bispecific binding molecule as described herein, or one or more composition as described herein. In certain embodiments, the kit comprises packaging material and at least one vial comprising a composition comprising a bispecific binding molecule or composition described herein. In certain embodiments, the vial comprises a solution of at least one bispecific binding molecule or composition as described herein with the prescribed buffers and/or preservatives, optionally in an aqueous diluents. In

certain embodiments, the packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater. In certain embodiments, the kit comprises two vials. In certain embodiments, the first vial comprises at least one lyophilized bispecific binding molecule or composition as described herein and the second vial comprises aqueous diluents of prescribed buffer or preservative. In certain embodiments, the packaging material comprises a label that instructs a subject to reconstitute the at least one lyophilized bispecific binding molecule in the aqueous diluents to form a solution that can be held over a period of twenty-four hours or greater. In certain embodiments, the packaging material comprises a label that indicates that such solution can be held over a period of 1, 2, 3, 4, 5, 6, 9, 12, 18, 20, 24, 30, 36, 40, 48, 54, 60, 66, 72 hours or greater.

[00213] In certain embodiments, the compositions provided herein can be provided to a subject as solutions or as dual vials comprising a vial of lyophilized at least one bispecific binding molecule or composition that is reconstituted with a second vial containing water, a preservative and/or excipients, preferably a phosphate buffer and/or saline and a chosen salt, in an aqueous diluent. Either a single solution vial or dual vial requiring reconstitution can be reused multiple times and can suffice for a single or multiple cycles of subject treatment and thus can provide a more convenient treatment regimen than currently available.

[00214] In certain embodiments, a kit comprising a bispecific binding molecule or composition described herein is useful for administration over a period of immediately to twenty-four hours or greater. Accordingly, the kit offers significant advantages to the patient. In certain embodiments, a kit comprising a bispecific binding molecule or composition described herein can optionally be safely stored at temperatures of from about 2 °C to about 40 °C and retain the biologically activity of the protein for extended periods of time, thus, allowing a package label indicating that the solution can be held and/or used over a period of 6, 12, 18, 24, 36, 48, 72, or 96 hours or greater. In certain embodiments, the kit comprises a

[00215] If preserved diluent is used, such label can include use up to 1-12 months, one-half, one and a half, and/or two years.

[00216] The kits can be provided indirectly to a subject, such as a subject as described in Section 5.7, by providing to pharmacies, clinics, or other such institutions and facilities, solutions or dual vials comprising a vial of lyophilized at least one bispecific binding molecule or

composition that is reconstituted with a second vial containing the aqueous diluent. The solution in this case can be up to one liter or even larger in size, providing a large reservoir from which smaller portions of the at least one antibody solution can be retrieved one or multiple times for transfer into smaller vials and provided by the pharmacy or clinic to their customers and/or patients.

[00217] Recognized devices comprising these single vial systems include those pen-injector devices for delivery of a solution such as BD Pens, BD Autojector®, Humaject®, *e.g.*, as made or developed by Becton Dickensen (Franklin Lakes, N.J.,), Disetronic (Burgdorf, Switzerland; Bioject, Portland, Oreg.; National Medical Products, Weston Medical (Peterborough, UK), Medi-Ject Corp (Minneapolis, Minn.). Recognized devices comprising a dual vial system include those pen-injector systems for reconstituting a lyophilized drug in a cartridge for delivery of the reconstituted solution such as the HumatroPen®.

[00218] In certain embodiments, the kits comprise packaging material. In certain embodiments, the packaging material provides, in addition to the information required by a regulatory agencies, the conditions under which the product can be used. In certain embodiments, the packaging material provides instructions to the subject to reconstitute the at least one bispecific binding molecule in the aqueous diluent to form a solution and to use the solution over a period of 2-24 hours or greater for the two vial, wet/dry, product. For the single vial, solution product, the label indicates that such solution can be used over a period of 2-24 hours or greater. In a preferred embodiment, the kit is useful for human pharmaceutical product use. In certain embodiments, the kit is useful for veterinarian pharmaceutical use. In a preferred embodiment, the kit is useful for canine pharmaceutical product use. In a preferred embodiment, the kit is useful for intravenous administration. In another preferred embodiment, the kit is useful for intraperitoneal, intrathecal, intraventricular in the brain, or intraparenchymal in the brain administration.

5.6 USES AND METHODS

5.6.1 THERAPEUTIC USES

[00219] In certain embodiments, provided herein are methods for treating a HER2-positive cancer cell in a subject comprising administering to the subject in need thereof a therapeutically effective amount of a bispecific binding molecule as described in Section 5.1 or in Section 5.2, a

therapeutically effective amount of a cell, polynucleotide, or vector encoding such a bispecific binding molecule as described in Section 5.3, or a therapeutically effective amount of a pharmaceutical composition as described in Section 5.5, or a therapeutically effective amount of T cells bound to a bispecific binding molecule as described in Section 5.4. In a specific embodiment, the subject is a subject as described in Section 5.7. In a specific embodiment, the bispecific binding molecule is administered at a dose as described in Section 5.8. In a specific embodiment, the bispecific binding molecule is administered according to the methods as described in Section 5.5. In a preferred embodiment, the bispecific binding molecule is administered intravenously. In another preferred embodiment, the bispecific binding molecule is administered intrathecally, intraventricularly in the brain, intraparenchymally in the brain, or intraperitoneally. In a specific embodiment, the bispecific binding molecule is administered in combination with one or more additional pharmaceutically active agents as described in Section 5.9.

[00220] In certain embodiments, provided herein are methods for treating a HER2-positive cancer cell in a subject comprising administering to the subject in need thereof a pharmaceutical composition as described in Section 5.1 or in Section 5.2. In a specific embodiment, the pharmaceutical composition is a composition as described in Section 5.5. In a specific embodiment, the subject is a subject as described in Section 5.7. In a specific embodiment, the pharmaceutical composition is administered at a dose as described in Section 5.8. In a specific embodiment, the pharmaceutical composition is administered according to the methods as described in Section 5.5. In a preferred embodiment, the pharmaceutical composition is administered intravenously. In another preferred embodiment, the bispecific binding molecule is administered intrathecally, intraventricularly in the brain, intraparenchymally in the brain, or intraperitoneally. In a specific embodiment, the pharmaceutical composition is administered in combination with one or more additional pharmaceutically active agents as described in Section 5.9.

[00221] For use of a bispecific binding molecule in a subject of a particular species, a bispecific binding molecule is used that binds to the HER2 and the CD3 of that particular species. For example, to treat a human, the bispecific binding molecule comprises an aglycosylated monoclonal antibody that is an immunoglobulin that binds to human HER2, comprising two identical heavy chains and two identical light chains, said light chains being a

first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to human CD3, and wherein the first and second light chain fusion polypeptides are identical. In another example, to treat a canine, the bispecific binding molecule comprises an aglycosylated monoclonal antibody that is an immunoglobulin that binds to canine HER2, comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to canine CD3, and wherein the first and second light chain fusion polypeptides are identical. Bispecific binding molecules that are cross-reactive with HER2 and/or CD3 of various species can be used to treat subjects in those species. For example, trastuzumab is expected to bind both human and canine HER2 due to the relative conservation of the epitope in HER2 recognized by trastuzumab. *See*, also, for example, Singer *et al.,* 2012, Mol Immunol, 50: 200-209.

[00222] In addition, for use of a bispecific binding molecule in a subject of a particular species, the bispecific binding molecule, preferably, the constant region of the immunoglobulin portion, is derived from that particular species. For example, to treat a human, the bispecific binding molecule can comprise an aglycosylated monoclonal antibody that is an immunoglobulin, wherein the immunoglobulin comprises a human constant region. In another example, to treat a canine, the bispecific binding molecule can comprise an aglycosylated monoclonal antibody that is an immunoglobulin, wherein the immunoglobulin comprises a canine constant region. In a specific embodiment, when treating a human, the immunoglobulin is humanized. In a specific embodiment, the subject is a human. In a specific embodiment, the subject is a canine.

[00223] In a specific embodiment, the HER2-positive cancer is breast cancer, gastric cancer, an osteosarcoma, desmoplastic small round cell cancer, squamous cell carcinoma of head and neck cancer, ovarian cancer, prostate cancer, pancreatic cancer, glioblastoma multiforme, gastric

junction adenocarcinoma, gastroesophageal junction adenocarcinoma, cervical cancer, salivary gland cancer, soft tissue sarcoma, leukemia, melanoma, Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma, or any other neoplastic tissue that expresses the HER2 receptor.

[00224] In a specific embodiment, the HER2-positive cancer cell is resistant to treatment with trastuzumab, cetuximab, lapatinib, erlotinib, or any other small molecule or antibody that targets the HER family of receptors. In a specific embodiment, the tumor that is resistant to treatment with trastuzumab, cetuximab, lapatinib, erlotinib, or any other small molecule or antibody that targets the HER family of receptors is responsive to treatment with a bispecific binding molecule to the invention.

[00225] In specific embodiments, treatment can be to achieve beneficial or desired clinical results including, but not limited to, alleviation of a symptom, diminishment of extent of a disease, stabilizing (i.e., not worsening) of state of a disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. In a specific embodiment, "treatment" can also be to prolong survival as compared to expected survival if not receiving treatment.

5.6.2 DIAGNOSTIC USES

[00226] In certain embodiments, bispecific binding molecules described herein can be used for diagnostic purposes to detect, diagnose, or monitor a condition described herein (*e.g.*, a condition involving HER2-positive cancer cells). In certain embodiments, bispecific binding molecules for use in diagnostic purposes are labeled as described in Section 5.2.

[00227] In certain embodiments, provided herein are methods for the detection of a condition described herein comprising (a) assaying the expression of HER2 in cells or a tissue sample of a subject using one or more bispecific binding molecules described herein; and (b) comparing the level of HER2 expression with a control level, for example, levels in normal tissue samples (*e.g.*, from a subject not having a condition described herein, or from the same patient before onset of the condition), whereby an increase or decrease in the assayed level of HER2 expression compared to the control level of HER2 expression is indicative of a condition described herein.

[00228] Antibodies described herein can be used to assay HER2 levels in a biological sample using classical immunohistological methods as described herein or as known to those of skill in the art (*e.g.*, *see* Jalkanen *et al.,* 1985, J. Cell. Biol. 101:976-985; and Jalkanen *et al.,* 1987, J. Cell . Biol. 105:3087-3096). Other antibody-based methods useful for detecting protein gene

expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{121}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[00229] In certain embodiments, monitoring of a condition described herein (*e.g.*, a HER2-positive cancer), is carried out by repeating the method for diagnosing for a period of time after initial diagnosis.

[00230] Presence of the labeled molecule can be detected in the subject using methods known in the art for in vivo scanning. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

5.7 PATIENT POPULATION

[00231] A subject treated in accordance with the methods provided herein can be any mammal, such as a rodent, a cat, a canine, a horse, a cow, a pig, a monkey, a primate, or a human, etc. In a preferred embodiment, the subject is a human. In another preferred embodiment, the subject is a canine.

[00232] In certain embodiments, a subject treated in accordance with the methods provided herein has been diagnosed with a HER2-positive cancer, including but not limited to, breast cancer, gastric cancer, an osteosarcoma, desmoplastic small round cell cancer, squamous cell carcinoma of head and neck cancer, ovarian cancer, prostate cancer, pancreatic cancer, glioblastoma multiforme, gastric junction adenocarcinoma, gastroesophageal junction adenocarcinoma, cervical cancer, salivary gland cancer, soft tissue sarcoma, leukemia, melanoma, Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma, or any other neoplastic tissue that expresses the HER2 receptor.

[00233] In certain embodiments, the subject is resistant to treatment with trastuzumab, cetuximab, lapatinib, erlotinib, or any other small molecule or antibody that targets the HER family of receptors. In a specific embodiment, the tumor that is resistant to treatment with trastuzumab, cetuximab, lapatinib, erlotinib, or any other small molecule or antibody that targets

the HER family of receptors is responsive to treatment with a bispecific binding molecule to the invention.

[00234] In certain embodiments, a subject treated in accordance with the methods provided herein has a HER2-positive cancer that is resistant to treatment with trastuzumab, cetuximab, lapatinib, erlotinib, or any other small molecule or antibody that targets the HER family of receptors. In certain embodiments, a subject treated in accordance with the methods provided herein has a HER2-positive cancer that is responsive to treatment with a bispecific binding molecule to the invention.

[00235] In certain embodiments, the subject treated in accordance with the methods provided herein has previously received one or more chemotherapy regimens for metastatic disease, *e.g.*, brain or peritoneal metastases. In certain embodiments, the subject has not previously received treatment for metastatic disease.

5.8 DOSES AND REGIMENS

[00236] In certain embodiments, the dose of a bispecific binding molecule as described in Section 5.1 administered to a subject according to the methods provided herein is a dose determined by the needs of the subject. In certain embodiments, the dose is determined by a physician according to the needs of the subject.

[00237] In a specific embodiment, the dose of a bispecific binding molecule provided herein is less than the dose of trastuzumab. *See*, for example, Trastuzumab [Highlights of Prescribing Information]. South San Francisco, CA: Genentech, Inc.; 2014. In a specific embodiment, the dose of a bispecific binding molecule provided herein is approximately between 20 and 40 fold less than an FDA-approved dose of trastuzumab.

[00238] In certain embodiments, the dose of a bispecific binding molecule as described in Section 5.1 administered to a subject according to the methods provided herein is between 0.01 mg/kg and 0. 025 mg/kg, is between 0.025 mg/kg and 0.05 mg/kg, is between 0.05 mg/kg and 0.1 mg/kg, is between 0.1 mg/kg and 0.5 mg/kg, between 0.1 mg/kg and 0.6 mg/kg, between 0.2 mg/kg and 0.7 mg/kg, between 0.3 mg/kg and 0.8 mg/kg, between 0.4 mg/kg and 0.8 mg/kg, between 0.5 mg/kg and 0.9 mg/kg, or between 0.6 mg/kg and 1.

[00239] In certain embodiments, the dose of a bispecific binding molecule as described in Section 5.1 administered to a subject according to the methods provided herein is an initial dose followed by an adjusted dose that is the maintenance dose. In certain embodiments the initial

dose is administered once. In certain embodiments the initial is between 0.01 mg/kg and 0. 025 mg/kg, is between 0.025 mg/kg and 0.05 mg/kg, is between 0.05 mg/kg and 0.1 mg/kg, is between 0.1 mg/kg and 0.5 mg/kg, between 0.1 mg/kg and 0.6 mg/kg, between 0.2 mg/kg and 0.7 mg/kg, between 0.3 mg/kg and 0.8 mg/kg, between 0.4 mg/kg and 0.8 mg/kg, between 0.5 mg/kg and 0.9 mg/kg, or between 0.6 mg/kg and 1. In certain embodiments, the initial dose is administered via intravenous infusion over 90 minutes. In certain embodiments, the adjusted dose is administered once every about 4 weeks. In certain embodiments, the adjusted dose is administered for at least 13, at least 26, or at most 52 weeks. In certain embodiments the adjusted dose is administered for 52 weeks. In certain embodiments, the adjusted dose is between 0.01 mg/kg and 0. 025 mg/kg, is between 0.025 mg/kg and 0.05 mg/kg, is between 0.05 mg/kg and 0.1 mg/kg, is between 0.1 mg/kg and 0.5 mg/kg, between 0.1 mg/kg and 0.6 mg/kg, between 0.2 mg/kg and 0.7 mg/kg, between 0.3 mg/kg and 0.8 mg/kg, between 0.4 mg/kg and 0.8 mg/kg, between 0.5 mg/kg and 0.9 mg/kg, or between 0.6 mg/kg and 1. In certain embodiments, the adjusted dose is administered via intravenous infusion over 30 minutes. In certain embodiments, the adjusted dose is administered via intravenous infusion over 30 to 90 minutes.

[00240] In another specific embodiment, a bispecific binding molecule as described in Section 5.1 for use with the methods provided herein is administered 1, 2, or 3 times a week, every 1, 2, 3, or 4 weeks. In certain embodiments, the bispecific binding molecule is administered according to the following regimen: (i) 1, 2, or 3 administrations in a first week; (ii) 1, 2, 3, or 4 administrations a week after the first week; followed by (iii) 1, 2, or 3 administrations in one week each month for a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. In certain embodiments, the bispecific binding molecule is administered according to the following regimen: (i) 3 administrations in a first week; (ii) 3 administrations a week after the first week; followed by (iii) 3 administrations in one week each month for a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. In certain embodiments, the bispecific binding molecule is administered according to the following regimen: (i) 3 administrations in a first week; (ii) 2 administrations a week after the first week; followed by (iii) 2 administrations in one week each month for a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. In certain embodiments, the bispecific binding molecule is administered according to the following regimen: (i) 3 administrations in a first week; (ii) 1 administrations a week after the first week; followed by (iii) 1 administrations in one week each month for a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. In certain

embodiments, the bispecific binding molecule is administered according to the following regimen: (i) 2 administrations in a first week; (ii) 2 administrations a week after the first week; followed by (iii) 2 administrations in one week each month for a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. In certain embodiments, the bispecific binding molecule is administered according to the following regimen: (i) 2 administrations in a first week; (ii) 1 administrations a week after the first week; followed by (iii) 1 administrations in one week each month for a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. In certain embodiments, the bispecific binding molecule is administered according to the following regimen: (i) 1 administrations in a first week; (ii) 1 administrations a week after the first week; followed by (iii) 1 administrations in one week each month for a total of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months.

[00241] In certain embodiments, a bispecific binding molecule as described in Section 5.1 is administered to a subject according to the methods provided herein in combination with a second pharmaceutically active agent as described in Section 5.9.

[00242] In another preferred embodiment, the bispecific binding molecule is administered intrathecally, intraventricularly in the brain, intraparenchymally in the brain, or intraperitoneally.

5.9 COMBINATION THERAPY

[00243] In certain embodiments, a bispecific binding molecule provided herein, or polynucleotide, vector, or cell encoding the bispecific binding molecule, may be administered in combination with one or more additional pharmaceutically active agents, *e.g.*, a cancer chemotherapeutic agent. In certain embodiments, such combination therapy may be achieved by way of simultaneous, sequential, or separate dosing of the individual components of the treatment. In certain embodiments, the bispecific binding molecule or polynucleotide, vector, or cell encoding the bispecific binding molecule, and one or more additional pharmaceutically active agents may be synergistic, such that the dose of either or of both of the components may be reduced as compared to the dose of either component that would be given as a monotherapy. Alternatively, In certain embodiments, the bispecific binding molecule or polynucleotide, vector, or cell encoding the bispecific binding molecule and the one or more additional pharmaceutically active agents may be additive, such that the dose of the bispecific binding molecule and of the one or more additional pharmaceutically active agents is similar or the same as the dose of either component that would be given as a monotherapy.

[00244] In certain embodiments, a bispecific binding molecule provided herein, or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered on the same day as one or more additional pharmaceutically active agents. In certain embodiments, the bispecific binding molecule or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 hours before the one or more additional pharmaceutically active agents. In certain embodiments, the bispecific binding molecule or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 hours after the one or more additional pharmaceutically active agents. In certain embodiments, the bispecific binding molecule or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered 1, 2, 3, or more days before the one or more additional pharmaceutically active agents. In certain embodiments, the bispecific binding molecule or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered 1, 2, 3 or more days after the one or more additional pharmaceutically active agents. In certain embodiments, the bispecific binding molecule or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered 1, 2, 3, 4, 5, or 6 weeks before the one or more additional pharmaceutically active agents. In certain embodiments, the bispecific binding molecule or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered 1, 2, 3, 4, 5, or 6 weeks after the one or more additional pharmaceutically active agents.

[00245] In certain embodiments, the additional pharmaceutically active agent is doxorubicin. In certain embodiments, the additional pharmaceutically active agent is cyclophosphamide. In certain embodiments, the additional pharmaceutically active agent is paclitaxel. In certain embodiments, the additional pharmaceutically active agent is docetaxel. In certain embodiments, the one or more additional pharmaceutically active agents is carboplatin.

[00246] In certain embodiments, the additional pharmaceutically active agent is a cytokine, such as, for example, IL15, IL15R/IL15 complex, IL2, or GMCSF.

[00247] In certain embodiments, the additional pharmaceutically active agent is an agent that increases cellular HER2 expression, such as, for example, external beam or radioimmunotherapy. *See*, for example, Wattenberg *et al.,* 2014, British Journal of Cancer, 110: 1472.

[00248] In certain embodiments, the additional pharmaceutically active agent is a radiotherapeutic agent.

[00249] In certain embodiments, the additional pharmaceutically active agent is an agent that directly controls the HER2 signaling pathway, *e.g.,* lapatinib. *See*, for example, Scaltiri *et al.,* 2012, 28(6): 803-814.

[00250] In certain embodiments, the additional pharmaceutically active agent is an agent that increases cell death, apoptosis, autophagy, or necrosis of tumor cells.

[00251] In certain embodiments, a bispecific binding molecule provided herein, or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered in combination with two additional pharmaceutically active agents, *e.g.*, those used in combination with trastuzumab (*see*, Trastuzumab [Highlights of Prescribing Information]. South San Francisco, CA: Genentech, Inc.; 2014). In certain embodiments, the two additional pharmaceutically active agents are doxorubicin and paclitaxel. In certain embodiments, the two additional pharmaceutically active agents are doxorubicin and docetaxel. In certain embodiments, the two additional pharmaceutically active agents are cyclophosphamid and paclitaxel. In certain embodiments, the two additional pharmaceutically active agents are cyclophosphamide and docetaxel. In certain embodiments, the two additional pharmaceutically active agents are docetaxel and carboplatin. In certain embodiments, the two additional pharmaceutically active agents are cisplatin and capecitabine. In certain embodiments, the two additional pharmaceutically active agents are cisplatin and 5-fluorouracil.

[00252] In certain embodiments, a bispecific binding molecule provided herein, or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered as a single agent following multi-modality anthracycline based therapy.

[00253] In certain embodiments, a bispecific binding molecule provided herein, or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered after one or more chemotherapy regimens for metastatic disease, *e.g.*, brain or peritoneal metastases. In specific embodiments, a bispecific binding molecule provided herein, or polynucleotide, vector, or cell encoding the bispecific binding molecule is administered in combination with cytoreductive chemotherapy. In a specific embodiment, the administering is performed after treating the subject with cytoreductive chemotherapy.

[00254] In specific embodiments, a bispecific binding molecule provided herein, polynucleotide, vector, or cell encoding the bispecific binding molecule, or a pharmaceutical composition comprising the bispecific binding molecule, is administered in combination with T cell infusion. In specific embodiments, the bispecific binding molecule is not bound to a T cell. In specific embodiments, the bispecific binding molecule is bound to a T cell. In specific embodiments, the binding of the bispecific binding molecule to the T cell is noncovalently. In a specific embodiment, the administering of a bispecific binding molecule provided herein, polynucleotide, vector, or cell encoding the bispecific binding molecule, or a pharmaceutical composition comprising the bispecific binding molecule is performed after treating the patient with T cell infusion. In specific embodiments the T cell infusion is performed with T cells that are autologous to the subject to whom the T cells are administered. In specific embodiments, the T cell infusion is performed with T cells that are allogeneic to the subject to whom the T cells are administered. In specific embodiments, the T cells can be bound to molecules identical to a bispecific binding molecule as described herein. In specific embodiments, the binding of the T cells to molecules identical to the bispecific binding molecule is noncovalently. In specific embodiments, the T cells are human T cells. Methods that can be used to bind bispecific binding molecules to T cells are known in the art. *See*, *e.g.*, Lum *et al.,* 2013, Biol Blood Marrow Transplant, 19:925-33, Janeway *et al.,* Immunobiology: The Immune System in Health and Disease, 5th edition, New York: Garland Science; Vaishampayan *et al.,* 2015, Prostate Cancer, 2015:285193, and Stromnes *et al.,* 2014, Immunol Rev. 257(1):145-164. *See*, *also*, Vaishampayan *et al.,* 2015, Prostate Cancer, 2015:285193, which describes the following exemplary, non-limiting method for binding bispecific binding molecules to T cells:

> Peripheral blood mononuclear cells (PBMCs) can be collected to obtain lymphocytes for activated T cell expansion from 1 or 2 leukopheresis. PBMCs can be activated with, for example, 20 ng/mL of OKT3 and expanded in 100 IU/mL of IL-2 to generate 40-320 billion activated T cells during a maximum of 14 days of culture under cGMP conditions as described in Ueda *et al.*, 1993, Transplantation, 56(2):351-356 and Uberti *et al.*, 1994, Clinical Immunology and Immunopathology, 70(3):234-240. Cells are grown in breathable flasks (FEP Bag Type 750-Cl, American Fluoroseal Corporation, Gaithersburg, MD) in RPMI 1640 medium (Lonza) supplemented with 2% pooled heat inactivated human

serum. Activated T cells are split approximately every 2-3 days based on cell counts. After 14 days, activated T cells are cultured with 50 ng of a bispecific binding molecule described herein per 10^6 activated T cells. The mixture is then washed and cryopreserved.

6. EXAMPLES

6.1 EXAMPLE 1

6.1.1 INTRODUCTION

[00255] This example describes a HER2 /CD3 bi-specific binding molecule (herein referred to as "HER2-BsAb") based on a IgG1 platform. This platform was utilized to allow for: (1) an optimal size to maximize tumor uptake, (2) bivalency towards the tumor target to maintain avidity, (3) a scaffold that is naturally assembled like any IgG (heavy chain and light chain) in CHO cells, purifiable by standard protein A affinity chromatography, (4) structural arrangement to render the anti-CD3 component functionally monovalent, hence reducing spontaneous activation of T cells, and (5) a platform with proven tumor targeting efficiency in animal models. This bispecific binding molecule has the same specificity as trastuzumab; but also recruits and activates CD3(+) T cells redirecting them against HER2 expressing tumor cells, generating robust anti-tumor responses. Without being bound by any theory, the effectiveness of this BsAb centers on the exploitation of the cytotoxic potential of polyclonal T cells, and its unique capacity to target tumor cells that express even low levels of HER2, independent of the activation status of the HER2 pathway.

6.1.2 MATERIALS AND METHODS

6.1.2.1 HER2-BsAb Design, Production, and Purification Analyses

[00256] The HER2-BsAb format was designed as a huOKT3 scFv fusion to the C-terminus of the light chain of a human IgG1. The V_H was identical to that of Trastuzumab IgG1, except N297A mutation in a standard human IgG1 Fc region for aglycosylated form (SEQ ID NO: 62), while the light chain is constructed as VL-Cκ-$(G_4S)_3$-scFv (SEQ ID NO: 60). Nucleotide sequences encoding VH and VL domains from Trastuzumab, and the huOKT3 scFv were synthesized by GenScript with appropriate flanking restriction enzyme sites, and were subcloned into a standard mammalian expression vector. HER2-C825 control BsAb (C825 is a murine

scFv antibody with high affinity for 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-metal complexes with lanthanides including lutetium and yttrium) was constructed in a similar way.

[00257] Linearized plasmid DNA was used to transfect CHO-S cells (Invitrogen) for stable production of BsAb. 2 x 10^6 cells were transfected with 5 μg of plasmid DNA by Nucleofection (Lonza) and then recovered in CD OptiCHO medium supplemented with 8 mM L-glutamine (Invitrogen) for 2 d at 37°C in 6-well culture plates. Stable pools were selected with 500 μg/mL hygromycin for approximately two weeks and single clones were then selected out with limited dilution. HER2-BsAb titer was determined by HER2(+) AU565 cell and CD3(+) Jurket cell ELISA, respectively, and stable clones with highest expression were selected.

[00258] The BsAb producer line was cultured in OptiCHO medium and the mature supernatant harvested. A protein A affinity column (GE Healthcare) was pre-equilibrated with 25 mM sodium citrate buffer with 0.15 M NaCl, pH 8.2. Bound BsAb was eluted with 0.1 M citric acid/sodium citrate buffer, pH 3.9 and neutralized with 25 mM sodium citrate, pH 8.5 (1:10 v/v ratio). For storage, BsAb was dialyzed into 25 mM sodium citrate, 0.15 M NaCl, pH 8.2 and frozen in aliquots at -80°C. Two micrograms of the protein was analyzed by SDS-PAGE under reducing conditions using 4–15% Tris-Glycine Ready Gel System (Bio-Rad). Invitrogen SeeBlue Plus2 Pre-Stained Standard was used as the protein MW marker. After electrophoresis, the gel was stained using Coomassie G-250 (GelCode Blue Stain Reagent; Pierce). The purity of HER2-BsAb was also evaluated by size-exclusion high-performance liquid chromatography (SE-HPLC). Approximately 20 μg of protein was injected into a TSK-GEL G3000SWXL 7.8 mm x 30 cm, 5 μm column (TOSOH Bioscience) with 0.4 M $NaClO_4$, 0.05 M NaH_2PO_4, pH 6.0 buffer at flow rate of 0.5 mL/min, and UV detection at 280 nm. Ten microliters of gel-filtration standard (Bio-Rad) was analyzed in parallel for MW markers.

6.1.2.2 FACS Analyses

[00259] Cells were incubated with 5 μg/mL of primary antibody (trastuzumab, HER2-BsAb, or cetuximab) for thirty minutes at 4°C in PBS, and a secondary phycoerythrin-labeled antibody specific for human Fc was used after wash of excess primary antibody. Cells were fixed with 1% paraformaldehyde (PFA) prior to analysis on FACSCalibur cytometer (BD biosciences). Controls were cells with secondary antibody only, for which the mean fluorescent intensity (MFI) was set to 5. FACS data display the MFI in the upper right panel of each plot.

6.1.2.3 ^{51}Cr Release Assay

[00260] The ^{51}Cr release assay was performed with effector T cells cultured in vitro in the presence of anti-CD3 and anti-CD28 for about 14 days. All target tumor cells were harvested with 2 mM EDTA in PBS, labeled with ^{51}Cr (Amersham, Arlington Height, IL) at 100 μCi/106 cells at 37°C for 1 h. 5000 target cells/well were mixed with 50,000 effector cells (E:T=10:1) and BsAb antibodies in 96-well polystyrene round-bottom plates (BD Biosciences) to a final volume of 250 μl/well. The plates were incubated at 37 °C for 4 h. The released ^{51}Cr in supernatant was counted in a γ-counter (Packed Instrument, Downers Grove, IL). Percentage of specific release was calculated using the formula: (experimental cpm - background cpm)/(total cpm - background cpm) x 100%, where cpm represented counts per minute of ^{51}Cr released. Total release was assessed by lysis with 10% SDS (Sigma, St Louis, Mo), and background release was measured in the absence of effector cells. EC50 was calculated using SigmaPlot software.

6.1.2.4 Competition Assay

[00261] To assess the ability of trastuzumab and/or huOKT3 to interfere with HER2-BsAb binding, the HER2-positive SKOV3 cell line was incubated for thirty minutes a 4 °C with PBS or with 10 μg/mL of trastuzumab or huOKT3. Cells were subsequently stained with 10 μg/mL of Alexa-Fluor 488-conjugated HER2-BsAb and analyzed by flow cytometry. Alexa-Fluor 488-conjugated HER2-BsAb was generated with the Zenon® Alexa Fluor® 488 Human IgG Labeling Kit (Life Technologies) according to the manufacturer's instructions.

6.1.2.5 Binding Assay

[00262] Binding assays were performed by Surface Plasmon Resonance using Biacore T100 similar as described in Okazaki *et al.,* 2004, J Mol Biol; 336(5): 1239-1249.

6.1.2.6 Avidity Assay

[00263] To compare the avidity of HER2-BsAb and trastuzumab, HER2-positive SKOV3 cells were incubated with 10 fold dilutions (from 10 to $1x10^{-5}$ μg/mL) of trastuzumab or HER2-BsAb and analyzed by flow cytometry with FITC-labeled human Fc-specific antibody as the secondary antibody. MFI was plotted against the antibody concentration and the curves were compared.

6.1.2.7 Proliferation Assay

[00264] To determine anti-proliferative effects, cells were treated with isotype control monoclonal antibody, 10 nM lapatinib (as a positive control), 10 μg/mL HER2-BsAb, 10 μg/mL Trastuzumab, 10 nM lapatinib, 10 nM erlotinib, 10 nM neratinib, or 10 μg/mL cetuximab for 72 hours and cell proliferation assayed. Cell proliferation was determined using an ELISA plate reader and the WST-8 kit (Dojindo technologies) following the manufacturer's instructions and using the formula: % survival rate = (Sample-Background)/(Negative control-Background). Lapatinib (MSKCC pharmacy) was ground using a mortar and pestle and suspended in DMSO as previously described. To determine statistical significance, the results were analyzed using one-way ANOVA using Prism 6.0.

6.1.2.8 qRT-PCR

[00265] RNA was extracted when cells were at 70% confluence and cDNA was analyzed in a prism 7700 sequence detection system using the HER2 specific, commercially available kit Hs01001580_m1 from Applied Biosciences.

6.1.2.9 Animals and *In Vivo* Assays

[00266] For *in vivo* studies, BALB-Rag2-KO-IL-2R-γc-KO (DKO) mice (derived from colony of Dr. Mamoru Ito, CIEA, Kawasaki, Japan). *See*, for example, Koo *et al.,* 2009, Expert Rev Vaccines, 8: 113-120 and Andrade *et al.,* 2011, Arthritis Rheum, 2011, 63: 2764-2773. MCF7 cells or HCC1954 were mixed at a 1:1 ratio with PMBCs (unactivated, from buffy coat) and implanted in DKO mice subcutaneously. Four days post implantation, mice were treated with PBS, 10 μg of trastuzumab, or 10 μg of HER2-BsAb twice a week for two weeks. Tumor size was measured at the indicated days post implantation. Tumor size was determined by either calipers with the formula $V = 0.5$ (length x width x width), or by using the Peira TM900 optical system.

[00267] For the metastatic model, MCF7 cells expressing luciferase were administered to DKO mice intravenously. Four days post administration, mice were treated with 100 ug of HER2-BsAb, 20 ug or HER2-BsAb, or 20 ug of a HER2-BsAb lacking CD3 targeting (HER2-C825) twice a week for three weeks, with or without intravenous administration fo $5x10^6$ PBMC. Tumor size was quantified at the indicated timepoints using IVIS 200 (Xenogen) to quantify luciferin bioluminescence.

6.1.3 RESULTS

6.1.3.1 HER2-BsAb binds to both tumor cells and T cells.

[00268] The HER2-BsAb was generated utilizing a trastuzumab variant comprising a N297A mutation in the human IgG1 Fc region to remove glycosylation (SEQ ID NO: 62). The BsAb light chain fusion polypeptide was generated by attaching the anti-CD3 humanized OKT3 (huOKT3) single chain Fv fragment (ScFv) to the carboxyl end of the trastuzumab IgG1 light chain via a C-terminal $(G_4S)_3$ linker (Fig. 1A and SEQ ID NO: 60). To avoid aggregation, a cysteine at position 105 of the variable heavy chain of huOKT3 was substituted with serine. A N297A mutation was also introduced into the HER2-BsAb Fc region to eliminate binding of HER2-BsAb to Fc receptors. This mutation has previously been shown to eliminate the capacity of human IgG1-Fc binding to CD16A (Fig. 1D) and CD32A Fc receptors (Fig. 1E).

[00269] To produce the HER2-BsAb, a mammalian expression vector encoding both the heavy chain and the light chain fusion polypeptide was transfected into CHO-S cells, stable clones were selected, supernatants collected, and the HER2-BsAb was purified by protein A affinity chromatography. Biochemical purity analysis of the BsAb is depicted in Fig. 1B and Fig. 1C. Under reducing SDS-PAGE conditions, HER2-BsAb gave rise to two bands at around 50 KDa, since the huOKT3 scFv fusion to trastuzumab light chain increased the MW to ~50 KDa. SEC-HPLC showed a major peak (97% by UV analysis) with an approximate MW of 210 KDa, as well as a minor peak of multimers removable by gel filtration. The HER2-BsAb was stable by SDS-PAGE and SEC-HPLC after multiple freeze and thaw cycles.

[00270] FACS and immunostaining were performed to assess the binding of HER2-BsAb to both target cells and effector cells. Trastuzumab and HER2-BsAb displayed comparable binding to the HER2-positive breast carcinoma cell line, AU565 (Fig. 2A). In contrast, HER2-BsAb demonstrated more than 20-fold less binding to CD3+ T cells than huOKT3 (Fig. 2B). This is consistent with the observation that light chain-anchored scFv had lower avidity for T cells than regular huOKT3 IgG1, purposely designed to minimize cytokine release in the absence of target tumor cells.

[00271] The lower avidity of HER2-BsAb for T cells was further confirmed by the binding affinity analysis by Biacore as described in Cheung et al. 2012, OncoImmunology, 1:477-486. For antigen CD3, HER2-BsAb had a k_{on} at $4.53x10^5$ $M^{-1}s^{-1}$, a k_{off} at $8.68x10^{-2}$ s^{-1}, and overall K_D at 192 nM; comparable to parental huOKT3 IgG1-aGlyco at k_{off} ($1.09x10^{-1}$ s^{-1}), but less at k_{on}

($1.68x10^6$ $M^{-1}s^{-1}$) and overall K_D (64.6 nM). In summary, HER2-BsAb had much lower k_{on} than its parental huOKT3-aGlyco, suggesting less chance of BsAb binding to and activating T cells under the same condition, hence less cytokine release.

6.1.3.2 HER2-BsAb redirected T cell killing of human tumor cell lines.

[00272] To evaluate whether HER2-BsAb could redirect T cells to kill tumor cells, T cell cytotoxicity on HER2(+) breast cancer AU565 cells was tested in a standard 4-hour ^{51}Cr release assay. Substantial killing of tumor cells was observed n the presence of HER2-BsAb, with an EC50 at 300 fM (Fig. 3). Moreover, the killing was effective for an extensive panel of human tumor cell lines including breast carcinoma, ovarian carcinoma, melanoma, osteosarcoma, Ewing's sarcoma, rhabdomyosarcoma, and neuroblastoma, wherein the killing potency correlated with the HER2 expression level in the cells by FACS (Fig. 4).

6.1.3.3 HER2-BsAb mediates tumor antigen specific T cell cytotoxicity.

[00273] To investigate the tumor antigen specificity of HER2-BsAb in T cell cytotoxicity, a cytotoxicity assay was performed in the HER2-positive UM SCC 47 cells (a model for head and neck cancer) and in the HER2-negative HTB-132 cells (a model for breast cancer). HER2-BsAb mediated T cell cytotoxicity against the HER2-positive UM-SCC47 cells (EC50 of 14.5 pM), but not against the HER2-negative HTB-132 cells (Fig. 5A).

[00274] To investigate the specificity of HER2-BsAb in the T cell cytotoxicity, HER2-positive cells were first blocked with huOKT3 or with trastuzumab. In the absence of HER2-BsAb, the T cells displayed minimal cytotoxicity, reassuring that T cells on their own have minimum non-specific cytotoxicity. Both huOKT3 and trastuzumab blocked the ability of HER2-BsAb to induce T cell cytotoxicity.

6.1.3.4 HER2-BsAb mediates T cell cytotoxicity against HER2-positive cells below the HER2 threshold of detection by flow cytometry.

[00275] The HER2+ ovarian carcinoma cell line SKOV3 was used in a ^{51}Cr cytotoxicity assay with 10 fold dilutions of HER2-BsAb in the presence of T cells. These same cells were stained using HER2-BsAb at the same concentrations and analyzed by flow cytometry, MFI was plotted over the same x-axis as cytotoxicity, and EC50 was calculated for both curves. HER2-BsAb mediated T cell cytotoxicity against HER2-positive cells even when HER2-BsAb binding was

not detected by flow cytometry (Fig. 6). Comparing the EC50 for the cytotoxicity assay (2pM) vs EC50 for flow cytometry curve (3.5 nM) suggests that T cells in the presence of HER2-BsAb were 2500x more effective in detecting HER2-positive cells than flow cytometry.

6.1.3.5 HER2-BsAb has the same specificity, affinity and antiproliferative effects as trastuzumab.

[00276] Prior to treatment with HER2-BsAb, HER2-positive cells were pre-incubated with trastuzumab to determine if HER2-BsAb shares the same antigen specificity as trastuzumab. Pre-incubation with trastuzumab blocked HER2-BsAb binding to the cells, demonstrating a shared specificity (Fig. 7A). To compare the affinity of HER2-BsAb to trastuzumab, HER2-positive cells were incubated with dilutions of trastuzumab or HER2-BsAb and analyzed by flow cytometry for cellular binding. Plotting of MFI against the antibody concentration revealed similar curves for trastuzumab and HER2-BsAb, demonstrating a similar binding affinity (Fig. 7B). Further, trastuzumab and HER2-BsAb demonstrated similar anti-proliferative effects against HER2-positive cells (Fig. 7C).

6.1.3.6 HER2-BsAb mediated T cell cytotoxicity against SCCHN with an EC50 in the picomolar range.

[00277] The level and frequency of HER2 in the previously characterized head and neck cancer cell lines 93-VU-147T, PCI-30, UD-SCC2, SCC90, UMSCC47 and PCI-15B were assessed via flow cytometry with trastuzumab. The cells were also tested for HER2 expression by qRT-PCR (Fig. 8). HER2 was comparably expressed in the panel of head and neck cancer cell lines. Finally, the level of cytotoxicity in the presence of T cells and HER2-BsAb was correlated with the level of HER2 in the cells, revealing HER2-BsAb displays an EC50 in the picomolar range for these head and neck cell lines (Fig. 8).

6.1.3.7 HER2-BsAb mediates T cell cytotoxicity against SCCHN resistant to other HER targeted therapies.

[00278] To determine the EGFR and HER2 status of the SCCHN cell line PCI-30, cells were stained with trastuzumab or cetuximab and analyzed by flow cytometry as previously described (Fig. 9A). A proliferation assay demonstrated that these cells are resistant to the HER-specific targeted therapies, trastuzumab, cetuximab, lapatinib, erlotinib and pan-HER inhibitor neratinib

(Fig. 9B). However, PCI-30 cells were sensitive to treatment with HER2-BsAb utilizing three different cytotoxicity assays (Fig. 9C). HER2-BsAb generated potent cytotoxic responses against PCI-30 independent of their sensitivity to other HER targeted therapies, even when these drugs target more than one of these receptors. These assays suggest that HER2-BsAb was able to generate powerful cytotoxic responses, regardless of target cell sensitivity to EGFR or HER2 targeted therapies.

6.1.3.8 HER2-BsAb mediated T cell cytotoxicity against osteosarcoma cell lines with an EC50 in the picomolar range.

[00279] The previously characterized osteosarcoma cell lines, RG-160, CRL 1427 and U2OS, were assessed for their HER2 expression by flow cytometry with trastuzumab (Fig. 10) and by qRT-PCR, and the levels of HER2 were correlated with cytotoxicity in the presence of T cells and HER2-BsAb (Fig. 10). All tested cell lines were positive for HER2, although the expression level varied. Further, all HER2-positive cells were sensitive to T cell cytotoxicity mediated by HER2 BsAb, with an EC50 ranging from 11-25 pM.

6.1.3.9 HER2-BsAb mediates T cell cytotoxicity against HER-therapy resistant osteosarcoma cell lines.

[00280] U2OS cells are a HER2-positive, EGFR-positive osteosarcoma cell line (Fig. 11A). U2OS cells were analyzed for their sensitivity to trastuzumab, cetuximab, lapatinib and the pan-HER inhibitor Neratinib by proliferation assay in the presence of each of the inhibitors. These cells were resistant to cetuximab and trastuzumab with minimal sensitivity to Lapatinib, erlotinib and neratinib (Fig. 11B). These same cells were tested for sensitivity for T cell cytotoxic responses mediated by HER2-BsAb. HER2-BsAb generated potent cytotoxic responses against U2OS cells using three different cytotoxicity assays, independent of its sensitivity to other HER targeted therapies (Fig. 11C).

6.1.3.10 HER2-BsAb mediates T cell cytotoxicity against HER-therapy resistant cervical cancer HeLa cells.

[00281] HeLa cells are a HER2-positive, EGFR-positive cervical carcinoma cell line (Fig. 12A). HeLa cells were analyzed for their sensitivitiy to HER family tyrosine kinase inhibitors, Erlotinib, Lapatinib or Neratinib, or to the HER specific antibodies, Cetuximab or trastuzumab.

These results demonstrated that HeLa cells are pan-resistant to these therapies (Fig. 12B). However, these same cells were tested for sensitivity for T cell cytotoxic responses mediated by HER2-BsAb. HER2-BsAb generated potent cytotoxic responses against HeLa cells using three different cytotoxicity assays, independent of its sensitivity to other HER targeted therapies (Fig. 12C). Interestingly, pretreatment with lapatinib increased sensitivity to HER2-BsAb mediated cytotoxicity, even when lapatinib alone had no effect on cell proliferation.

6.1.3.11 HER2-BsAb is effective against human breast cancer in humanized mice.

[00282] For *in vivo* therapy studies, BALB-*Rag2*-KO-IL-2R-γc-KO (DKO) mice (derived from colony of Dr. Mamoru Ito, CIEA, Kawasaki, Japan) were used. *See*, for example, Koo et al. 2009, Expert Rev Vaccines 8: 113-120 and Andrade et al. 2011, Arthritis Rhem 63: 2764-2773. MCF7-Luciferase breast cancer cells were mixed with peripheral blood mononuclear cells (PBMC) and planted subcutaneously. Four days post cell implantation, the mice were treated with HER2-BsAb or with trastuzumab and the tumor size was analyzed over time (Fig. 13). HER2-BsAb demonstrated a significant suppression of tumor progression. HER2-BsAb was also effective against tumor progression when the trastuzumab resistant HCC1954 breast cancer cells (*See*, for example, Huang *et al.,* 2011, Breast Cancer Research, 13: R84) were planted subcutaneously with PBMCs (Fig. 14).

[00283] To assess a metastatic tumor model, MCF7-Luciferace cells were inoculated intravenously. HER2-BsAb was administered and subsequently in combination with PBMC. Tumor luciferin bioluminescence signal demonstrated HER2-BsAb plus PBMC showed complete suppression of tumor progression (Fig. 15, Fig. 16A, Fig. 16B, Fig. 16C, and Fig. 16D).

6.1.4 CONCLUSIONS

[00284] The aglycosylated HER2-BsAb allowed for minimized Fc functions and avoidance of a cytokine storm and elimination of all complement activation, complement mediated and complement receptor mediated immune adherence. In addition, despite bivalency of huOKT3 in the IgG-scFv platform, binding to CD3 was functionally monovalent; hence there was no spontaneous activation of T cells in the absence of tumor target. HER2-BsAb displayed potent cytotoxicity against HER2-positive tumor cells in vitro, even against cells with low antigen expression, or cells that are resistant to trastuzumab, cetuximab, lapatinib, erlotinib or the pan-

HER inhibitor neratinib. HER2-BsAb also displayed potent cytotoxicity against breast cancer, ovarian cancer, SCCHN, osteosarcomas, and sarcomas. Finally, HER2-BsAb displayed strong *in vivo* efficacy against tumor xenografts, substantially better than the trastuzumab hIgG1 counterpart.

6.2 EXAMPLE 2

[00285] This example provides (a) a more detailed description of certain of the experiments described in Example 1 (Section 6.1); and (b) additional experiments as compared to Example 1 (Section 6.1).

6.2.1 INTRODUCTION

[00286] Trastuzumab has significantly improved patient outcomes in breast cancer and has also been key in the design and implementation of other targeted therapies (Singh *et al.,* 2014, Br J Cancer 111:1888-98). However, HER2 expression does not guarantee a clinical response to trastuzumab or other HER2 targeted therapies (Gajria *et al.,* 2011, Expert Review of Anticancer Therapy, 11(2):263-75; Lipton *et al.,* 2013, Breast Cancer Research and Treatment, 141(1):43-53). Less than 35% of patients with HER2 positive breast cancer initially respond to trastuzumab and 70% of the initial responders will ultimately progress with metastatic disease within a year (Vu and Claret., 2011, Frontiers in Oncology 2:62). In osteosarcoma and Ewing's sarcoma, where high levels of HER2 expression are associated with decreased survival (Gorlick *et al.,* 1999, Journal of Clinical Oncology: Official Journal of The American Society of Clinical Oncology 17:2781-2788), trastuzumab has not shown any benefit even when used in conjunction with cytotoxic chemotherapy (Ebb *et al.,* 2012, Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology 30:2545-2551). Furthermore, trastuzumab, like other HER targeted therapies, has shown modest or no benefit against HER2-positive head and neck cancer (Pollock *et al.,* 2014, Clinical Cancer Research, 21(3):526-33).

[00287] The reasons for these failures are complex and only partially understood. The genomic diversity and constant evolution of malignancies make them less prone to oncogene addiction, a requirement for the success of targeted therapy. Furthermore, even when oncogene addiction is present, resistance can emerge from selection pressure induced by the use of targeted therapies (Lipton *et al.,* 2013, Breast Cancer Research and Treatment, 141(1):43-53). In fact, despite the initial enthusiasm received, the majority of targeted therapies have not produced a

significant benefit in the overall cure of patients receiving it (Nathanson *et al.,* 2014, Science, 343:72-76). A different approach, one that selectively targets malignant cells that overexpress HER family receptors, and that can generate cytotoxic anti-tumor responses independently of the receptor activation status can be beneficial.

[00288] Blinatumomab – a CD19/CD3 BsAb was approved in 2014 for treating Acute Lymphoplastic Leukemia (Sanford, 2015, Drugs 75:321-7). However, despite its promising results, the unfavorable PK of these small size molecules necessitates prolonged infusions, complicating their administration (Shalaby *et al.,* 1995, Clin Immunol Immunopathol 74:185-92, 1995; Portell *et al.,* 2013, Clin Pharmacol 5:5-11). Furthermore, the resulting cytokine release syndrome (CRS) still poses costly and often life-threatening complications. Importantly, despite the ability of bispecific antibodies to activate T cells, the same inhibitory pathways that regulate classic T cell function might still limit their effectiveness. For example, the heterodimeric design of a monovalent binding HER2/CD3 bispecific antibody was inhibited by the PD-1/PD-L1 inhibitory axis (Junttila *et al.,* 2014, Cancer Res 74:5561-71).

[00289] The present example provides a bispecific binding molecule (herein referred to as "HER2-BsAb") that offers two distinct advantages over the existing technologies: (1) it is based on the fully humanized HER2 specific IgG1 mAb Trastuzumab, preserving its pharmacologic advantages (Wittrup *et al.,* 2012, Methods Enzymol 503:255-68) and bivalent binding to HER2; maximizing tumor avidity; and (2) its binding to CD3 is functionally monovalent through the scFv derived from the humanized huOKT3 mAb sequence. Thus, HER2-BsAb is built on two mAbs with extensive records of clinical safety. Furthermore, this is a platform with its Fc function deleted to eliminate all antibody-dependent cell-mediated cytotoxicity (ADCC) and CMC activities in order to reduce the cytokine release syndrome.

[00290] The data presented in this example demonstrate the ability of HER2-BsAb to produce potent anti-tumor responses, both *in vitro* and *in vivo*, against tumor cells that are resistant to HER2 targeted therapy or trastuzumab.

6.2.2 MATERIALS AND METHODS

6.2.2.1 Cell Lines

[00291] All cell lines were purchased from ATCC (Manassas Va) except: UM-SCC47, obtained from Dr. Carey at the University of Michigan; SCC-90, PCI-30 and PCI-15B, obtained

from Dr. Robert Ferris at the University of Pittsburgh; HCC1954, obtained from Dr. Sarat Chandarlapaty at Memorial Sloan Kettering Cancer Center; 93-VU-147T and HeLa, obtained from Dr. Luc Morris; and UD-SCC2, obtained from Henning Bier at Hals-Nasen-Ohrenklinik und Poliklinik. All cells were authenticated by short tandem repeat profiling using PowerPlex 1.2 System (Promega), and periodically tested for mycoplasma using a commercial kit (Lonza). The luciferase-labeled tumor cell lines MCF7-Luc were generated by retroviral infection with a SFG-GFLuc vector.

6.2.2.2 HER2-BsAb design and expression in CHO-S cells

[00292] In the HER2-BsAb IgG-scFv format (Fig. 17A, "HER2-BsAb"), the V_H was identical to that of the trastuzumab IgG1 V_H, except that an N297A mutation in the Fc region was introduced into the HER2-BsAb to remove glycosylation, thereby depleting Fc function (SEQ ID NO: 62). The light chain fusion polypeptide was constructed by extending the trastuzumab IgG1 light chain with a C-terminal $(G_4S)_3$ linker followed by huOKT3 scFv (SEQ ID NO: 60). The DNA encoding both the heavy chain and the light chain was inserted into a mammalian expression vector, transfected into CHO-S cells, and stable clones of the highest expression were selected. Supernatants were collected from shaker flasks and the HER2-BsAb was purified by protein A affinity chromatography. The control BsAb, HER2-C825 (composed of SEQ ID NOS: 71 and 72), was generated as previously described (Xu *et al.,* 2015, Cancer Immunol Res 3:266-77; Cheal *et al.,* 2014, Mol Cancer Ther 13:1803-12).

6.2.2.3 Other Antibodies and Small molecules

[00293] Fluorophore-labeled HER2-BsAb was generated with the Zenon® Alexa Fluor® 488 Human IgG Labeling Kit from Life Technologies following the manufacturer's instructions. Pembrolizumab, cetuximab, trastuzumab, Erlotinib, Lapatinib and Neratinib were purchased from the Memorial Sloan Kettering Cancer Center pharmacy. Small molecules were re-suspended in DMSO. The CD4, CD8, CD16 and CD56 antibodies were purchased from BD Biosciences (San Jose CA). The commercially available PE labeled PD-L1 specific mAb 10F.9G2 was purchased from BioLegend.

6.2.2.4 Cell proliferation assays

[00294] For cell proliferation assays, 5,000 tumor cells were plated using RPMI-1640 supplemented with 10% FBS in a 96 well plate for 36 hours before being treated with lapatinib or the antibodies at the specified concentrations. Cell proliferation was determined using an ELISA plate reader and the WST-8 kit (Dojindo technologies) following the manufacturer's instructions and using the formula: % survival rate = (Sample-Background)/(Negative control-Background). Lapatinib (Memorial Sloan Kettering Cancer Center pharmacy) was ground using a mortar and pestle and suspended in DMSO as previously described (Chen *et al.,* 2012, Molecular cancer therapeutics 11:660-669). To determine statistical significance, the results were analyzed using one-way ANOVA using Prism 6.0.

6.2.2.5 Cytotoxicity Assays (^{51}chromium release assay)

[00295] Cell cytotoxicity was assayed by ^{51}Cr release as previously described (Xu *et al.,* 2015, Cancer Immunol Res 3:266-77), and EC50 was calculated using SigmaPlot software. Effector T cells were purified from human PBMC using Pan T cell isolation kit (Miltenyi Biotec), and then activated and expanded with CD3/CD28 Dynabeads (Invitrogen) according to the manufacturer's protocol.

6.2.2.6 PD-1/PD-L1 expression

[00296] To overexpress PD-L1 in HEK293 cells, cells were cultured in DMEM (Cellgro) supplemented with 10% heat-inactivated FBS and Penicillin (100 IU/ml) and streptomycin (100 µg/ml). On Day(-1), HEK293 cells were trypsinized, counted and plated into 6 well plates at 0.5 M cells/well and kept in 2 mL of fresh media. On the day of transfection, Day(0), the media was exchanged with 2 mL of fresh media. Transfection reagents were prepared as follows for both hPD-L1 and control plasmids: 2.5 µg of DNA was diluted into 250 µl of unsupplemented DMEM (no serum). 5 µl of Lipofectamine 2000 (Invitrogen) was diluted into a separate 250 µl of DMEM (no serum), and incubated for 5 minutes at room temperature. After 5 minutes, the diluted DNA was combined with the diluted Lipofectamine 2000 (Invitrogen) and incubated for another 30 minutes at room temperature. After 30 minutes, the entire 500 µl reaction was added, dropwise, onto a single well of HEK293 cells. The plate was rocked back and forth briefly to help mix the reagents. For the untransfected control, 500 µl of unsupplemented DMEM without DNA or Lipofectamine 2000 was added to one well. Cells were incubated at 37°C for 24-48

hours before harvesting. On Day(1) or Day(2), cells were lifted from the plate using 2 mM EDTA in PBS, and counted. 100,000-200,000 cells were used for FACS analysis and the rest were used for the killing assays.

[00297] To induce PD-1 expression of activated T cells (ATCs), effector cells were incubated in a 3:1 ratio for 24 hours with the HER2-high Breast Carcinoma Cell line HCC1954 after these target cells were incubated with HER2-BsAb at a concentration of 10 μg/mL for 30 minutes and antibody excess was removed. Cells were harvested and used in cytotoxicity assays as previously described against the HEK293 cells transfected with PD-L1.

6.2.2.7 *In vivo* experiments

[00298] For *in vivo* therapy studies, BALB-Rag2-/-IL-2R-γc-KO ("DKO") mice (derived from colony of Dr. Mamoru Ito, CIEA, Kawasaki, Japan; *see*, *e.g.*, Koo *et al.,* 2009, Expert Rev Vaccines 8:113-20 and Andrade *et al.,* 2011, Arthritis Rheum 63:2764-73) were used. Three humanized mouse xenograft models were used: (1) intravenous tumor plus intravenous effector cells; (2) subcutaneous tumor plus subcutaneous effector cells; and (3) subcutaneous tumor plus intravenous effector cells. Subcutaneous xenografts were created with $5x10^6$ cells suspended in Matrigel (Corning Corp, Tewksbury MA) and implanted in the flank of DKO mice. Effector peripheral blood mononuclear cell (PBMC) cells were purified from buffy coats purchased from the New York Blood Center. Prior to every experimental procedure, PBMCs were analyzed for their percentage of CD3, CD4, CD8 and CD56 cells to ensure consistency. HER2-BsAb was injected intravenously twice a week at 100 μg/injection, beginning two days before effectors cells for three weeks, given as $5\text{-}10x10^6$ PBMC per injection, once a week for 2 weeks. Tumor size was measured using (1) hand-held TM900 scanner (Pieira, Brussels, BE); (2) Calipers; or (3) bioluminescence. Bioluminescence imaging was conducted using the Xenogen In Vivo Imaging System (IVIS) 200 (Caliper LifeSciences). Briefly, mice were injected intravenously with 0.1 mL solution of D-luciferin (Gold Biotechnology; 30 mg/mL stock in PBS). Images were collected 1 to 2 minutes after injection using the following parameters: a 10- to 60-second exposure time, medium binning, and an 8 f/stop. Bioluminescence image analysis was performed using Living Image 2.6 (Caliper LifeSciences).

6.2.3 RESULTS

6.2.3.1 HER2-BsAb

[00299] HER2-BsAb was designed using an IgG-scFv format (Fig. 17A). The VH was identical to that of trastuzumab IgG1, except for the N297A mutation in the Fc region of HER2-BsAb to remove glycosylation (SEQ ID NO: 62). The light chain fusion polypeptide was constructed by extending the trastuzumab IgG1 light chain with a C-terminal $(G_4S)_3$ linker followed by huOKT3 scFv (Xu *et al.,* 2015, Cancer Immunol Res 3:266-77) (SEQ ID NO: 60). The DNAs encoding both heavy chain and light chain were inserted into a mammalian expression vector, transfected into CHO-S cells, and stable clones of highest expression were selected. Supernatants were collected from shaker flasks and purified on protein A affinity chromatography.

[00300] SEC-HPLC and SDS-PAGE of the HER2-BsAb is shown in Fig. 17B and Fig. 17C, respectively. Under reducing SDS-PAGE conditions, HER2-BsAb gave rise to two bands at around 50 kDa, since the huOKT3 scFv fusion to trastuzumab light chain increased the molecular weight to approximately 50 kDa. SEC-HPLC showed a major peak (97% by UV analysis) with an approximate molecular weight of 200 KDa, as well as a minor peak of multimers removable by gel filtration. The BsAb remained stable by SDS-PAGE and SEC-HPLC after multiple freeze and thaw cycles.

6.2.3.2 HER2-BsAb retained specificity, affinity and anti-proliferative effects of trastuzumab

[00301] To determine if HER2-BsAb retained the specificity and anti-proliferative effects of trastuzumab, the HER2-positive-high SKOV3 ovarian carcinoma cell line was pre-incubated with 10 μg/mL of trastuzumab for 30 minutes and then immunostained using HER2-BsAb labeled with Alexa 488 (Fig. 18A). Incubation with trastuzumab prevented HER2-BsAb binding to SKOV3 cells, demonstrating that these antibodies shared the same specificity. To compare the avidity of HER2-BsAb to trastuzumab, the same cell line was incubated with 10-fold downward dilutions (from 10 μg/ml to $1x10^{-5}$ μg/mL) of trastuzumab or HER2-BsAb and analyzed by flow cytometry. The mean fluorescence intensity (MFI) was plotted against the antibody concentration in μM. The similarity in the binding curves confirmed that trastuzumab and HER2-BsAb had similar binding avidities for their common HER2 target (Fig. 18B).

[00302] Finally, the trastuzumab-sensitive breast carcinoma cell line SKBR3 was treated with isotype control mAb, 10 mM Lapatinib (as a positive control), 10 μg/mL HER2-BsAb, or 10 μg/mL trastuzumab for 72 hours and cell proliferation was assayed. As shown in Fig. 18C, trastuzumab and HER2-BsAb had similar anti-proliferative effects that were significant as compared to the negative control. As expected, lapatinib showed the strongest inhibition of cell proliferation.

6.2.3.3 HER2-BsAb redirected T cell cytotoxicity was HER2-specific and dependent on CD3

[00303] To establish the specificity of cytotoxic responses by T cells in the presence of HER2-BsAb; HER2-negative and HER2-positive cell lines were assayed in a cytotoxicity assays using ATCs (effector:T cell ("E:T") ratio of 10:1) and HER2-BsAb at decreasing concentrations (Fig. 19A and Fig. 20). Cytotoxicity was absent for HER2-negative cell lines. To demonstrate the dependency of cytotoxicity on CD3, HER2-BsAb cytotoxicity was tested in the presence of the CD3 specific blocking mAb OKT3 (Fig. 19B). Pre-incubation with either trastuzumab or OKT3 prevented HER2-BsAb T cell mediated cytotoxicity.

6.2.3.4 HER2-BsAb mediated Cytotoxicity against HER2-positive cell lines that were resistant to other HER2 targeted therapies.

[00304] Several cell lines from different tumor systems (*e.g.*, head and neck, breast, and sarcoma) were characterized for their HER2 level of expression by flow cytometry (Fig. 20). In this panel, 75% of these cells tested positive for HER2 expression by flow cytometry. Representative cell lines were assayed for their sensitivity to tyrosine kinase inhibitors (*e.g.*, erlotinib, lapatinib, and neratinib), or HER antibodies (*e.g.*, trastuzumab and cetuximab), as well as HER2-BsAb mediated T cell cytotoxicity. Fig. 21 shows representative examples of these experiments from three different lines from three different tumor systems. As shown, HER2 expression–even in low quantities–was sufficient to mediate T cell cytotoxicity in the presence of ATC and HER2-BsAb in cell lines otherwise resistant *in vitro* to HER-targeted therapies. When these cell lines were tested for cytotoxicity in the presence of ATC and HER2-BsAb, sensitivity to HER2-BsAb, expressed as EC50, strongly correlated with surface HER2 expression (Fig. 22)

6.2.3.5 HER2-BsAb mediated T cell cytotoxicity was relatively insensitive to PD-L1 expression on the tumor target or PD-1 expression on T cells.

[00305] Activation of tumor-specific CTL in the tumor microenvironment is known to promote expression of PD-1/PD-L1, leading to T cell exhaustion or suppression, a phenomenon termed "adaptive immune resistance" (Tumeh *et al.,* 2014, Nature 515:568-71). The presence of the PD-1/PD-L1 pathway has also been reported to limit the anti-tumor effects of T cell engaging bispecific antibodies (Junttila *et al.,* 2014, Cancer Res 74:5561-71). To determine if HER2-BsAb had the same limitations, PD-1-positive ATCs were used against the HER2-positive, PD-L1-positive breast carcinoma cell line HCC1954, with or without the PD-1-specific mAb pembrolizumab. As shown in Fig. 23A, Fig. 23B, and Fig. 23C, PD-1-positive T cells generated similar cytotoxic responses in the presence of HER2-BsAb, independently of the presence of pembrolizumab. When HER2-positive human embryonic kidney cells (HEK-293) were transfected with the full sequence of PD-L1 and used as targets, cytotoxicity against cells expressing PD-L1 was not significantly different to the cytotoxicity observed in non-transfected HEK-293 cells (although maximal cytotoxicity was slightly less with PD-L1-positive HEK-293 versus PD-L1-negative HEK-293) (Fig. 24A and Fig. 24B shows the average of six experiments, and error bars represent standard error).

6.2.3.6 HER2-BsAb was effective against HER2-positive xenografts

[00306] To determine the *in vivo* efficacy of HER2-BsAb, the breast carcinoma cell lines HCC1954 (HER2-high) and MCF-7 (HER2-low) were used in xenograft models in DKO mice. Three tumor models differing in tumor locations and effector routes were used: (1) intravenous tumor cells and intravenous effector PBMCs; (2) subcutaneous tumor cells and SC PBMCs; and (3) subcutaneous tumor cells and intravenous PBMCs. Fig. 25 summarizes the results of these experiments. The HER2-low MCF-7-luc (carrying luciferase reporter) cells were inoculated via tail vein injection into DKO. When tumor presence was confirmed by bioluminescence, mice were treated with six doses of intravenous HER2-BsAb or control BsAb twice a week for 3 weeks. Intravenous effector PBMCs were administered 48 hours after the first dose of HER2-BsAb, and again (one week later). Mice were evaluated for tumor burden using luciferin bioluminescence every week. In this hematogenous disease model, MCF-7 cells were completely eradicated without disease progression (Fig. 25B). This same cell line was implanted

subcutaneously mixed with effector PBMCs subcutaneously and treated with four injections of HER2-BsAb twice a week for 2 weeks (totaling 4 injections in the first experiment) or twice a week for 3 weeks (totaling 6 injections in 2nd experiment). In both experiments, HER2-BsAb caused a significant delay in tumor progression while PBMC+trastuzumab or PBMC alone were ineffective (Fig. 25A). In two other separate experiments, subcutaneous HER2-positive breast carcinoma cell line HCC1954 was mixed with subcutaneous PBMCs. Again, both 4 or 6 injections of HER2-BsAb resulted in a complete suppression of tumor growth, while trastuzumab or control BsAb HER2-C825 had no effect (Fig. 25C). In the third model, where subcutaneous HCC1954 xenografts were treated with intravenous PBMC (once a week for 3 weeks), and intravenous HER2-BsAb twice a week for 3 weeks, tumor growth was substantially delayed (in 2 separate experiments), in contrast to only modest effects for trastuzumab + huOKT3 + PBMC, control antibody (HER2-C825) + PBMC, huOKT3 + PBMC, or HER2-BsAb alone without PBMC (Fig. 25D). The following observation were made: when effector PBMCs were mixed with tumor cells subcutaneously, complete tumor regression without recurrence was seen for mice over 90 days post-tumor implantation. When effector PBMCs were administered intravenously, there was significant reduction in the size of the tumors, but complete regression was only observed in a subset of animals.

6.2.4 CONCLUSIONS

[00307] This example describes a HER2-specific BsAb that has been shown to have potent T cell-mediated anti-tumor activity *in vitro* and *in vivo*, ablating tumors or delaying tumor growth in 3 separate tumor models in the presence of human PBMCs. Unlike monovalent bispecific antibodies, this HER2-BsAb had identical anti-proliferative capacity as trastuzumab. In addition, the serum half-life and area under the curve of HER2-BsAb were similar to IgG. Unlike other bispecific antibodies, which tended to aggregate, HER2-BsAb was stable at -20°C and at 37°C, despite long term storage. Most importantly, the T cell-mediated cytotoxicity it induced was relatively insensitive to inhibition by the PD-1/PD-L1 pathway.

[00308] When compared to the existing platforms that target HER2, HER2-BsAb offers advantages. The F(ab) x F(ab) format, though effective *in vitro*, was similar in size to Blinatumomab (Sanford, 2015, Drugs, 75:321-7) and was expected to share similar pharmacokinetic and toxicity profiles (Shalaby *et al.,* 1995, Clin Immunol Immunopathol 74:185-92, 1995), having a short half-life, thus requiring daily infusions, potential leakage into

the central nervous system (CNS), potential CNS toxicity, and potential significant cytokine release syndrome. In addition, the anti-proliferative capacity of this F(ab) x F(ab) univalent system was 10-fold lower than trastuzumab. The IgG x IgG chemical conjugate between trastuzumab and OKT3 was useful for arming T cells *ex vivo*, but was not useful as an injectable, likely due to impurities associated with chemical conjugates (Lum and Thakur, 2011, BioDrugs 25:365-79; Lum *et al.*, Clin Cancer Res 21:2305, 2015); in contrast, the HER2-BsAb provided herein is tolerated as an injectable. A heterodimer format was recently described using a monovalent system (Junttila *et al.,* 2014, Cancer Res 74:5561-71) that does not preserve trastuzumab's anti-proliferative effects retained in HER2-BsAb.

[00309] There are other design features that distinguish HER2-BsAb from other known candidates of this class. Unlike most bispecific antibodies, HER2-BsAb's bivalent binding to the HER2 target was preserved, providing anti-proliferative activity similar to that of trastuzumab IgG1. Unlike F(ab) x F(ab) (Shalaby *et al.,* 1995, Clin Immunol Immunopathol 74:185-92) or tandem scFv constructs (Sanford, 2015, Drugs, 75:321-7), HER2-BsAb had a molecular weight high enough to behave in pharmacokinetic analyses like a wild-type IgG. Unlike other bivalent bispecifics (Reusch *et al.*, MAbs, 7:584, 2015), HER2-BsAb's reaction with CD3 was functionally monovalent. HER2-BsAb also differed from man heterodimeric bispecifics in its modified Fc, where aglycosylation removed both ADCC and CMC functions, thereby reducing cytokine release syndrome without affecting serum pharmacokinetics or compromising T cell activation. The other advantage is manufacturability; HER2-BsAb was produced in CHO cells and purified using procedures standard for IgG, without significant aggregation despite prolonged incubation at 37°C. HER2-BsAb is an important salvage option for patients who progress on standard HER2-based therapies, or a replacement for trastuzumab given its dual anti-proliferative and T cell retargeting properties.

7. EQUIVALENTS

[00310] The invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

[00311] All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent

application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

WHAT IS CLAIMED:

1. A bispecific binding molecule comprising a monoclonal antibody that is an immunoglobulin that binds to HER2, wherein said immunoglobulin is mutated in its Fc region to destroy a glycosylation site, said immunoglobulin comprising two identical heavy chains and two identical light chains, said light chains being a first light chain and a second light chain, wherein the first light chain is fused to a first single chain variable fragment (scFv), via a peptide linker, to create a first light chain fusion polypeptide, and wherein the second light chain is fused to a second scFv, via a peptide linker, to create a second light chain fusion polypeptide, wherein the first and second scFv (i) are identical, and (ii) bind to CD3, and wherein the first and second light chain fusion polypeptides are identical,

wherein the heavy chains comprise a V_H domain present in any of SEQ ID NOs: 23, 27, 62 or 63;

wherein the light chains comprise a V_L domain present in SEQ ID NO: 25;

wherein the first and second scFvs comprise a V_H domain having a sequence selected from the group consisting of SEQ ID NOs: 15, 17 and 64, and a V_L domain having a sequence selected from the group consisting of SEQ ID NOs: 16 and 65; and

wherein the first scFv is fused to the carboxyl end of the first light chain, and wherein the second scFv is fused to the carboxyl end of the second light chain.

2. The bispecific binding molecule of claim 1, wherein

(a) the sequence of each heavy chain is SEQ ID NO: 23, 27 or 62;

(b) the sequence of the first light chain is SEQ ID NO: 25;

(c) the sequence of the peptide linker is any one of SEQ ID NOs: 14 and 35-41; and/or

(d) the sequence of an intra-scFv peptide linker between the V_H domain and the V_L domain in the first scFv is any of SEQ ID NOs: 14 and 35-41.

3. The bispecific binding molecule of any of claims 1-2, wherein the sequence of the first scFv is any of SEQ ID NOs: 19 and 48-59 or wherein the sequence of the first light chain fusion polypeptide is any of SEQ ID NOs: 29, 34, 42-47, and 60.

4. The bispecific binding molecule of claim 1, wherein the sequence of each heavy chain is SEQ ID NO: 27, and wherein the sequence of each light chain is SEQ ID NO: 25, optionally wherein the sequence of the scFv is SEQ ID NO: 19 or wherein the sequence of the peptide linker is SEQ ID NO: 14.

5. The bispecific binding molecule of any of claims 1, 3, or 4, wherein the peptide linker is 5-30, 5-25, 5-15, 10-30, 10-20, 10-15, 15-30, or 15-25 amino acids in length.

6. The bispecific binding molecule of claim 1, wherein (a) the sequence of the heavy chain is SEQ ID NO: 62 and wherein the sequence of each light chain fusion polypeptide is SEQ ID NO: 60 or (b) wherein the sequence of the heavy chain is SEQ ID NO: 27 and wherein the sequence of each light chain fusion polypeptide is SEQ ID NO: 47.

7. The bispecific binding molecule of claim 1, wherein the sequence of the heavy chain is SEQ ID NO: 27 and wherein the sequence of each light chain fusion polypeptide is SEQ ID NO: 29.

8. The bispecific binding molecule of any of the preceding claims, wherein the K_D is between 70nM and 1 μM for CD3.

9. The bispecific binding molecule of any of the preceding claims, wherein the bispecific binding molecule does not bind an Fc receptor in its soluble or cell-bound form.

10. The bispecific binding molecule of any of the preceding claims, wherein the destroyed glycosylated site is an N-linked glycosylation site.

11. The bispecific binding molecule of claim 10, wherein an asparagine at the N-linked glycosylation site is replaced with an amino acid that does not function as a glycosylation site.

12. The bispecific binding molecule of any of the preceding claims, wherein the heavy chain has been mutated to destroy a C1q binding site.

13. The bispecific binding molecule of any of the preceding claims, wherein the bispecific binding molecule does not activate complement.

14. The bispecific binding molecule of any of the preceding claims, wherein the scFv is disulfide stabilized.

15. A polynucleotide comprising nucleotide sequences encoding a light chain fusion polypeptide comprising an immunoglobulin light chain fused to a scFv, via a peptide linker, wherein the scFv is fused to the C-terminus of the light chain; wherein the light chain binds to HER2 and comprises a V_L domain present in SEQ ID NO: 25; and wherein the scFv binds to CD3 and comprises a V_H domain having a sequence selected from the group consisting of SEQ ID NOs: 15, 17 and 64, and a V_L domain having a sequence selected from the group consisting of SEQ ID NOs: 16 and 65; and

optionally wherein

(a) the sequence of the light chain is SEQ ID NO: 25, wherein optionally the nucleotide sequence encoding the light chain is SEQ ID NO: 24;

(b) the sequence of the scFv is SEQ ID NO: 52;

(c) the sequence of the scFv is SEQ ID NO: 19, wherein optionally the nucleotide sequence encoding the scFv is SEQ ID NO: 18;

(d) the sequence of the light chain is SEQ ID NO: 25, and the sequence of the scFv is SEQ ID NO: 52;

(e) the sequence of the light chain is SEQ ID NO: 25, and the sequence of the scFv is SEQ ID NO: 19, wherein optionally the nucleotide sequence encoding the light chain is SEQ ID NO: 24, and the nucleotide sequence encoding the scFv is SEQ ID NO: 18;

(f) the peptide linker is 5-30, 5-25, 5-15, 10-30, 10-20, 10-15, 15-30, or 15-25 amino acids in length, wherein optionally the sequence of the peptide linker is SEQ ID NO: 14, wherein optionally the nucleotide sequence encoding the peptide linker is SEQ ID NO: 13; or

(g) the sequence of the light chain fusion polypeptide is SEQ ID NO: 34 or SEQ ID NO: 29, wherein optionally the nucleotide sequence encoding the light chain fusion polypeptide of SEQ ID NO: 29 is SEQ ID NO: 28.

16. A vector comprising (i) a first polynucleotide of claim 15 operably linked to a first promoter, and (ii) a second polynucleotide encoding an immunoglobulin heavy chain that binds to HER2 operably linked to a second promoter.

17. A mixture of polynucleotides comprising (i) a first polynucleotide of claim 15 operably linked to a first promoter, and (ii) a second polynucleotide encoding an immunoglobulin heavy chain that binds to HER2 operably linked to a second promoter.

18. An *ex vivo* cell comprising the vector of claim 16 or the mixture of polynucleotides of any of claims 17.

19. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount to:

the bispecific binding molecule of any of claims 1-14;

the vector of claim 16; or

the mixture of polynucleotides of claim 17.

20. A method fortreating a HER2-positive cancer in a subject in need thereof comprising administering to the subject an effective amount of the pharmaceutical composition or the bispecific binding molecule of any of claims 1-14.

21. The method of claim 20, wherein the HER2-positive cancer is breast cancer, gastric cancer, an osteosarcoma, desmoplastic small round cell cancer, squamous cell carcinoma of head and neck cancer, ovarian cancer, prostate cancer, pancreatic cancer, glioblastoma multiforme, gastric junction adenocarcinoma, gastroesophageal junction adenocarcinoma, cervical cancer, salivary gland cancer, soft tissue sarcoma, leukemia, melanoma, Ewing's sarcoma, rhabdomyosarcoma, neuroblastoma, a primary tumor, a metastic tumor or any other neoplastic tissue that expresses the HER2 receptor.

22. The method of any of claims 20 or 21, wherein the administering is intravenous, intraperitoneal, intrathecal, intraventricular in the brain, or intraparenchymal in the brain.

23. The method of any of claims 20, 21 or 22, wherein the method further comprises administering to the subject

(a) doxorubicin, cyclophosphamide, paclitaxel, docetaxel, and/or carboplatin;

(b) radiotherapy;

(c) multi-modality anthracycline-based therapy;

(d) external beam or radioimmunotherapy; and/or

(e) T cells, that are either bound or not bound to the bispecific binding molecule.

24. The method of any of claims 20-23, wherein the HER2-positive cancer is resistant to treatment with trastuzumab, cetuximab, lapatinib, erlotinib, or any other small molecule or antibody that targets the HER family of receptors.

25. The method of any of claims 20-23 or 24, wherein the subject is a human or canine.

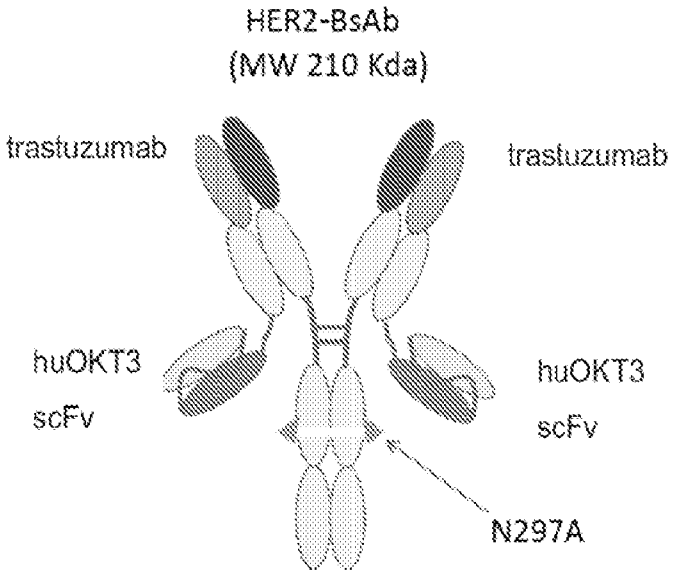


Fig. 1A

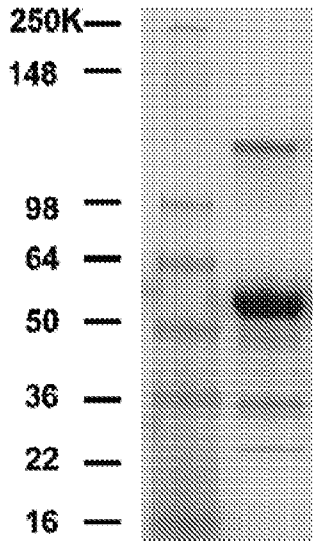


Fig. 1B

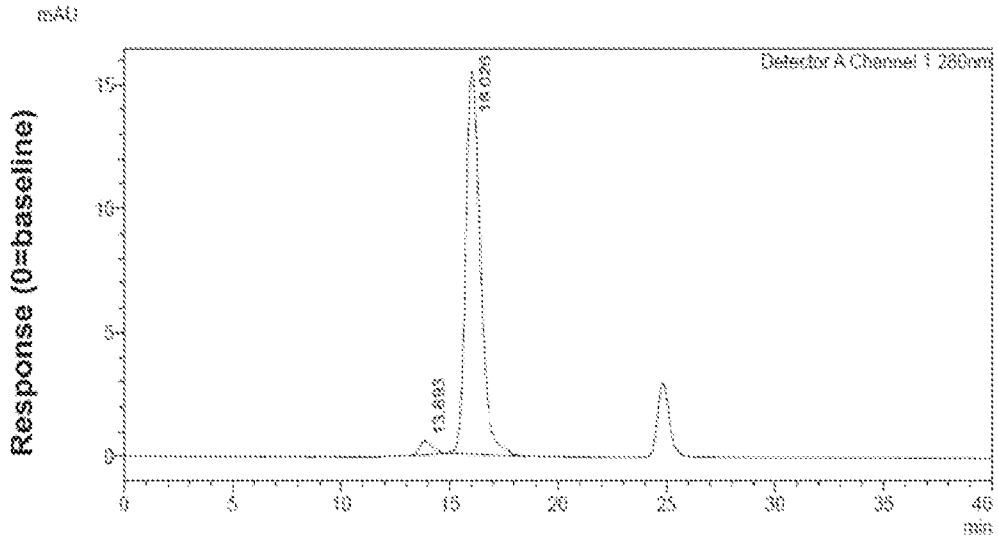


Fig. 1C

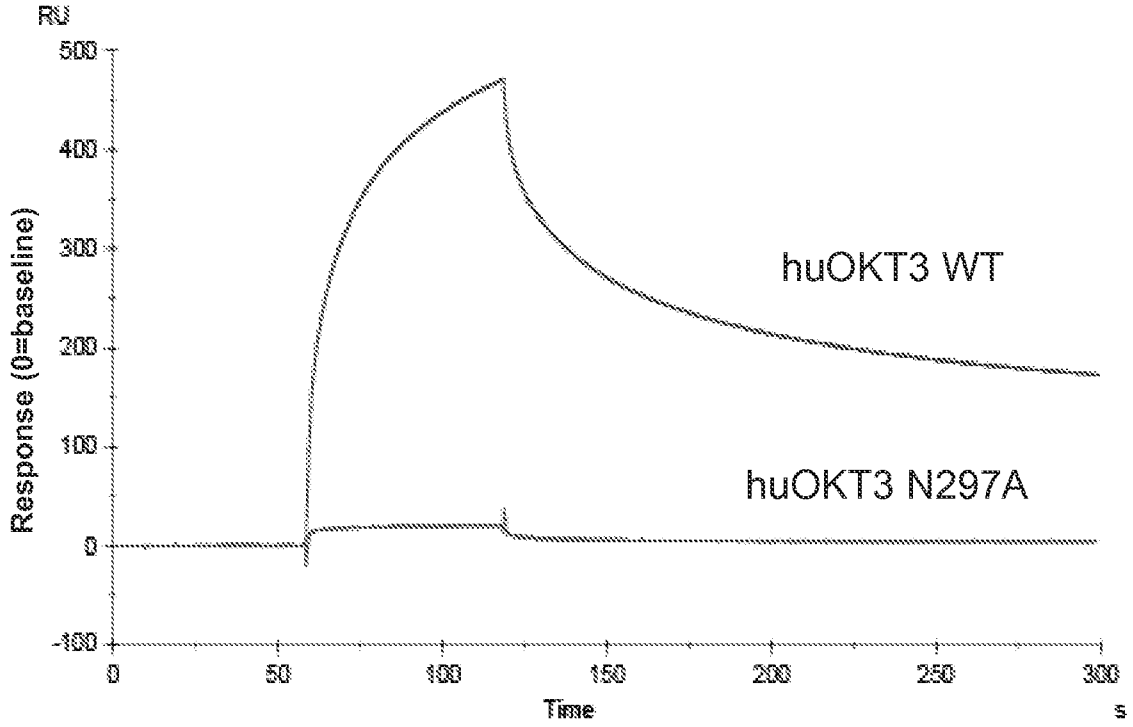
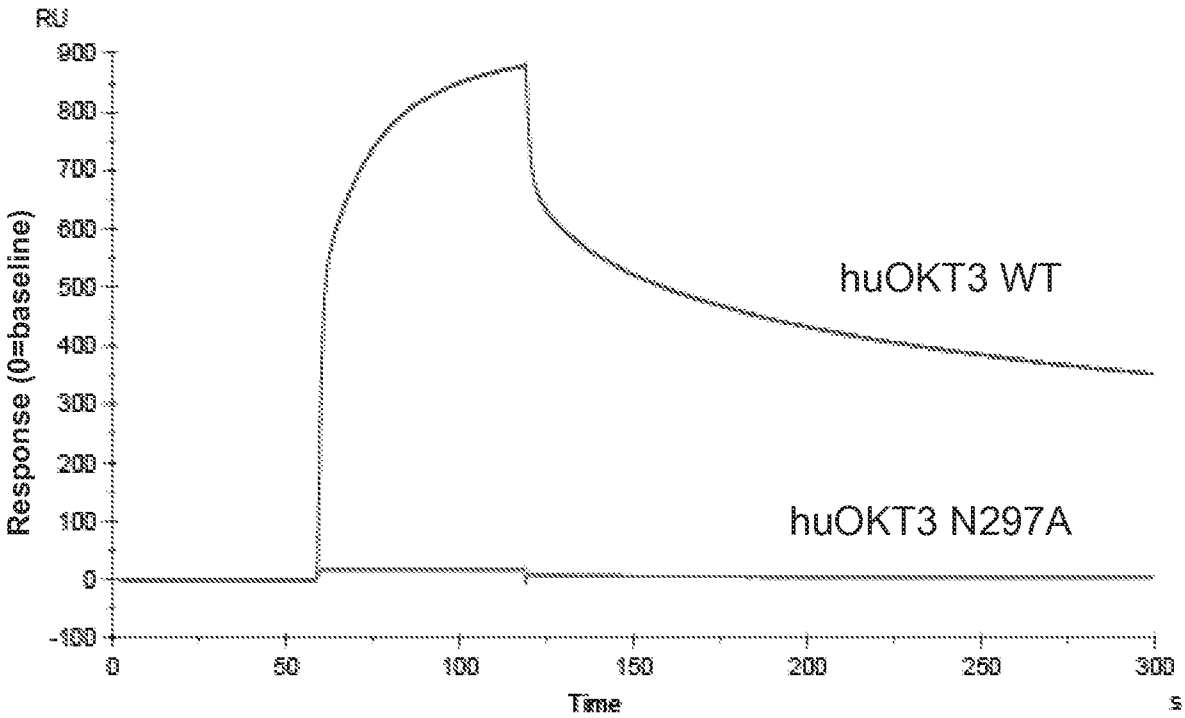

RU
500
400
300
200
100
0
-100
Response (0=baseline)
huOKT3 WT
huOKT3 N297A
0
50
100
150
200
250
300
Time
s

Fig. 1D


RU
900
800
700
600
500
400
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200
100
0
-100
Response (0=baseline)
huOKT3 WT
huOKT3 N297A
0
50
100
150
200
250
300
Time
s

Fig. 1E

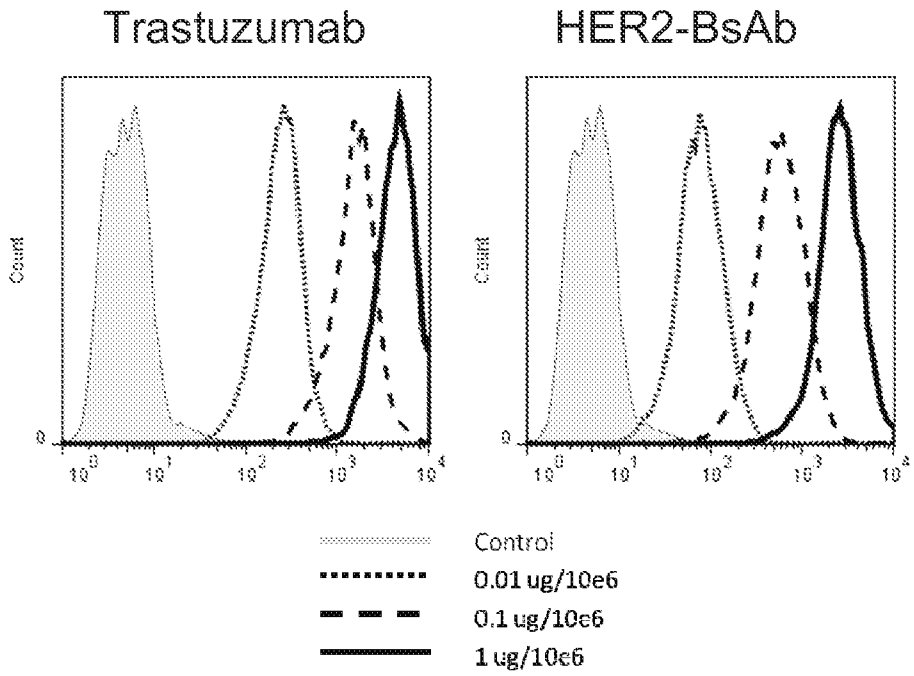

Trastuzumab
HER2-BsAb
Count
0
Control
0.01 ug/10e6
0.1 ug/10e6
1 ug/10e6

Fig. 2A

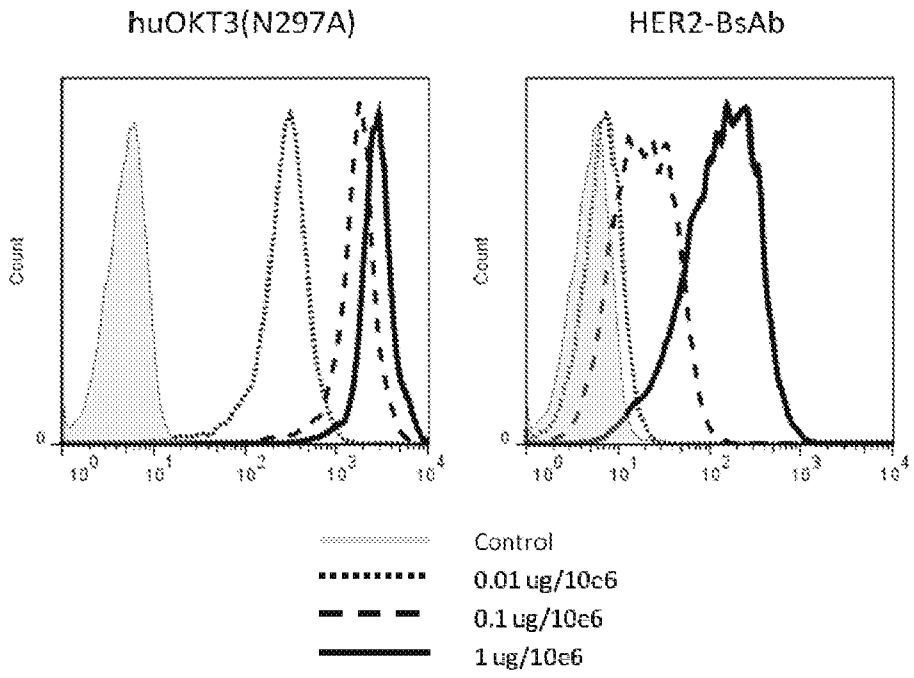

huOKT3(N297A)
HER2-BsAb
Count
0
Control
0.01 ug/10e6
0.1 ug/10e6
1 ug/10e6

Fig. 2B

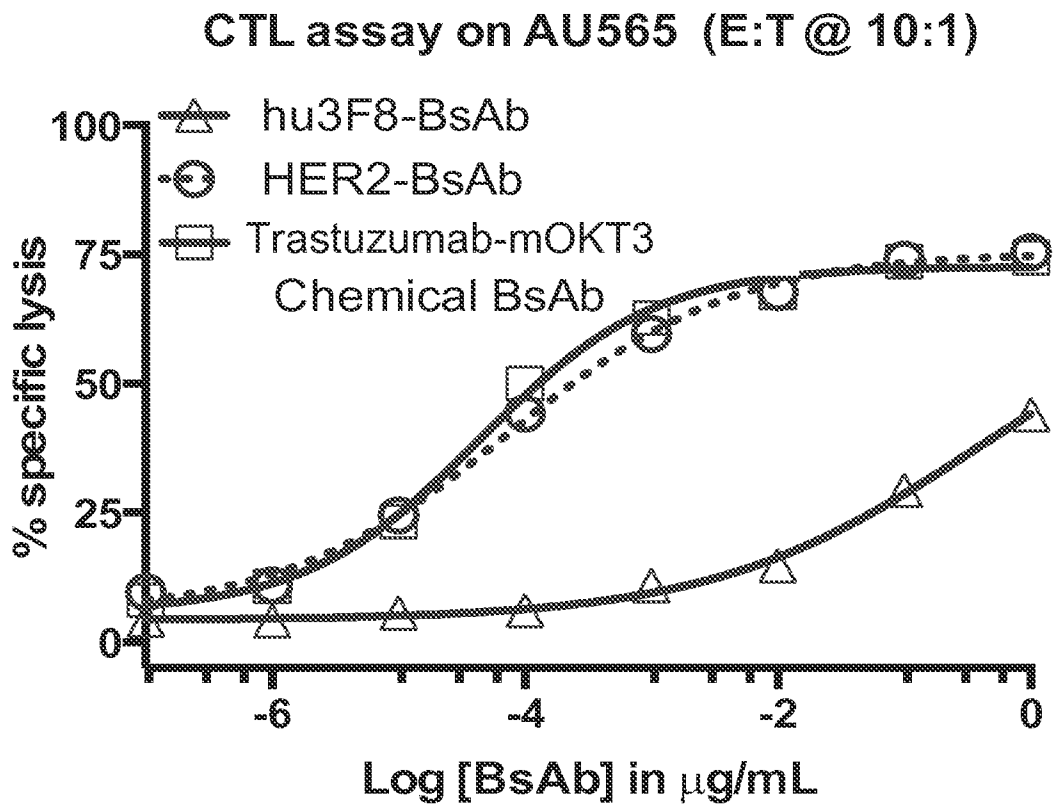

CTL assay on AU565 (E:T @ 10:1)
hu3F8-BsAb
HER2-BsAb
Trastuzumab-mOKT3
Chemical BsAb
% specific lysis
100
75
50
25
0
-6
-4
-2
0
Log [BsAb] in μg/mL

Fig. 3

Tumor Type	Cell Line Name	HER2 Expression (MFI)	EC50 (ng/ml)	EC50 (pM)
Breast Carcinoma	AU565	1175	0.06	0.3
Breast Carcinoma	SKBR3	760	0.2	1
Breast Carcinoma	MCF7	296	0.32	1.6
Ovarian Carcinoma	OVCAR3	183	0.36	1.8
Breast Carcinoma	MDA-MB-361 (HTB27)	777	0.5	2.5
Melanoma	SKMEL28	190	0.6	3
Osteosarcoma	CRL1427	108	2	10
Ewings	SKEAW	246	2	10
Rhabdomyosarcoma	HTB82	204	2	10
Melanoma	HT-144 (HTB63)	156	3	15
Neuroblastoma	NB5	66	3.1	15.5
Breast Carcinoma	MDA-MB-231 (HTB26)	68	4	20
Osteosarcoma	U2OS	90	4.5	22.5
Ewings	SKES-1	146	10	50
Melanoma	M14	57	26	130
Neuroblastoma	NMB7	12	>1000	>5000
Neuroblastoma	IMR32	6	>1000	>5000
Small Cell lung Cancer	NCI-H524	14	>1000	>5000
Neuroblastoma	SKNBE(1)N	3	>1000	>5000
Neuroblastoma	SKNBE(2)C	8	>1000	>5000
Small Cell lung Cancer	NCI-H69	10	>1000	>5000
Neuroblastoma	SKNBE(2)S	4	>1000	>5000
Small Cell lung Cancer	NCI-H345	6	>1000	>5000
Breast Carcinoma	MDA-MB-468 (HTB132)	6	>1000	>5000

Fig. 4

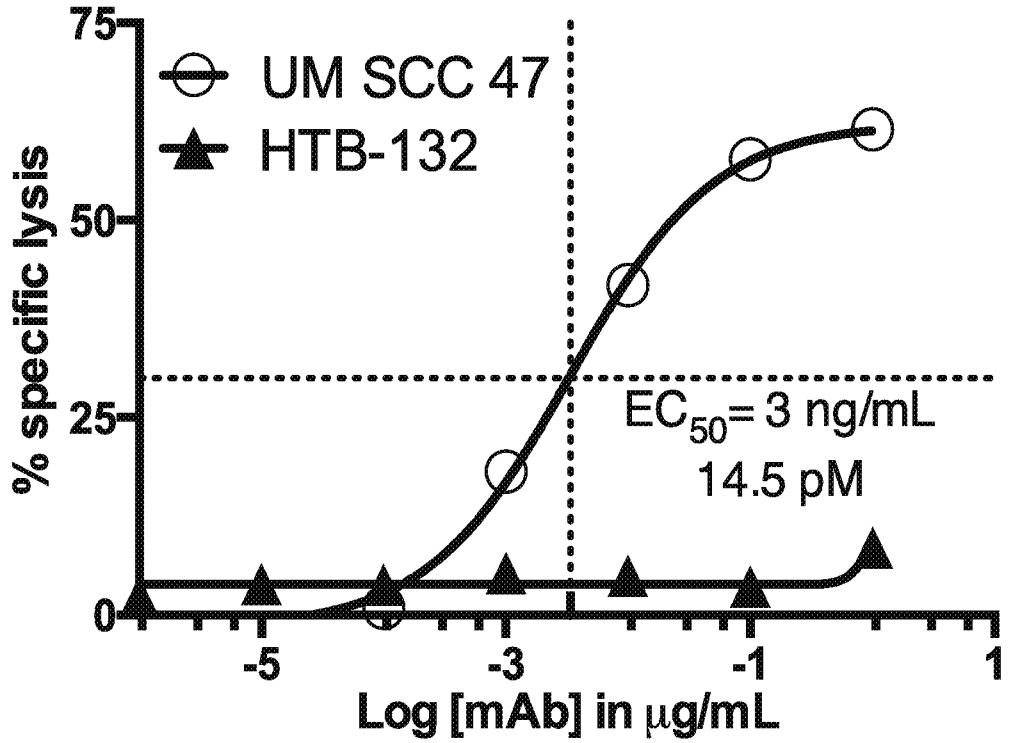

75
50
25
0
% specific lysis
UM SCC 47
HTB-132
EC50= 3 ng/mL
14.5 pM
-5
-3
-1
1
Log [mAb] in μg/mL

Fig. 5A

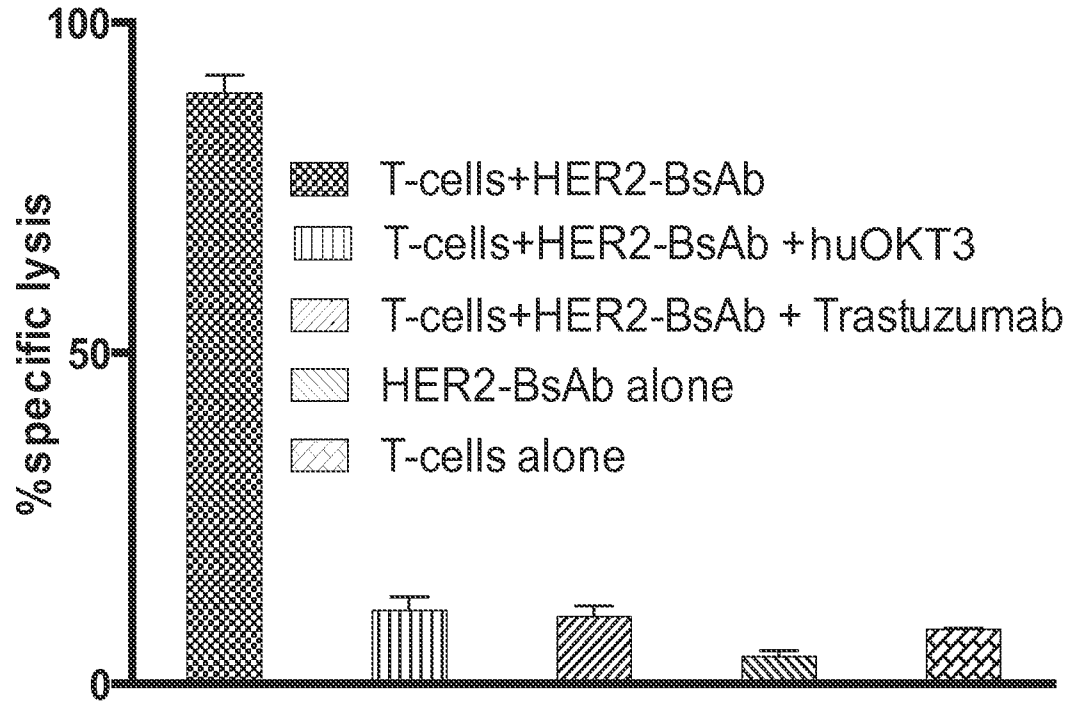

100
50
0
%specific lysis
T-cells+HER2-BsAb
T-cells+HER2-BsAb +huOKT3
T-cells+HER2-BsAb + Trastuzumab
HER2-BsAb alone
T-cells alone

Fig. 5B

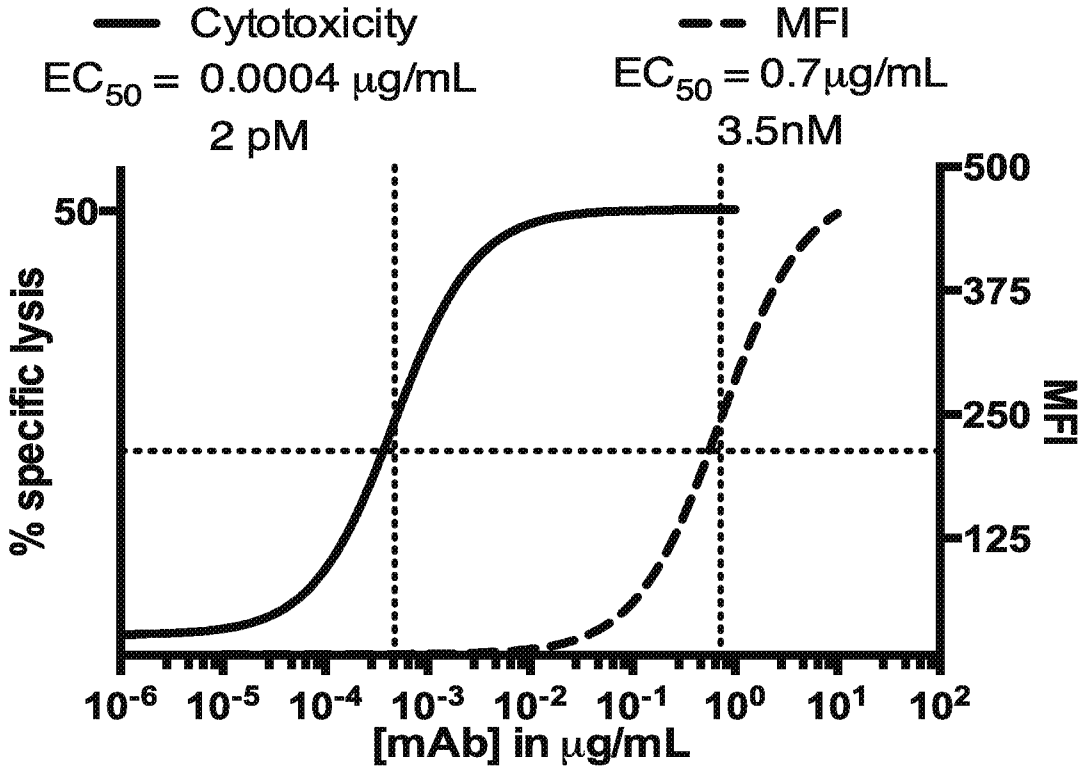

Cytotoxicity
EC50 = 0.0004 µg/mL
2 pM
MFI
EC50 = 0.7µg/mL
3.5nM
50
% specific lysis
500
375
250
125
MFI
10-6 10-5 10-4 10-3 10-2 10-1 100 101 102
[mAb] in µg/mL

Fig. 6

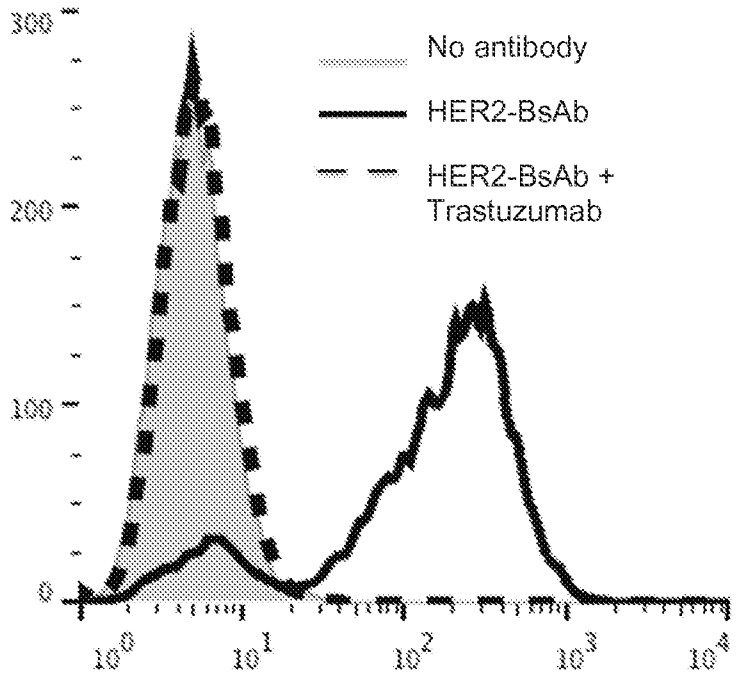


Fig. 7A

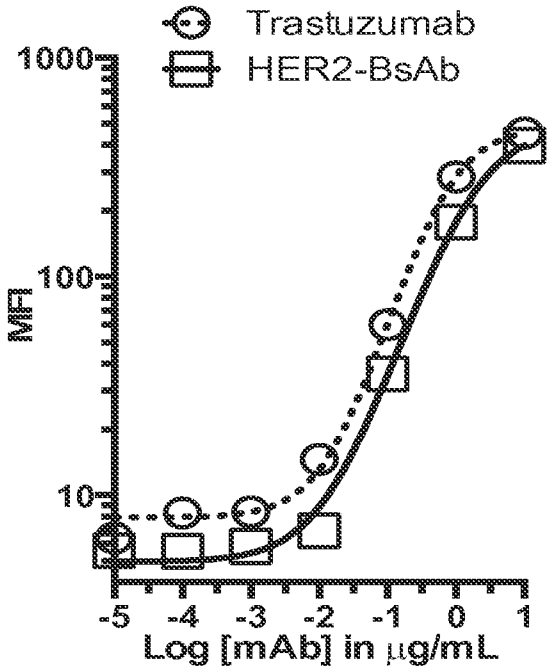


Fig. 7B

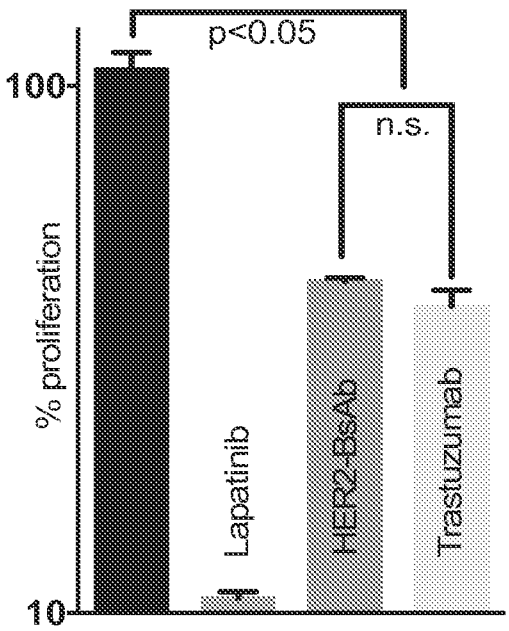


Fig. 7C

SCCHN cell lines	% Max Lysis	Flow HER2 (MFI)	qPCR (relative to MCF7)	Number of Experiments	EC50 ng/mL	EC50 pM
15B	47	305	121	2	13	63
93VU147T	45	127	151	3	6	32
PCI-30	53	359	237	3	2	12
SCC90	46	274	578	3	1	6
UDSCC2	42	178	139	5	5	27
UMSCC47	57	302	49	3	4	20

Fig. 8

Fig. 9A

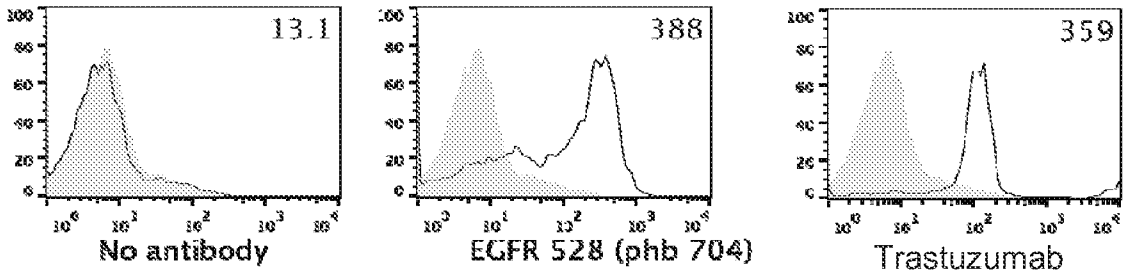


Fig. 9B

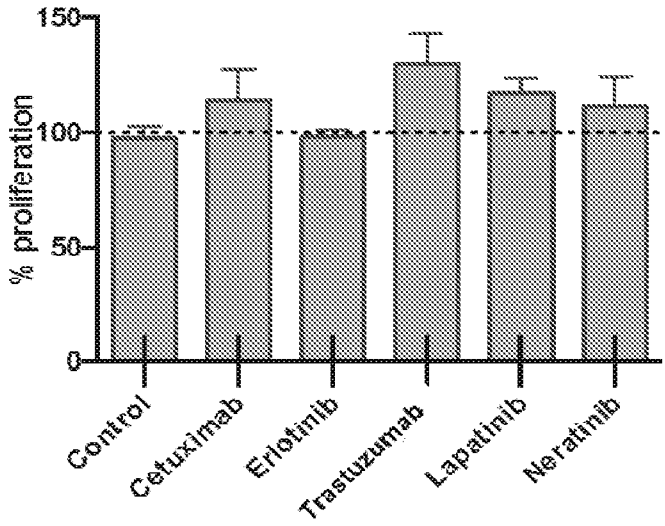
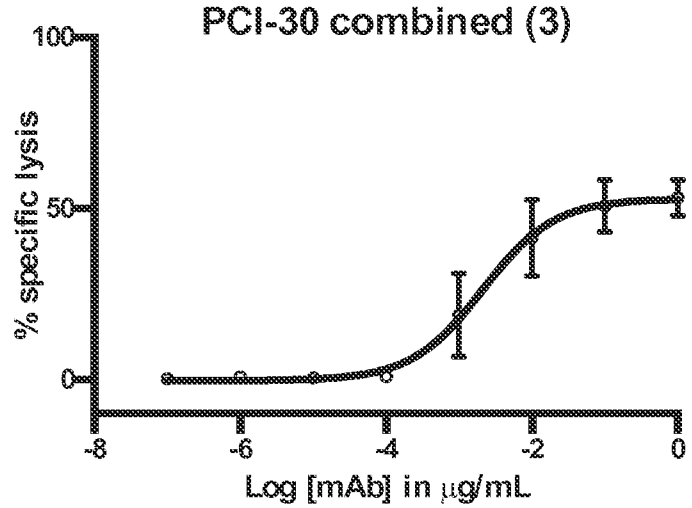


Fig. 9C



Osteosarcoma cell lines	% Max Lysis	Flow HER2 (MFI)	qtPCR (relative to MCF7)	Number of Experiments	EC50 ng/mL (average)	EC50 pM
U2OS	49	53	713	3	5	25
RG 160	62	563	1881	3	2	11
RG 164	68	439	5510	4	4	18
CRL 1427	46	81	52	2	3	16

Fig. 10

Fig. 11A

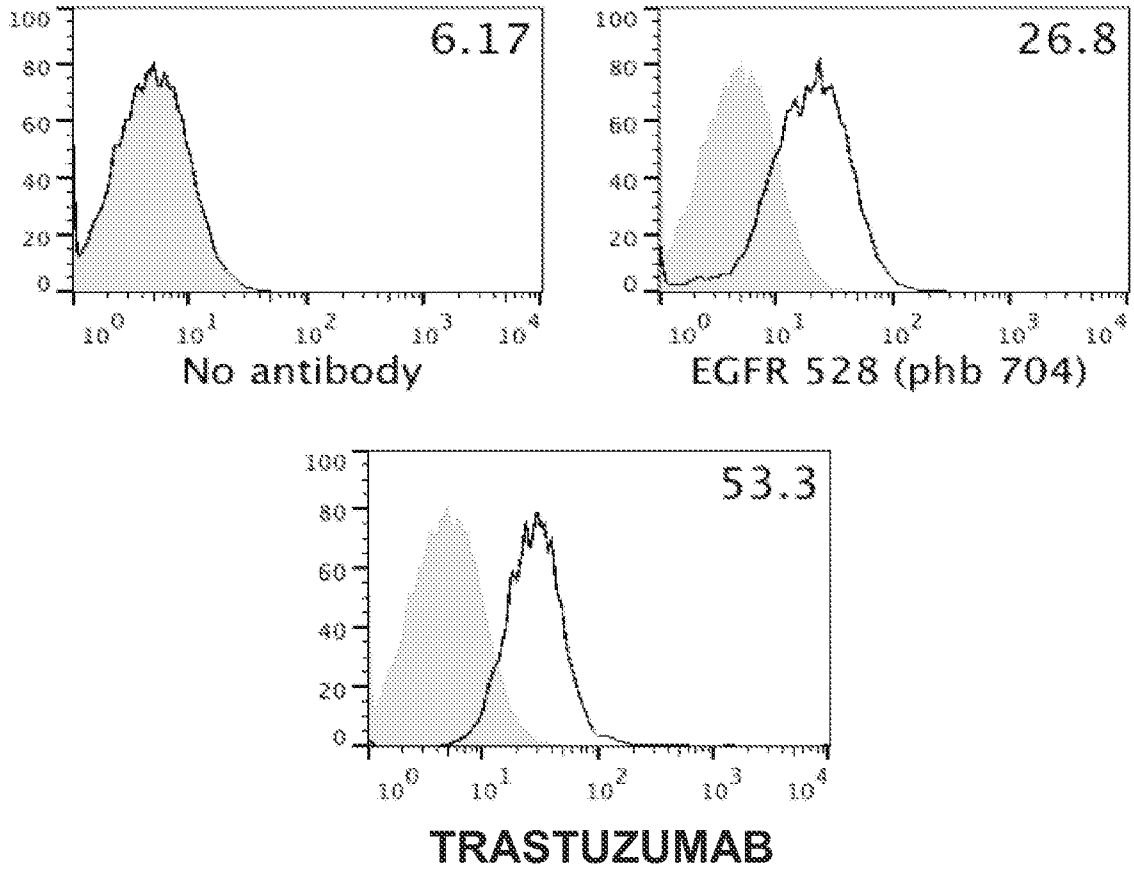


Fig. 11B

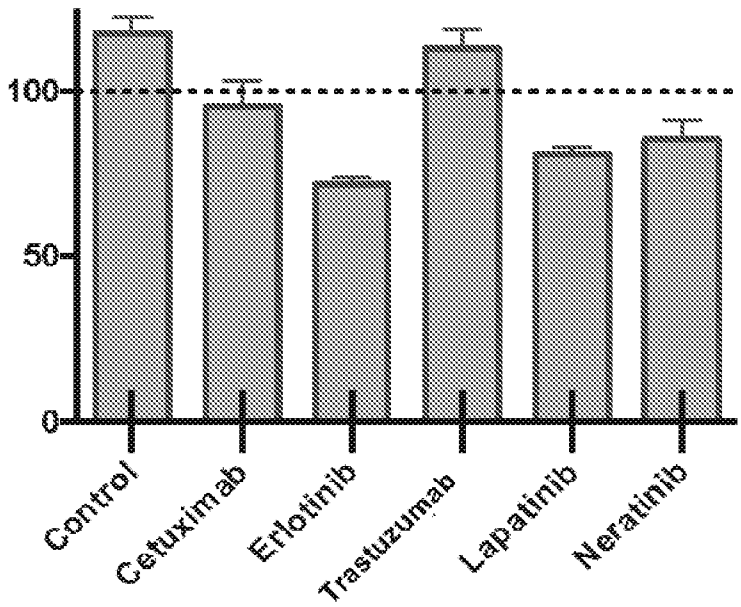
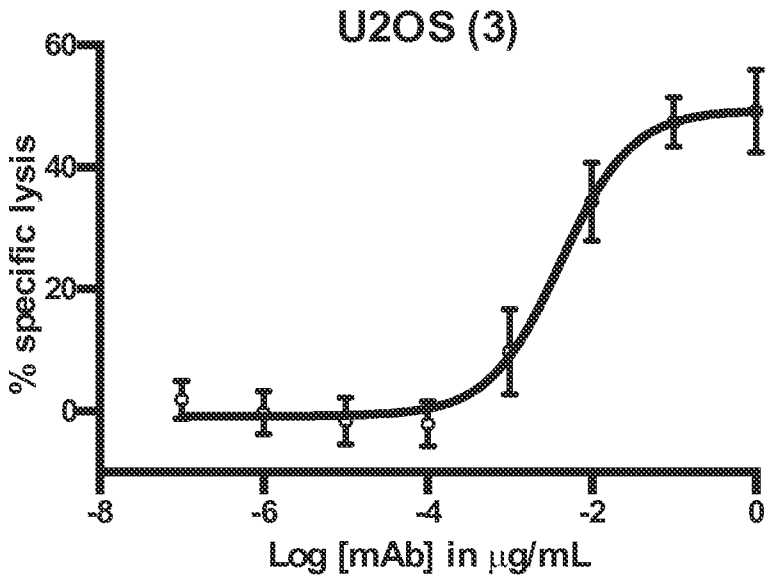


Fig. 11C



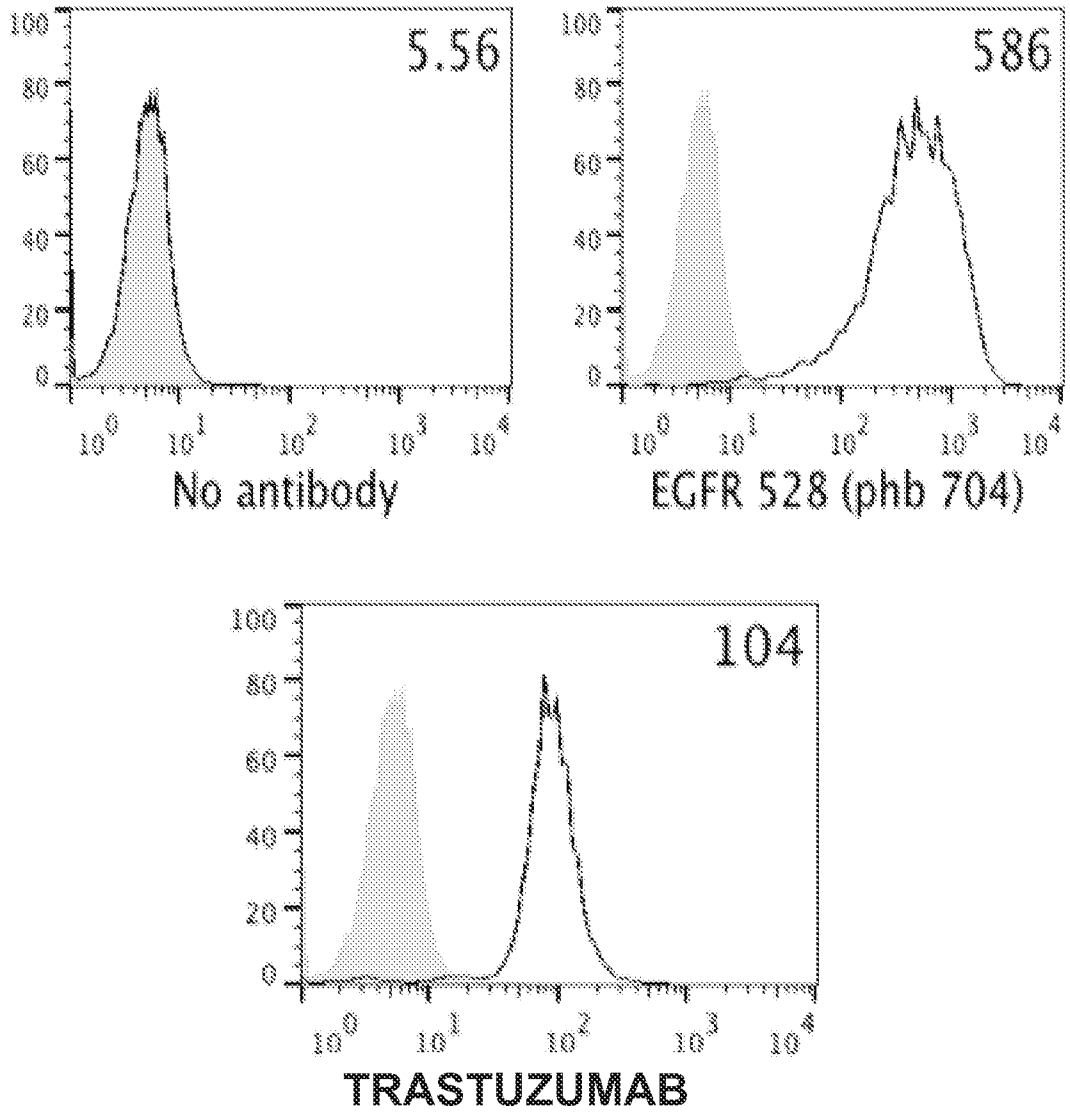


Fig. 12A

Fig. 12B

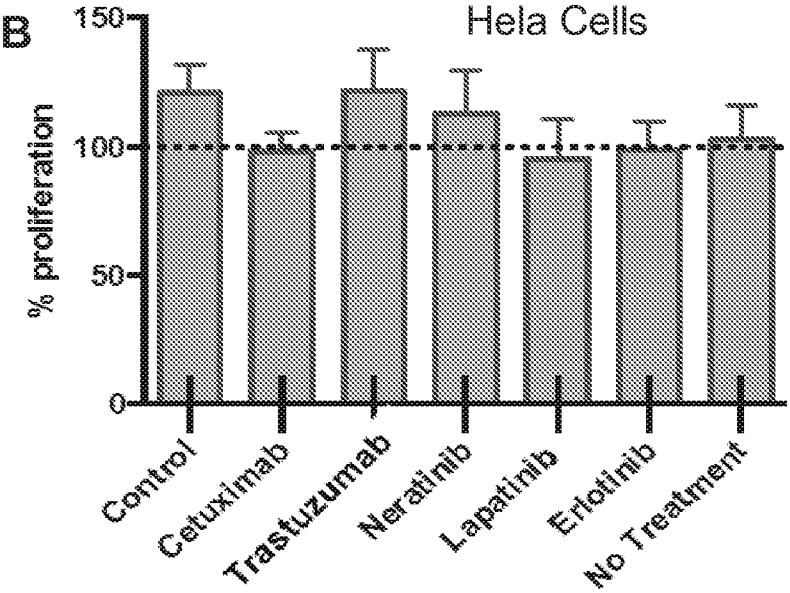


Fig. 12C

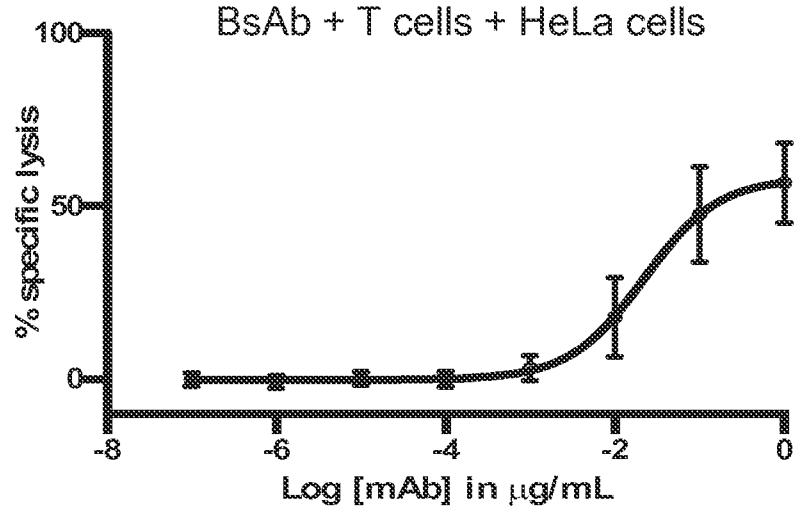
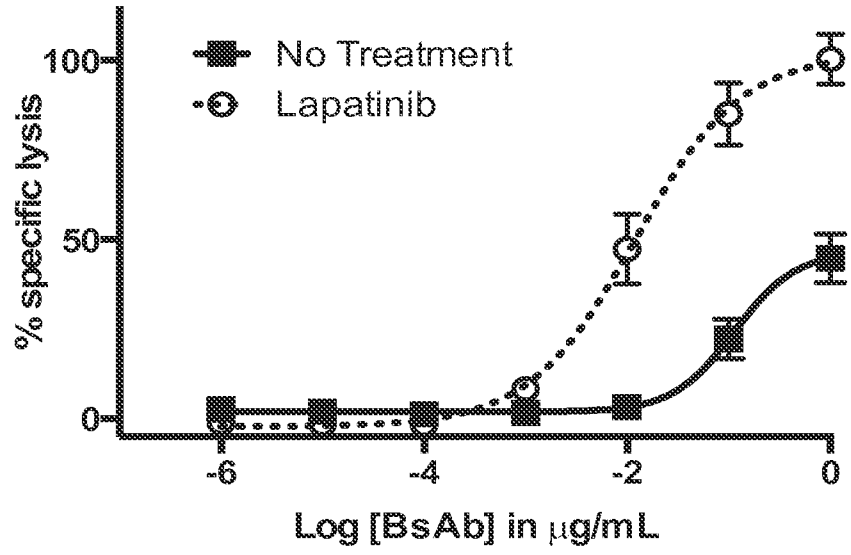


Fig. 12D



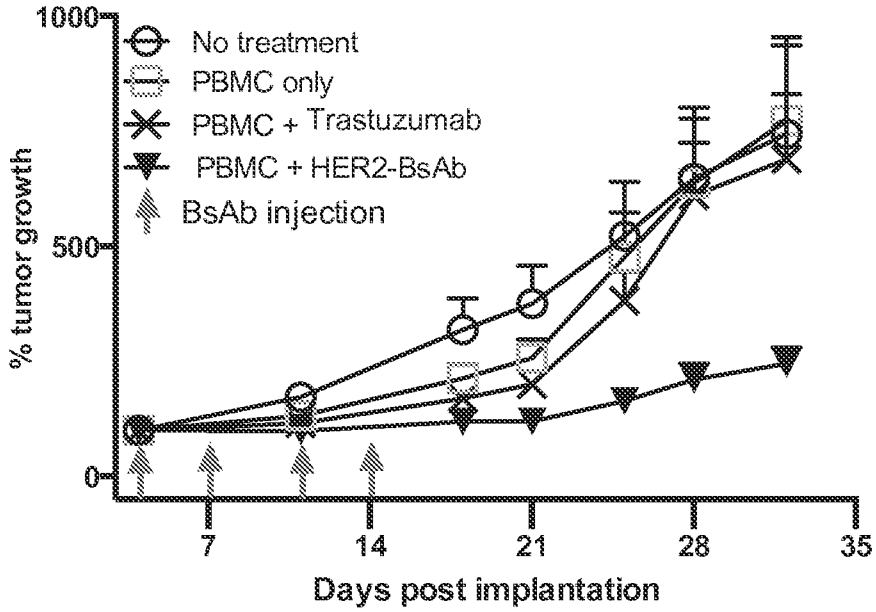

1000
500
0
% tumor growth
No treatment
PBMC only
PBMC + Trastuzumab
PBMC + HER2-BsAb
BsAb injection
7
14
21
28
35
Days post implantation

Fig. 13

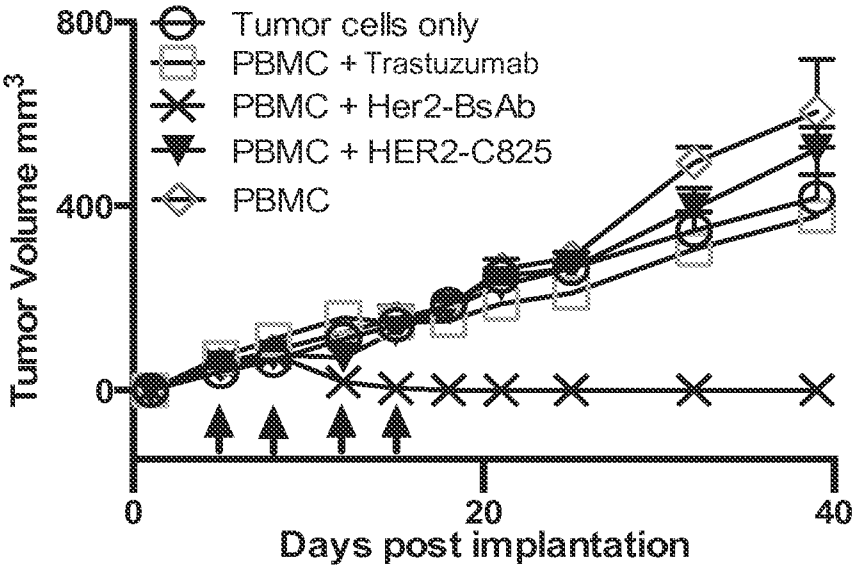

800
400
0
Tumor Volume mm3
Tumor cells only
PBMC + Trastuzumab
PBMC + Her2-BsAb
PBMC + HER2-C825
PBMC
0
20
40
Days post implantation

Fig. 14

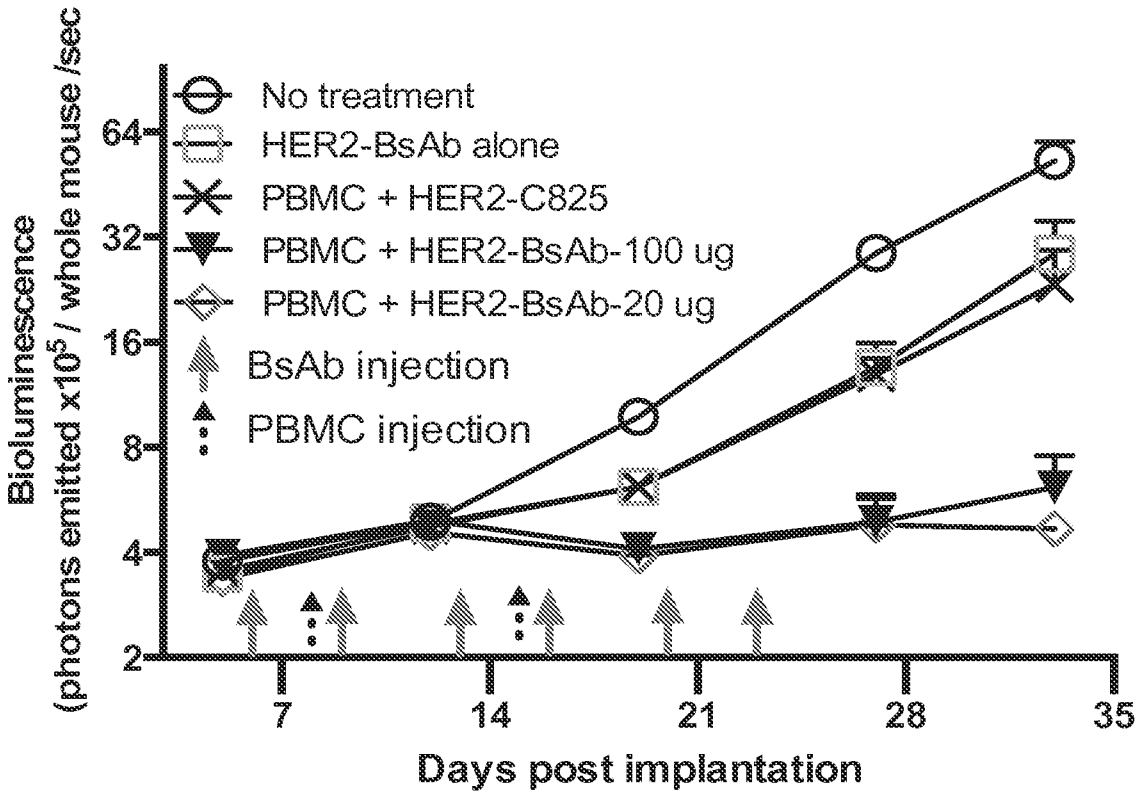

Bioluminescence
(photons emitted x10^5 / whole mouse /sec
64
32
16
8
4
2
No treatment
HER2-BsAb alone
PBMC + HER2-C825
PBMC + HER2-BsAb-100 ug
PBMC + HER2-BsAb-20 ug
BsAb injection
PBMC injection
7
14
21
28
35
Days post implantation

Fig. 15

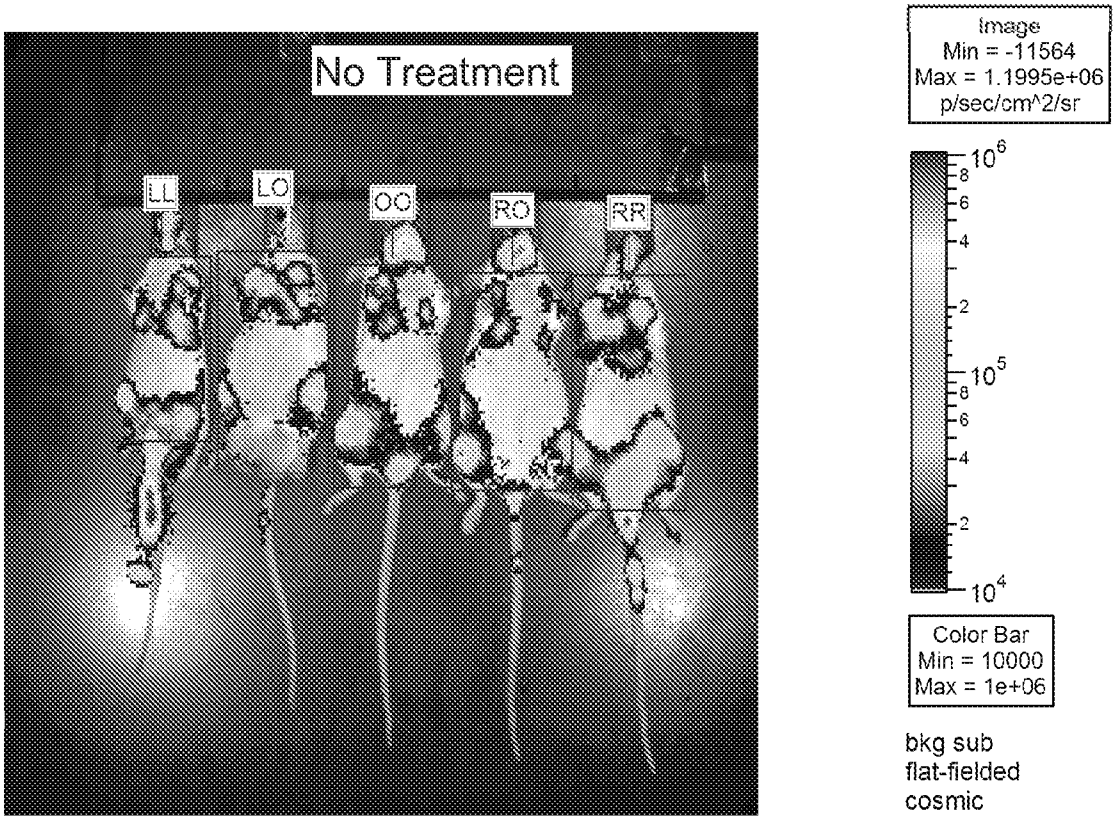


Fig. 16A

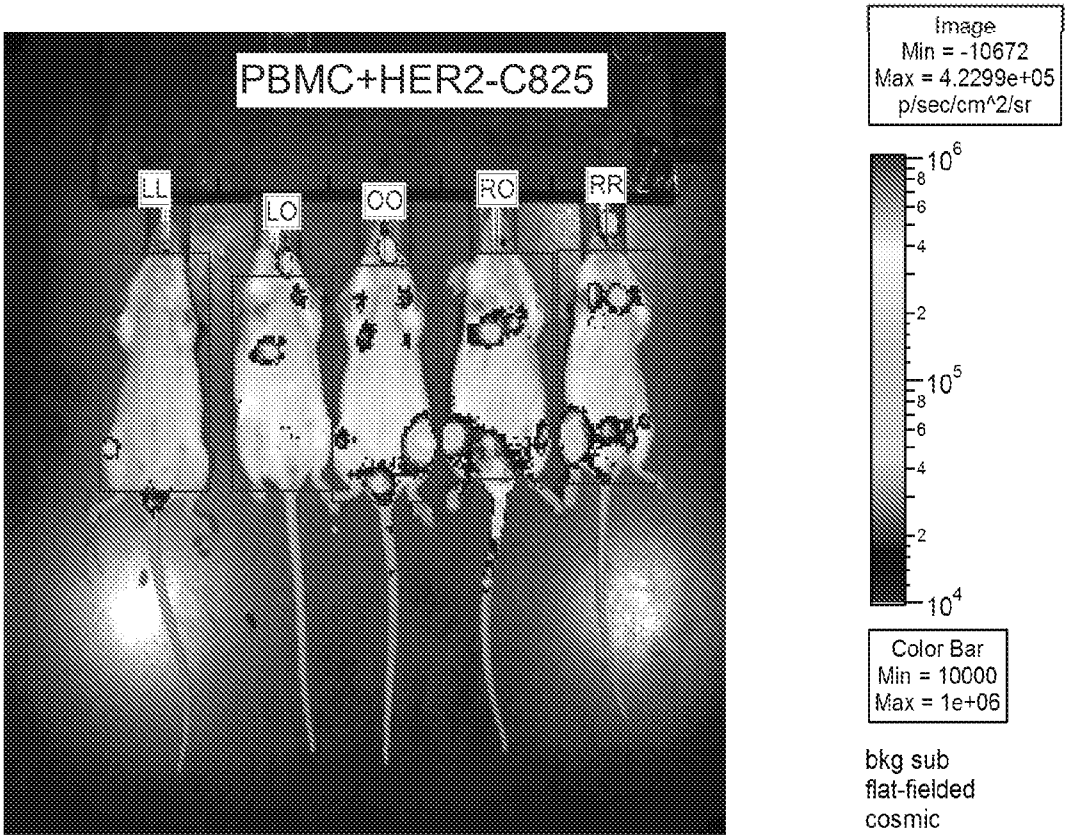


Fig. 16B

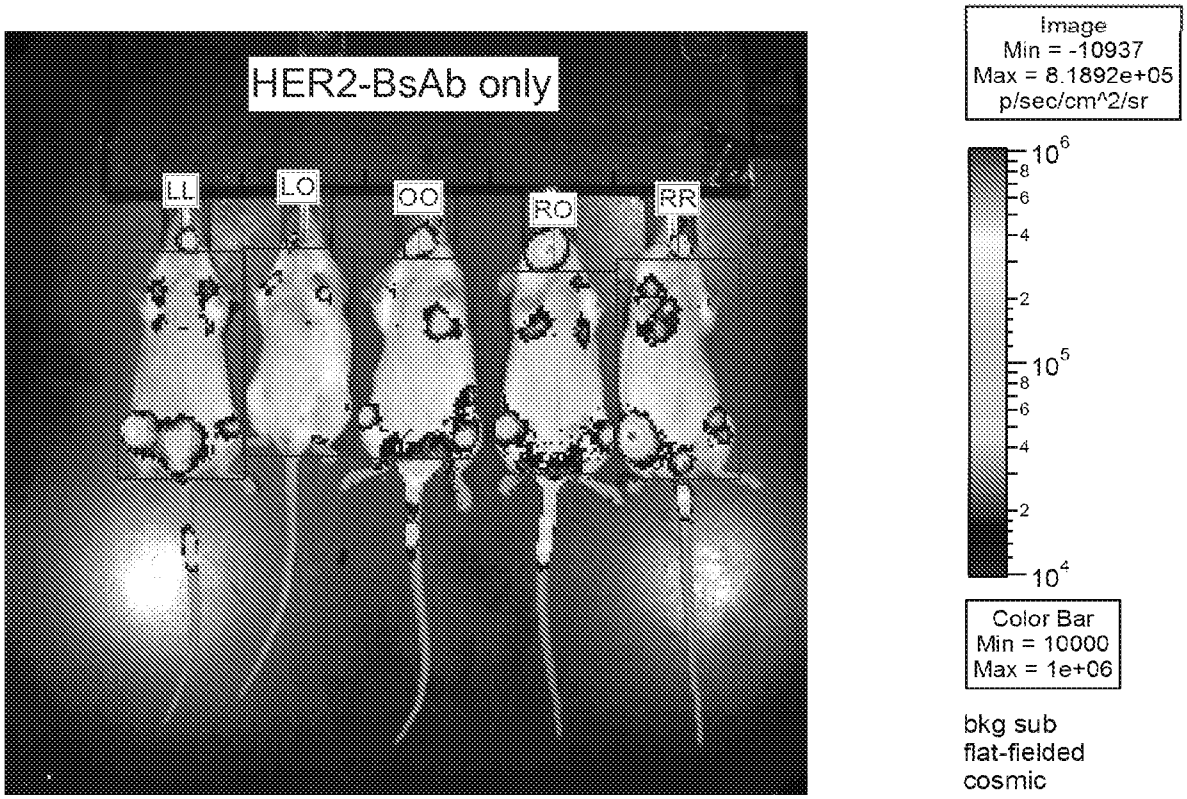


Fig. 16C

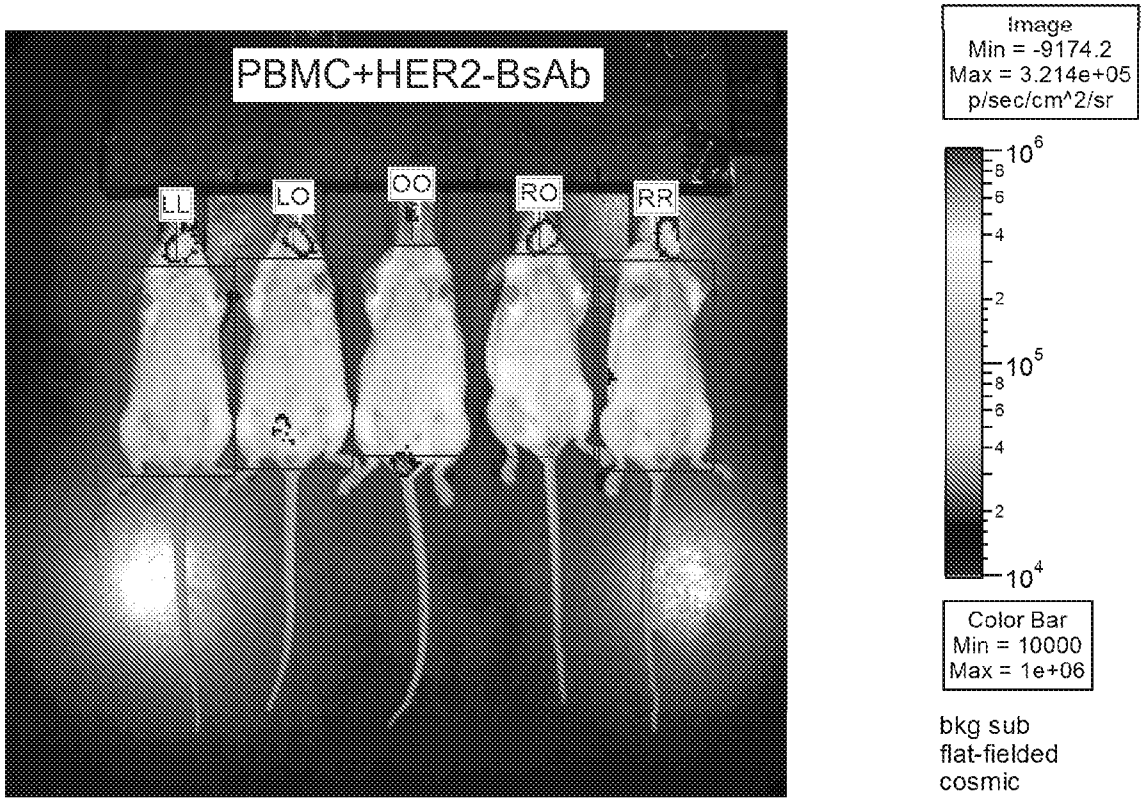


Fig. 16D

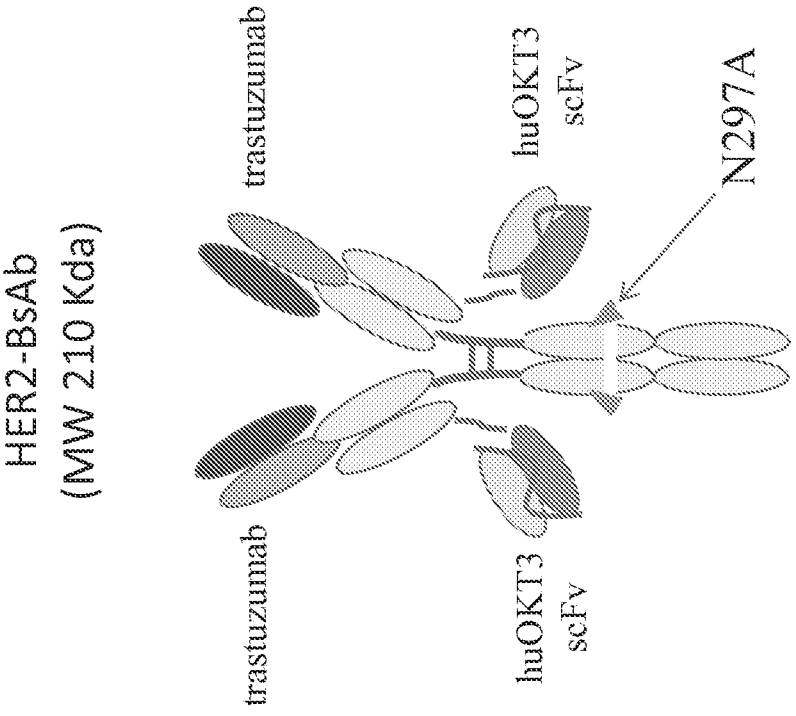


Fig. 17A

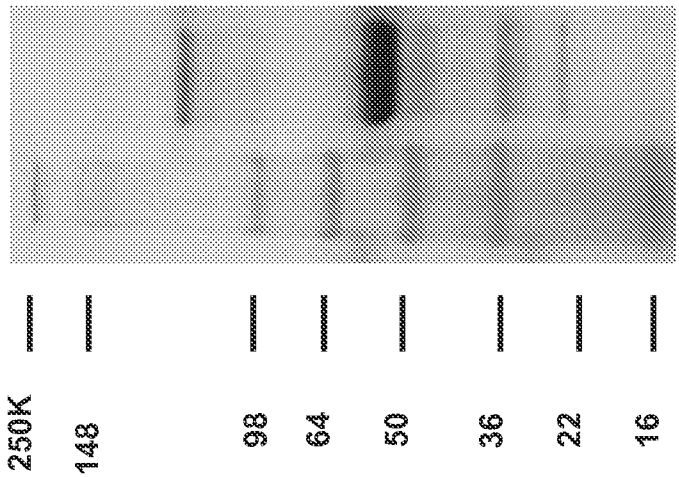


Fig. 17B

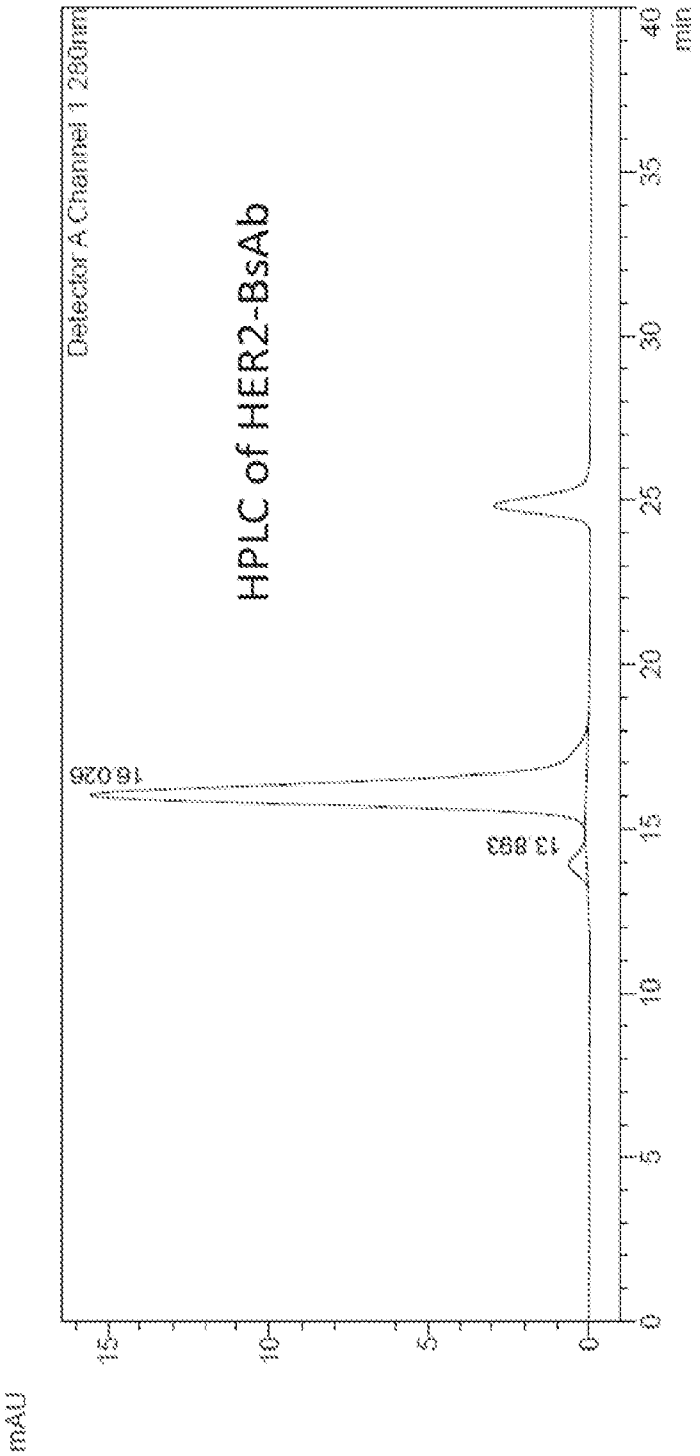

HPLC of HER2-BsAb
Detector A Channel 1 280nm
mAU
min
16.026
13.983
0
5
10
15
20
25
30
35
40

Fig. 17C

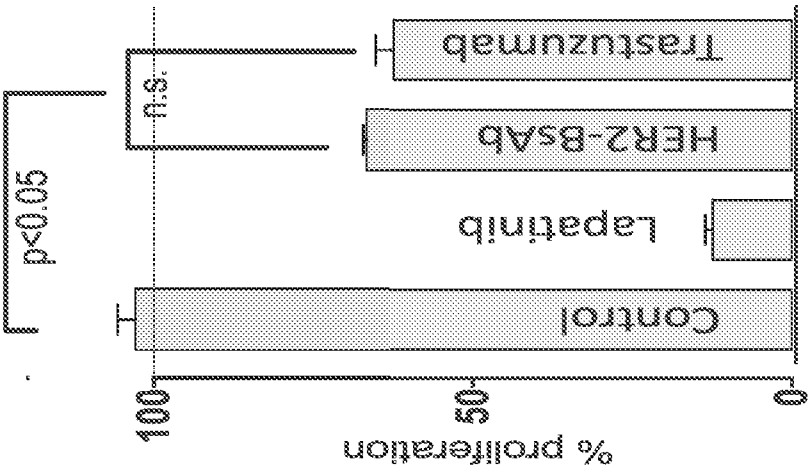


Fig. 18C

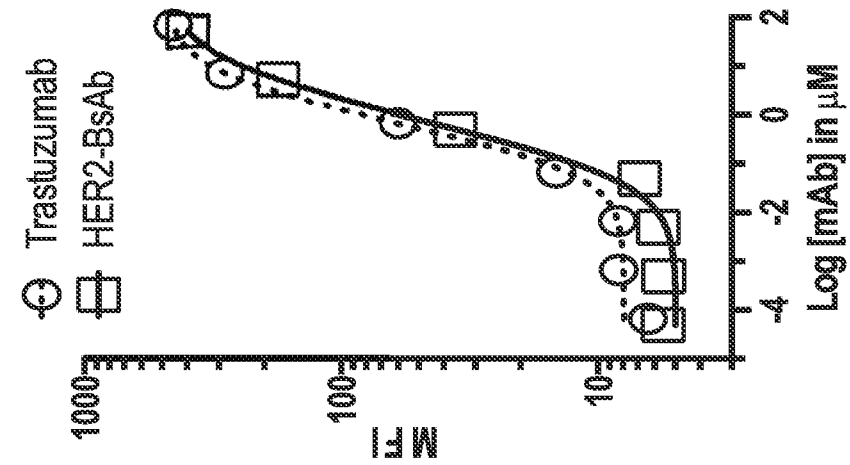


Fig. 18B

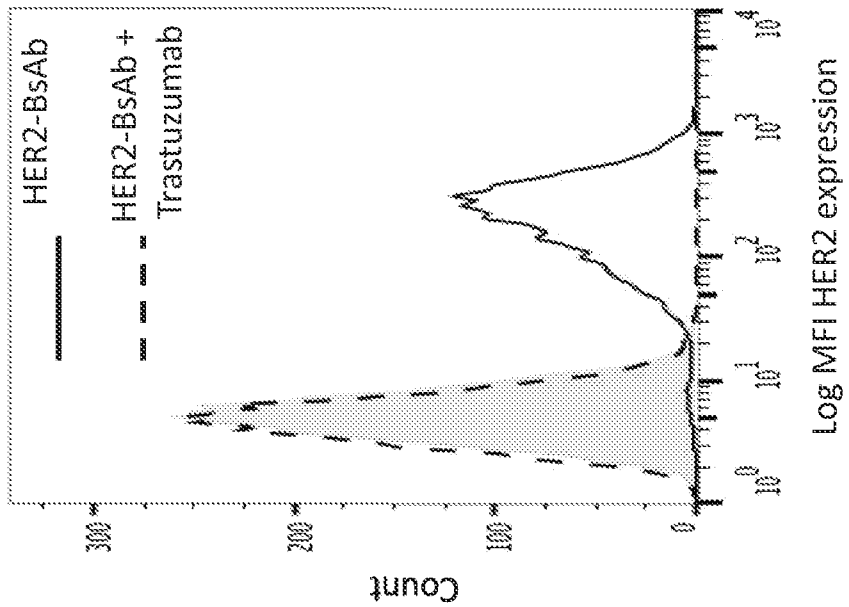


Fig. 18A

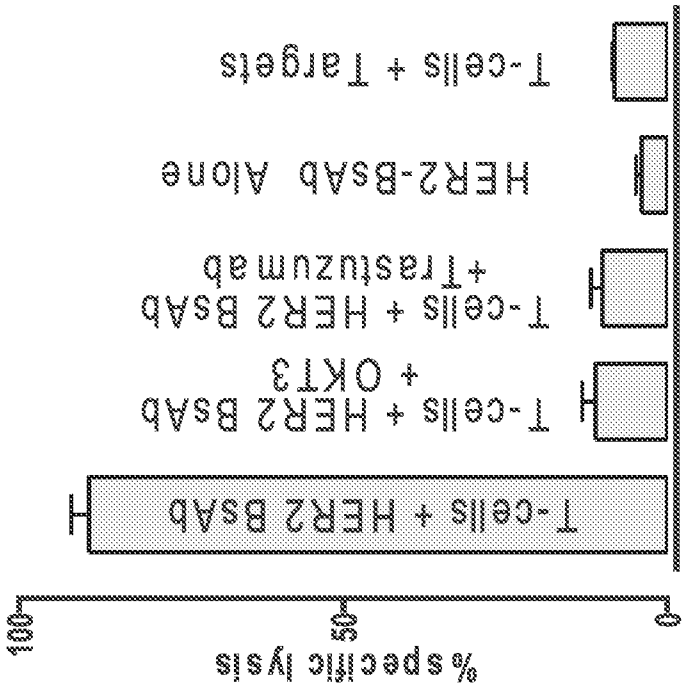


Fig. 19B

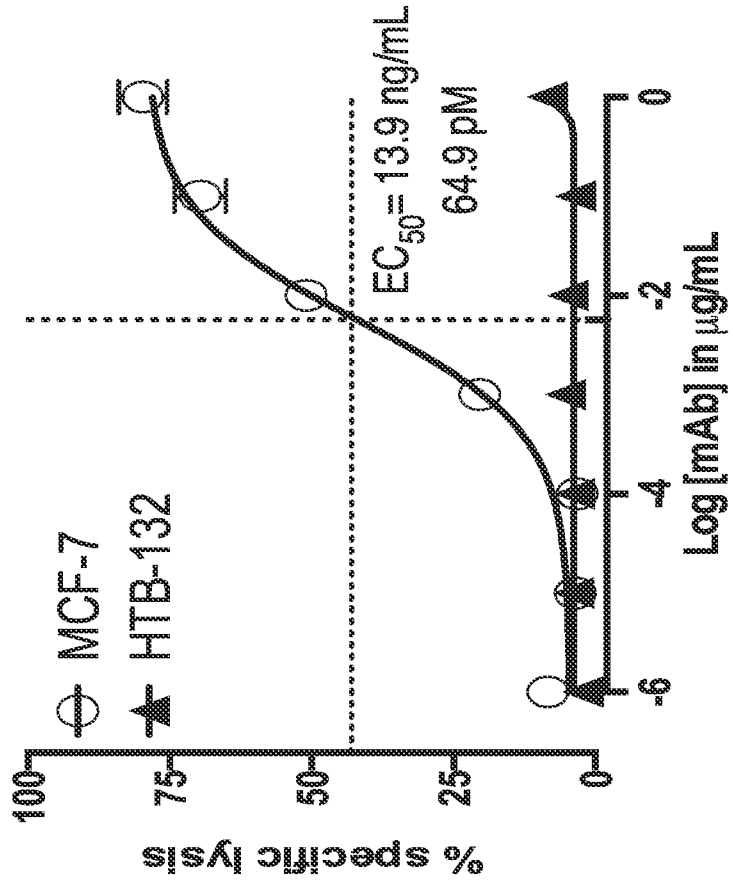


Fig. 19A

Tumor Type	Cell Line	HER2 Expression (MFI)	EC50 (pM)
Breast Carcinoma	AU565	1175	0.3
Ovarian Carcinoma	OVCAR3	183	1.8
Breast Carcinoma	MDA-MB-361	777	2.5
Ovarian Carcinoma	SKOV3	1577	2.8
Melanoma	SKMEL28	190	3
Breast Carcinoma	SKBR3	2506	4.1
Breast Carcinoma	HCC1954	1597	5.5
Head and Neck Cancer	SCC90	274	5.7
Ewings	SKEAW	246	10
Osteosarcoma	CRL1427	108	10
Rhabdomyosarcoma	HTB82	204	10
Osteosarcoma	RG 160	563	11
Head and Neck Cancer	PCI-30	359	12.2
Melanoma	HT-144	156	15
Neuroblastoma	NB5	66	15.5
Osteosarcoma	RG 164	439	17.7
Head and Neck Cancer	UM SCC47	302	19.8
Osteosarcoma	U2OS	90	22.5
Head and Neck Cancer	UDSCC2	178	26.9
Head and Neck Cancer	93VU147T	127	32.4
Ewings	SKES-1	146	50
Breast Carcinoma	HTB-26	76	50.2
Head and Neck Cancer	15B	305	62.8
Breast Carcinoma	MCF7	398	64.9
Cervical Cancer	HeLA	104	120.7
Melanoma	M14	57	130
Breast Carcinoma	MDA-MB-468	6	>5000
Neuroblastoma	NMB7	12	>5000
Neuroblastoma	SKNBE(2)C	8	>5000
Neuroblastoma	IMR32	6	>5000
Neuroblastoma	SKNBE(2)S	4	>5000
Neuroblastoma	SKNBE(1)N	3	>5000
Small Cell lung Cancer	NCI-H524	14	>5000
Small Cell lung Cancer	NCI-H69	10	>5000
Small Cell lung Cancer	NCI-H345	6	>5000

Fig. 20

Fig. 21A

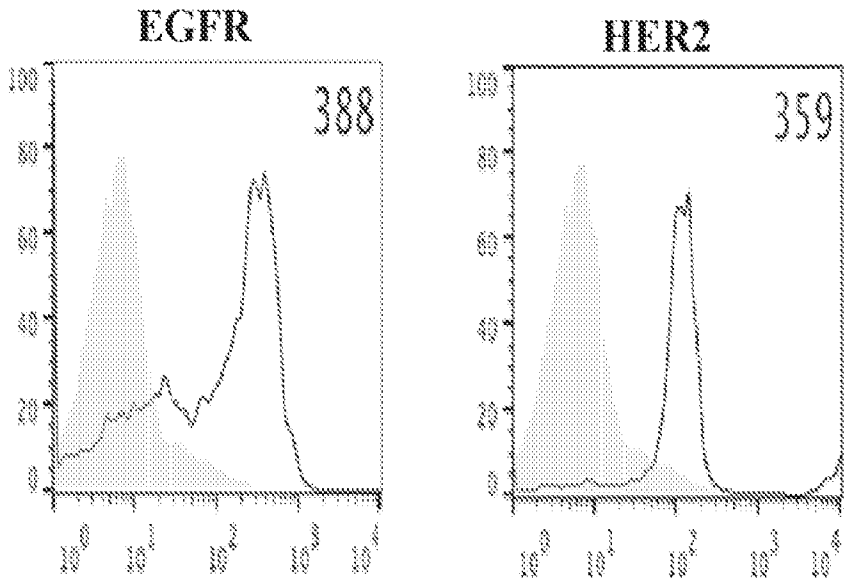


Fig. 21B

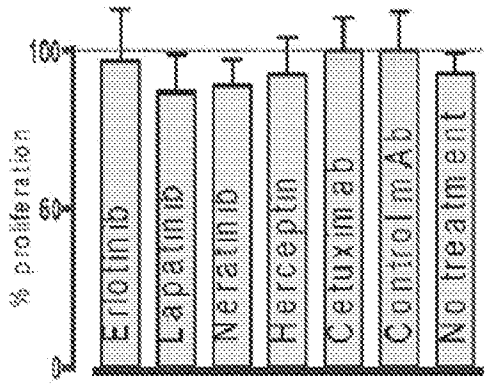


Fig. 21C

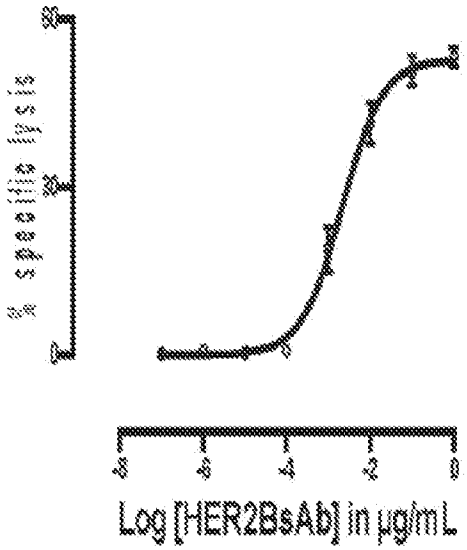


Fig. 21D

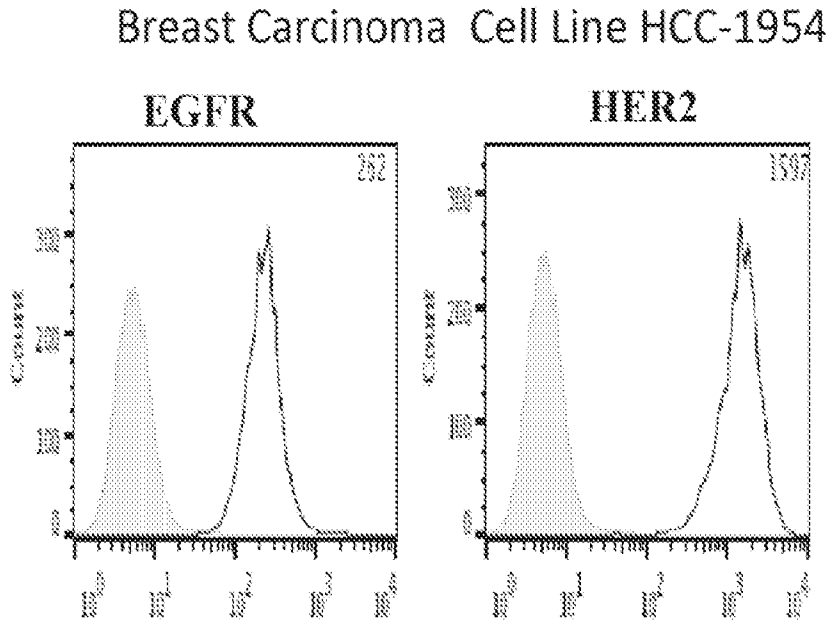


Fig. 21E

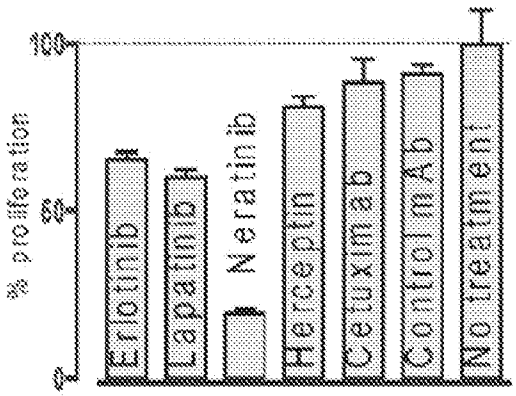


Fig. 21F

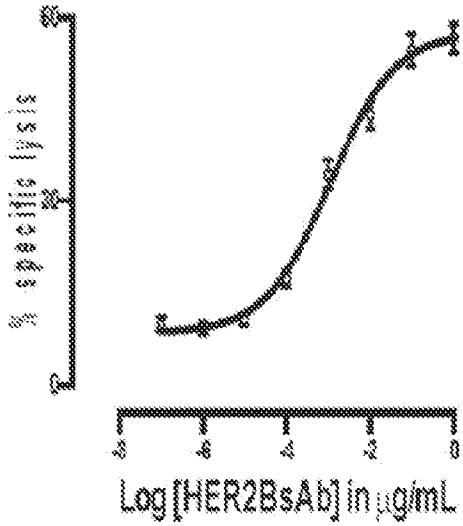


Fig. 21G

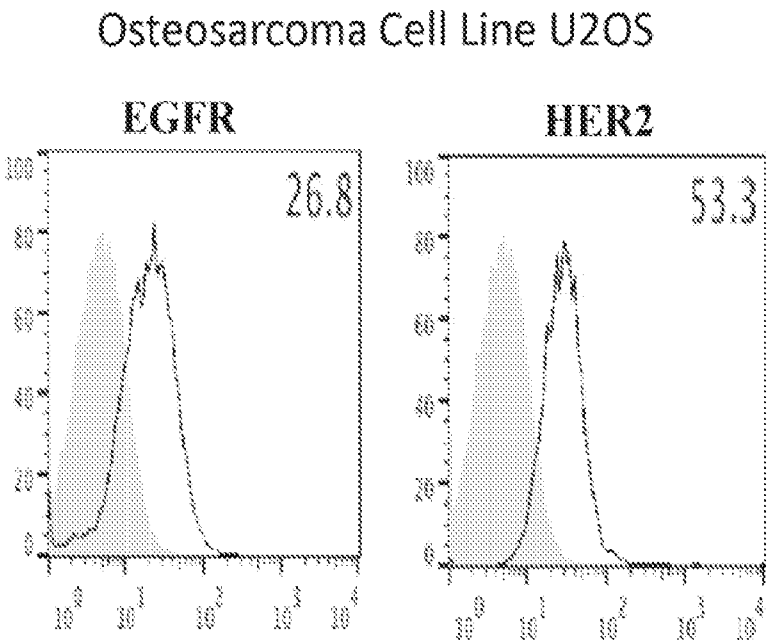


Fig. 21H

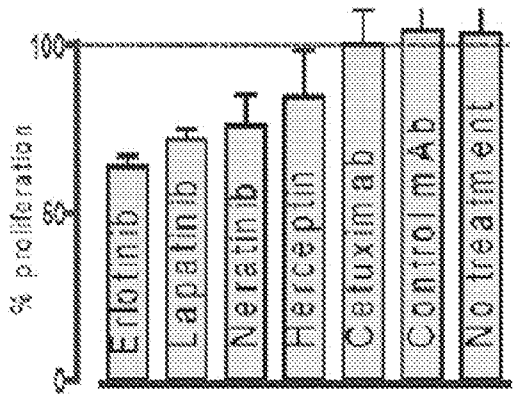
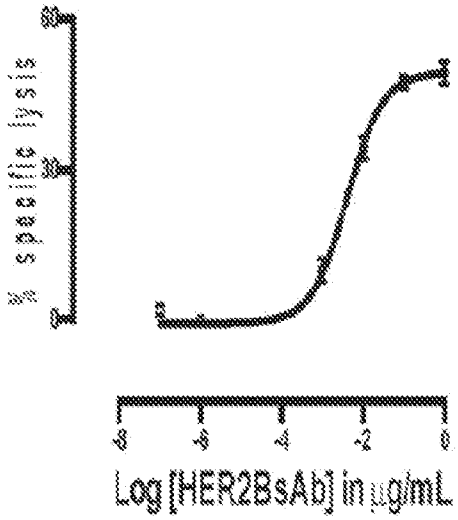


Fig. 21I



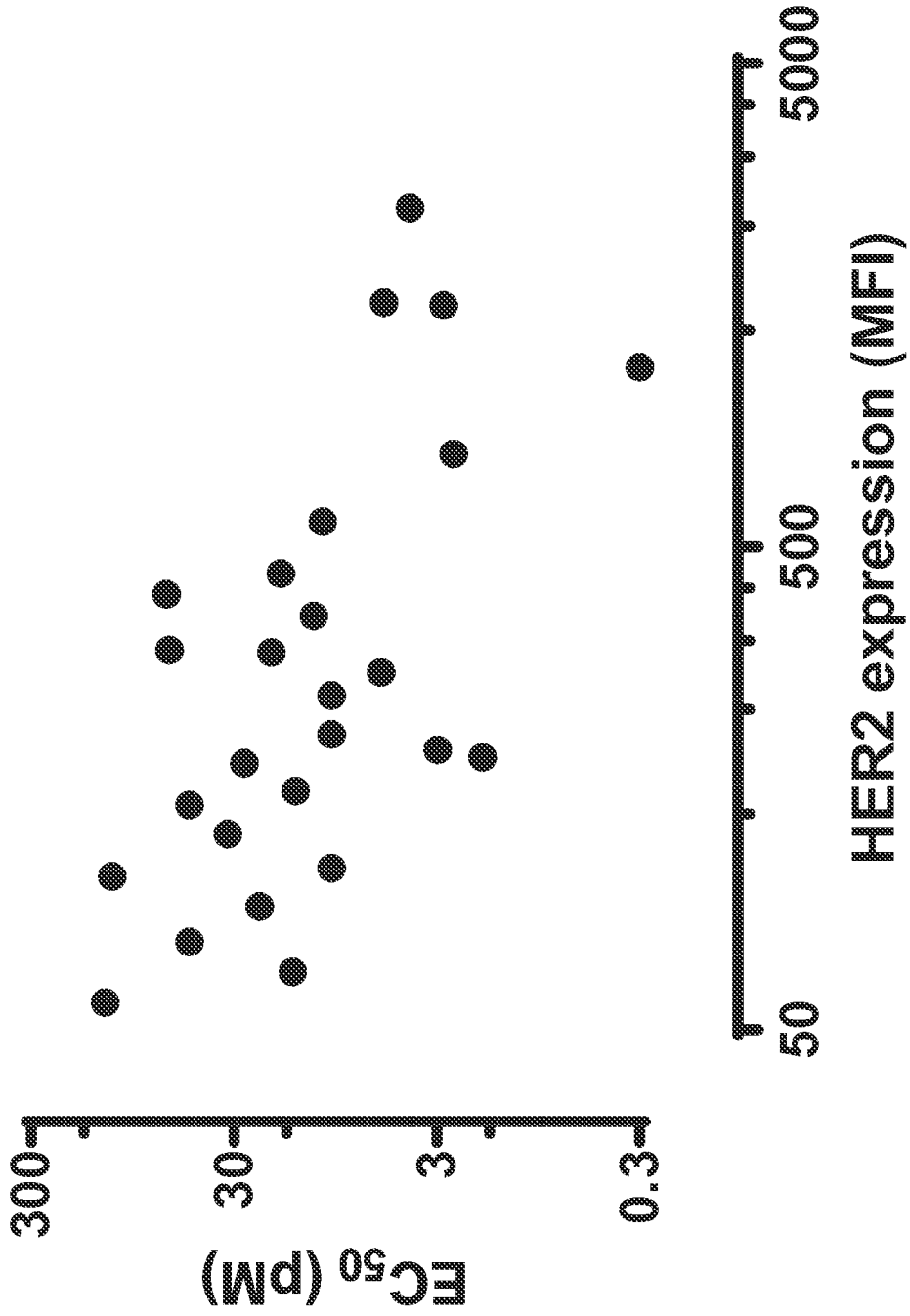


Fig. 22

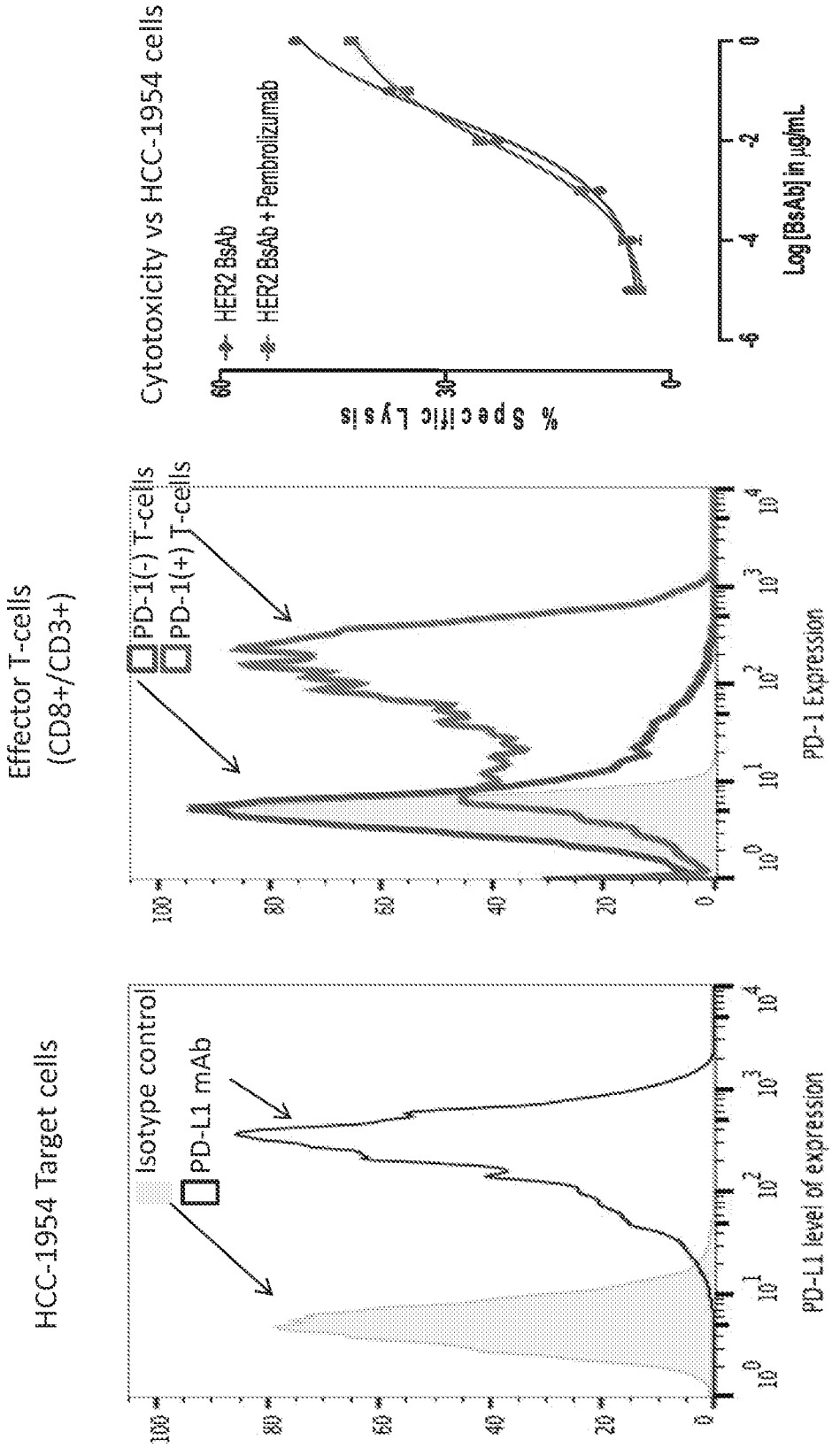


Fig. 23A

Fig. 23B

Fig. 23C

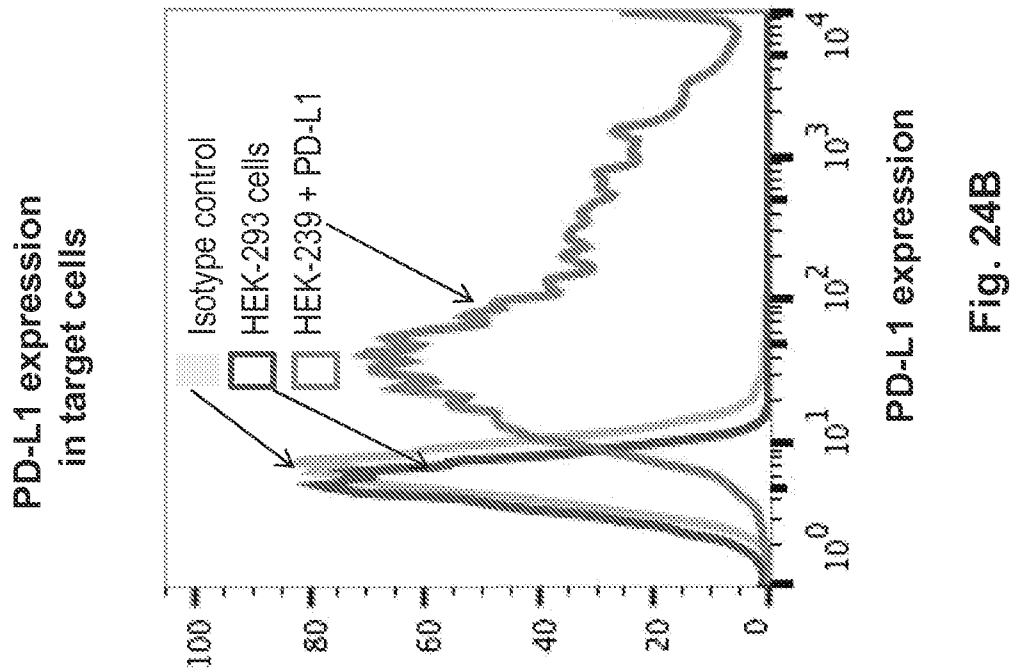


Fig. 24B

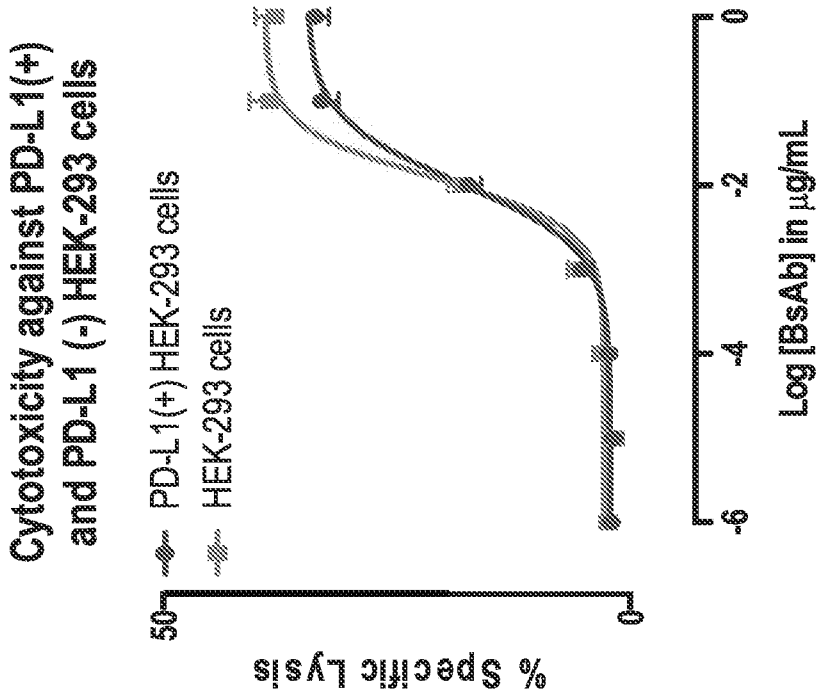


Fig. 24A

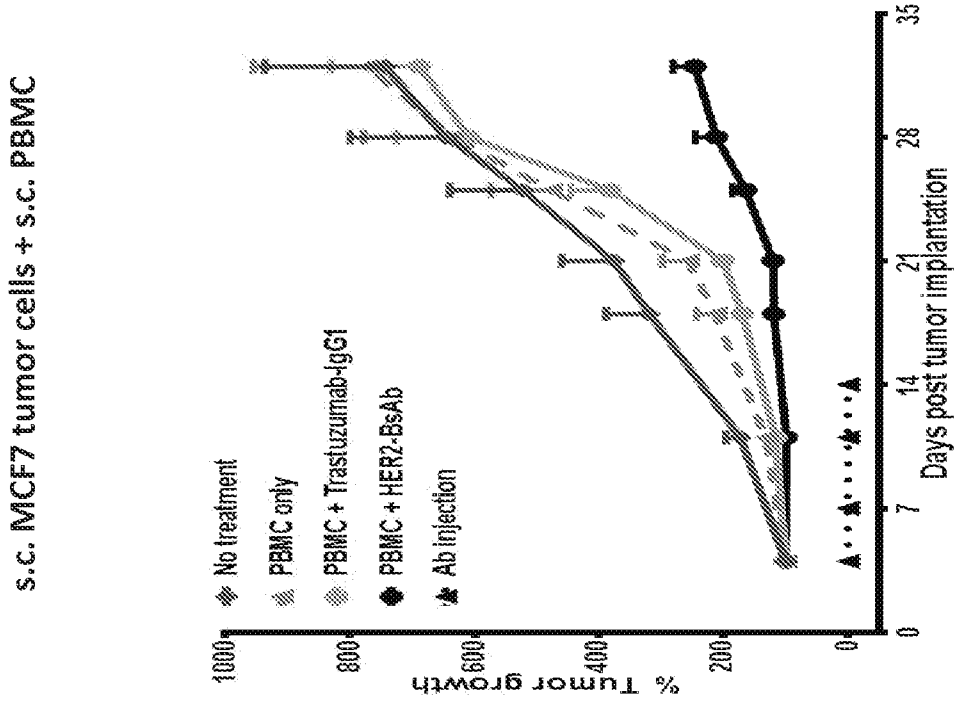


Fig. 25A

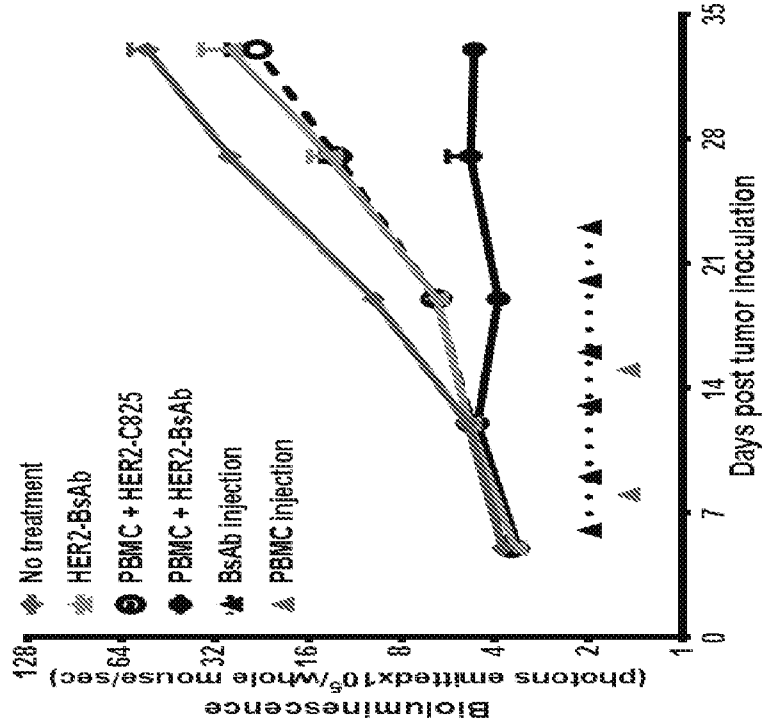


Fig. 25B

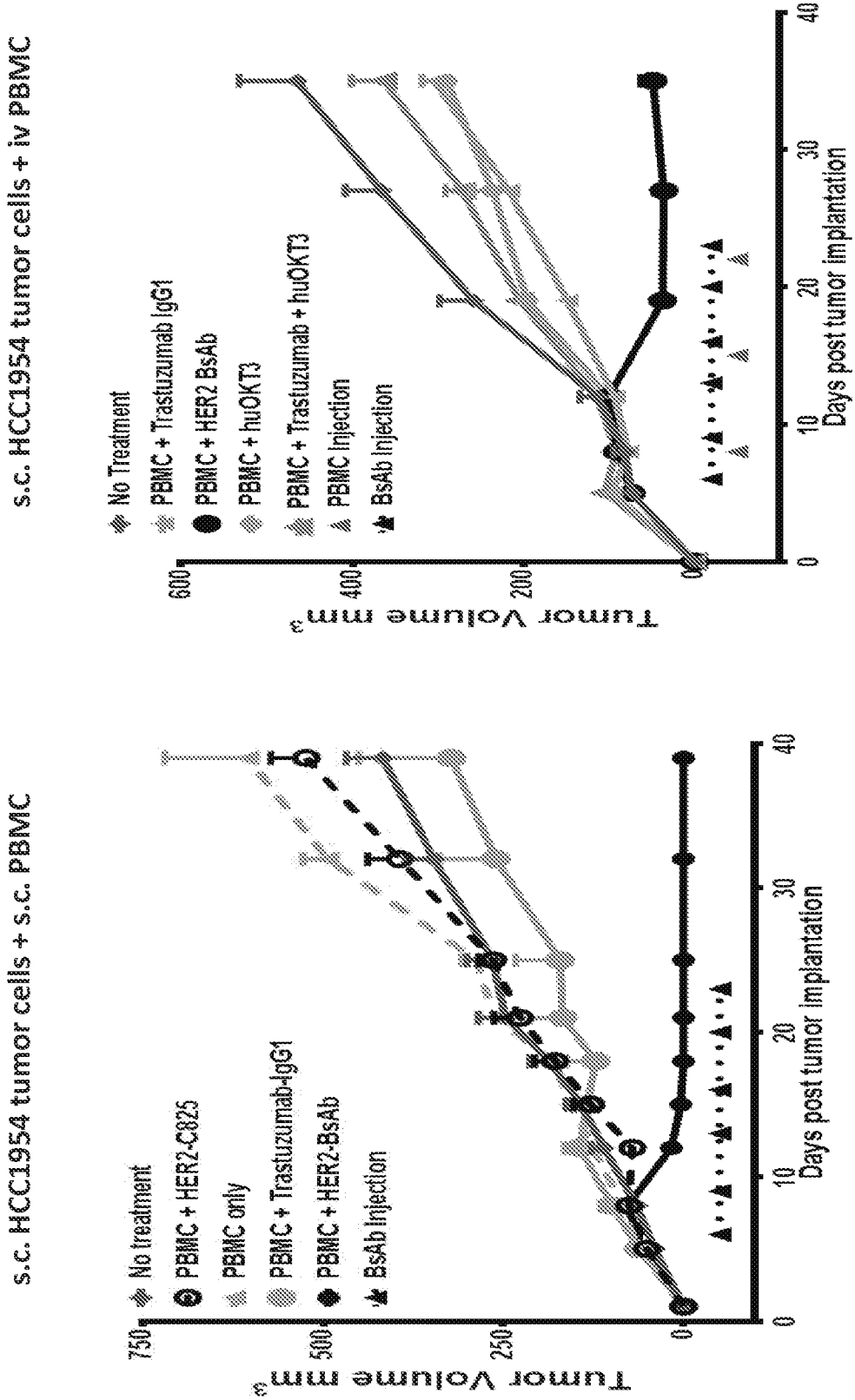


Fig. 25C

Fig. 25D

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Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
                85                  90                  95

Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
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Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
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Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
    130                 135                 140

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

Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
            180                 185                 190

His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
        195                 200                 205

Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
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Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
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Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
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His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
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Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
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Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
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Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
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Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
            340                 345                 350

Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
        355                 360                 365

Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
    370                 375                 380

Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
385                 390                 395                 400

Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
                405                 410                 415

Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
            420                 425                 430

Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
        435                 440                 445

Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
    450                 455                 460

Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
465                 470                 475                 480

Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
                485                 490                 495

Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
            500                 505                 510

Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
        515                 520                 525

Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
    530                 535                 540

Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
545                 550                 555                 560

Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
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Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
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Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
    610                 615                 620

Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
625                 630                 635                 640

Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser
                645                 650                 655

Ala Val Val Gly Ile Leu Leu Val Val Val Leu Gly Val Val Phe Gly
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Ile Leu Ile Lys Arg Arg Gln Gln Lys Ile Arg Lys Tyr Thr Met Arg
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Arg Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly
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Ala Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu
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Arg Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys
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Gly Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile
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Lys Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu
        755                 760                 765

Asp Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg
    770                 775                 780

Leu Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu
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Met Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu Asn Arg Gly Arg
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Leu Gly Ser Gln Asp Leu Leu Asn Trp Cys Met Gln Ile Ala Lys Gly
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Met Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala
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Arg Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe
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Gly Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp
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Gly Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Leu Arg
                885                 890                 895

Arg Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val
            900                 905                 910

Trp Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala
        915                 920                 925

Arg Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro
    930                 935                 940

Pro Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met
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Ile Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ser Glu Phe
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Ser Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu
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Asp Leu Gly Pro Ala Ser Pro Leu  Asp Ser Thr Phe Tyr  Arg Ser Leu
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Leu Glu  Asp Asp Asp Met Gly  Asp Leu Val Asp Ala  Glu Glu Tyr
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Leu Val  Pro Gln Gln Gly Phe  Phe Cys Pro Asp Pro  Ala Pro Gly
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Ala Gly  Gly Met Val His His  Arg His Arg Ser Ser  Ser Thr Arg
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Ser Gly  Gly Gly Asp Leu Thr  Leu Gly Leu Glu Pro  Ser Glu Glu
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Glu Ala  Pro Arg Ser Pro Leu  Ala Pro Ser Glu Gly  Ala Gly Ser
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Asp Val  Phe Asp Gly Asp Leu  Gly Met Gly Ala Ala  Lys Gly Leu
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Gln Ser  Leu Pro Thr His Asp  Pro Ser Pro Leu Gln  Arg Tyr Ser
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Glu Asp Pro Thr Val Pro Leu Pro Ser Glu Thr Asp Gly Tyr Val
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Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu Arg Pro Lys Thr Leu
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Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val Phe Ala Phe Gly
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Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln Gly Gly Ala
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Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala Phe Asp
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Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala Pro
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Gln Gly Tyr Val Leu Ile Ala His Ser Gln Val Arg Gln Ile Pro Leu
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Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
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Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Glu Gly Gly Ile Pro
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Ala Pro Gly Ala Ala Gln Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
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Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Ser Pro Gln
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Leu Cys His Gln Asp Thr Ile Leu Trp Lys Asp Val Phe His Lys Asn
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Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Phe Ser Ala Cys
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Pro Pro Cys Ser Pro Ala Cys Lys Asp Ala His Cys Trp Gly Ala Ser
        195                 200                 205

Ser Gly Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
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Ala Arg Cys Lys Gly Pro Gln Pro Thr Asp Cys Cys His Glu Gln Cys
225                 230                 235                 240

Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
                245                 250                 255

His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
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Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
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Tyr Thr Phe Gly Ala Ser Cys Val Thr Ser Cys Pro Tyr Asn Tyr Leu
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Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu Asn Asn Gln
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Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
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Glu Ala Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
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Gly Arg Val Leu His Asp Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
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Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
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Leu Ala Leu Ile His Arg Asn Ala Arg Leu Cys Phe Val His Thr Val
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Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Ser
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Ala Asn Arg Pro Glu Glu Glu Cys Val Gly Glu Gly Leu Ala Cys Tyr
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Pro Cys Ala His Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys Val
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Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys Arg
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Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Lys Asp Arg Tyr Cys Leu
545                 550                 555                 560

Pro Cys His Ser Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys Phe
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Gly Ser Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp Pro
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Cys Pro Ile Asn Cys Thr His Ser Cys Ala Asp Leu Asp Glu Lys Gly
625                 630                 635                 640

Cys Pro Ala Glu Gln Arg Ala Ser Pro Val Thr Ser Ile Ile Ala Ala
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Val Val Gly Ile Leu Leu Ala Val Val Val Gly Leu Val Leu Gly Ile
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Leu Ile Lys Arg Arg Arg Gln Lys Ile Arg Lys Tyr Thr Met Arg Arg
        675                 680                 685

Leu Leu Gln Glu Thr Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Ala
    690                 695                 700

Met Pro Asn Gln Ala Gln Met Arg Ile Leu Lys Glu Thr Glu Leu Arg
705                 710                 715                 720

Lys Val Lys Val Leu Gly Ser Gly Ala Phe Gly Thr Val Tyr Lys Gly
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Ile Trp Ile Pro Asp Gly Glu Asn Val Lys Ile Pro Val Ala Ile Lys
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Val Leu Arg Glu Asn Thr Ser Pro Lys Ala Asn Lys Glu Ile Leu Asp
        755                 760                 765

Glu Ala Tyr Val Met Ala Gly Val Gly Ser Pro Tyr Val Ser Arg Leu
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Leu Gly Ile Cys Leu Thr Ser Thr Val Gln Leu Val Thr Gln Leu Met
785                 790                 795                 800

Pro Tyr Gly Cys Leu Leu Asp His Val Arg Glu His Arg Gly Arg Leu
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Gly Ser Gln Asp Leu Leu Asn Trp Cys Val Gln Ile Ala Lys Gly Met
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Ser Tyr Leu Glu Asp Val Arg Leu Val His Arg Asp Leu Ala Ala Arg
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Asn Val Leu Val Lys Ser Pro Asn His Val Lys Ile Thr Asp Phe Gly
    850                 855                 860
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Leu Ala Arg Leu Leu Asp Ile Asp Glu Thr Glu Tyr His Ala Asp Gly
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Gly Lys Val Pro Ile Lys Trp Met Ala Leu Glu Ser Ile Pro Pro Arg
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Arg Phe Thr His Gln Ser Asp Val Trp Ser Tyr Gly Val Thr Val Trp
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Glu Leu Met Thr Phe Gly Ala Lys Pro Tyr Asp Gly Ile Pro Ala Arg
        915                 920                 925

Glu Ile Pro Asp Leu Leu Glu Lys Gly Glu Arg Leu Pro Gln Pro Pro
    930                 935                 940

Ile Cys Thr Ile Asp Val Tyr Met Ile Met Val Lys Cys Trp Met Ile
945                 950                 955                 960

Asp Ser Glu Cys Arg Pro Arg Phe Arg Glu Leu Val Ala Glu Phe Ser
                965                 970                 975

Arg Met Ala Arg Asp Pro Gln Arg Phe Val Val Ile Gln Asn Glu Asp
            980                 985                 990

Leu Gly Pro Ala Ser Pro Leu Asp  Ser Thr Phe Tyr Arg  Ser Leu Leu
        995                 1000                1005

Glu Asp  Asp Asp Met Gly Asp  Leu Val Asp Ala Glu  Glu Tyr Leu
    1010                1015                1020

Val Pro  Gln Gln Gly Phe Phe  Cys Pro Glu Pro Thr  Pro Gly Ala
    1025                1030                1035

Gly Gly  Thr Ala His Arg Arg  His Arg Ser Ser Ser  Thr Arg Asn
    1040                1045                1050

Gly Gly  Gly Glu Leu Thr Leu  Gly Leu Glu Pro Ser  Glu Glu Glu
    1055                1060                1065

Pro Pro  Lys Ser Pro Leu Ala  Pro Ser Glu Gly Ala  Gly Ser Asp
    1070                1075                1080

Val Phe  Asp Gly Asp Leu Gly  Met Gly Ala Ala Lys  Gly Leu Gln
    1085                1090                1095

Ser Leu  Pro Ser Gln Asp Pro  Ser Pro Leu Gln Arg  Tyr Ser Glu
    1100                1105                1110

Asp Pro  Thr Val Pro Leu Pro  Pro Glu Thr Asp Gly  Lys Val Ala
    1115                1120                1125
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Pro Leu Thr Cys Ser Pro Gln Pro Glu Tyr Val Asn Gln Pro Glu
1130 1135 1140

Val Trp Pro Gln Pro Pro Leu Ala Leu Glu Gly Pro Leu Pro Pro
1145 1150 1155

Ser Arg Pro Ala Gly Ala Thr Leu Glu Arg Pro Lys Thr Leu Ser
1160 1165 1170

Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
1175 1180 1185

Phe Ala Phe Gly Ser Ala Val Glu Asn Pro Glu Tyr Leu Ala Pro
1190 1195 1200

Arg Gly Arg Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser
1205 1210 1215

Pro Ala Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Ser Glu
1220 1225 1230

Arg Gly Ser Pro Pro Ser Thr Phe Glu Gly Thr Pro Thr Ala Glu
1235 1240 1245

Asn Pro Glu Tyr Leu Gly Leu Asp Val Pro Val
1250 1255

<210> 5
<211> 1311
<212> DNA
<213> Homo sapiens

<300>
<308> NM_000073.2
<309> 2014-05-03
<313> (1)..(1311)

<400> 5
agtctagctg ctgcacaggc tggctggctg gctggctgct aagggctgct ccacgctttt 60

gccggaggac agagactgac atggaacagg ggaagggcct ggctgtcctc atcctggcta 120

tcattcttct tcaaggtact ttggcccagt caatcaaagg aaaccacttg gttaaggtgt 180

atgactatca agaagatggt tcggtacttc tgacttgtga tgcagaagcc aaaaatatca 240

catggtttaa agatgggaag atgatcggct tcctaactga agataaaaaa aaatggaatc 300

tgggaagtaa tgccaaggac cctcgaggga tgtatcagtg taaaggatca cagaacaagt 360

caaaaccact ccaagtgtat tacagaatgt gtcagaactg cattgaacta aatgcagcca 420

ccatatctgg ctttctcttt gctgaaatcg tcagcatttt cgtccttgct gttggggtct 480

acttcattgc tggacaggat ggagttcgcc agtcgagagc ttcagacaag cagactctgt 540

tgcccaatga ccagctctac cagcccctca aggatcgaga agatgaccag tacagccacc 600

ttcaaggaaa ccagttgagg aggaattgaa ctcaggactc agagtagtcc aggtgttctc 660

ctcctattca gttcccagaa tcaaagcaat gcattttgga aagctcctag cagagagact 720

ttcagcccta aatctagact caaggttccc agagatgaca aatggagaag aaaggccatc 780

agagcaaatt tgggggtttc tcaaataaaa taaaaataaa aacaaatact gtgtttcaga 840

agcgccacct attggggaaa attgtaaaag aaaaatgaaa agatcaaata acccccctgga 900

tttgaatata atttttgtg ttgtaatttt tatttcgttt ttgtataggt tataattcac 960

atggctcaaa tattcagtga aagctctccc tccaccgcca tcccctgcta cccagtgacc 1020

ctgttgccct cttcagagac aaattagttt ctctttttt ttttttttt ttttttttg 1080

agacagtctg gctctgtcac ccaggctgaa atgcagtggc accatctcgg ctcactgcaa 1140

cctctgcctc ctgggttcaa gcgattctcc tgcctcagcc tcccgggcag ctgggattac 1200

aggcacacac taccacacct ggctaatttt tgtatttta gtagagacag ggttttgctc 1260

tgttggccaa gctggtctcg aactcctgac ctcaagtgat ccgcccgcct c 1311

<210> 6
<211> 182
<212> PRT
<213> Homo sapiens

<300>
<308> NP_000064.1
<309> 2014-05-03
<313> (1)..(182)

<400> 6

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

Leu Gln Gly Thr Leu Ala Gln Ser Ile Lys Gly Asn His Leu Val Lys
20 25 30

Val Tyr Asp Tyr Gln Glu Asp Gly Ser Val Leu Leu Thr Cys Asp Ala
35 40 45

Glu Ala Lys Asn Ile Thr Trp Phe Lys Asp Gly Lys Met Ile Gly Phe
50 55 60

Leu Thr Glu Asp Lys Lys Lys Trp Asn Leu Gly Ser Asn Ala Lys Asp
65 70 75 80

Pro Arg Gly Met Tyr Gln Cys Lys Gly Ser Gln Asn Lys Ser Lys Pro
85 90 95

Leu Gln Val Tyr Tyr Arg Met Cys Gln Asn Cys Ile Glu Leu Asn Ala
100 105 110

Ala Thr Ile Ser Gly Phe Leu Phe Ala Glu Ile Val Ser Ile Phe Val
115 120 125

Leu Ala Val Gly Val Tyr Phe Ile Ala Gly Gln Asp Gly Val Arg Gln
130 135 140

Ser Arg Ala Ser Asp Lys Gln Thr Leu Leu Pro Asn Asp Gln Leu Tyr
145 150 155 160

Gln Pro Leu Lys Asp Arg Glu Asp Asp Gln Tyr Ser His Leu Gln Gly
165 170 175

Asn Gln Leu Arg Arg Asn
180

<210> 7
<211> 771
<212> DNA
<213> Homo sapiens

<300>
<308> NM_000732.4
<309> 2014-05-03
<313> (1)..(771)

<400> 7
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gctaggggag ggcagctctc acccaggctg atagttcggt gacctggctt tatctactgg 120

atgagttccg ctgggagatg gaacatagca cgtttctctc tggcctggta ctggctaccc 180

ttctctcgca agtgagcccc ttcaagatac ctatagagga acttgaggac agagtgtttg 240

tgaattgcaa taccagcatc acatgggtag agggaacggt gggaacactg ctctcagaca 300

ttacaagact ggacctggga aaacgcatcc tggacccacg aggaatatat aggtgtaatg 360

ggacagatat atacaaggac aaagaatcta ccgtgcaagt tcattatcga atgtgccaga 420

gctgtgtgga gctggatcca gccaccgtgg ctggcatcat tgtcactgat gtcattgcca 480

ctctgctcct tgctttggga gtcttctgct ttgctggaca tgagactgga aggctgtctg 540

gggctgccga cacacaagct ctgttgagga atgaccaggt ctatcagccc ctccgagatc 600

gagatgatgc tcagtacagc caccttggag gaaactgggc tcggaacaag tgaacctgag 660

actggtggct tctagaagca gccattacca actgtacctt cccttcttgc tcagccaata 720

aatatatcct ctttcactca gaaaaaaaaa aaaaaaaaaa aaaaaaaaaa a 771

<210> 8
<211> 171
<212> PRT
<213> Homo sapiens

<300>
<308> NP_000723.1
<309> 2014-05-03
<313> (1)..(171)

<400> 8

Met Glu His Ser Thr Phe Leu Ser Gly Leu Val Leu Ala Thr Leu Leu
1 5 10 15

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Ser Gln Val Ser Pro Phe Lys Ile Pro Ile Glu Glu Leu Glu Asp Arg
            20                  25                  30

Val Phe Val Asn Cys Asn Thr Ser Ile Thr Trp Val Glu Gly Thr Val
        35                  40                  45

Gly Thr Leu Leu Ser Asp Ile Thr Arg Leu Asp Leu Gly Lys Arg Ile
    50                  55                  60

Leu Asp Pro Arg Gly Ile Tyr Arg Cys Asn Gly Thr Asp Ile Tyr Lys
65                  70                  75                  80

Asp Lys Glu Ser Thr Val Gln Val His Tyr Arg Met Cys Gln Ser Cys
                85                  90                  95

Val Glu Leu Asp Pro Ala Thr Val Ala Gly Ile Ile Val Thr Asp Val
            100                 105                 110

Ile Ala Thr Leu Leu Leu Ala Leu Gly Val Phe Cys Phe Ala Gly His
        115                 120                 125

Glu Thr Gly Arg Leu Ser Gly Ala Ala Asp Thr Gln Ala Leu Leu Arg
    130                 135                 140

Asn Asp Gln Val Tyr Gln Pro Leu Arg Asp Arg Asp Asp Ala Gln Tyr
145                 150                 155                 160

Ser His Leu Gly Gly Asn Trp Ala Arg Asn Lys
                165                 170
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<210> 9
<211> 1534
<212> DNA
<213> Homo sapiens

<300>
<308> NM_000733.3
<309> 2014-05-03
<313> (1)..(1534)

<400> 9

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ccagtccagg tggaggcctc tgccttgaac gtttccaagt gaggtaaaac ccgcaggccc      120

agaggcctct ctacttcctg tgtggggttc agaaaccctc ctcccctccc agcctcaggt      180

gcctgcttca gaaaatgaag tagtaagtct gctggcctcc gccatcttag taaagtaaca      240

gtcccatgaa acaaagatgc agtcgggcac tcactggaga gttctgggcc tctgcctctt      300

atcagttggc gtttgggggc aagatggtaa tgaagaaatg ggtggtatta cacagacacc      360

atataaagtc tccatctctg gaaccacagt aatattgaca tgccctcagt atcctggatc      420

tgaaatacta tggcaacaca atgataaaaa cataggcggt gatgaggatg ataaaaacat      480
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aggcagtgat gaggatcacc tgtcactgaa ggaattttca gaattggagc aaagtggtta 540

ttatgtctgc taccccagag gaagcaaacc agaagatgcg aacttttatc tctacctgag 600

ggcaagagtg tgtgagaact gcatggagat ggatgtgatg tcggtggcca caattgtcat 660

agtggacatc tgcatcactg ggggcttgct gctgctggtt tactactgga gcaagaatag 720

aaaggccaag gccaagcctg tgacacgagg agcgggtgct ggcggcaggc aaaggggaca 780

aaacaaggag aggccaccac ctgttcccaa cccagactat gagcccatcc ggaaaggcca 840

gcgggacctg tattctggcc tgaatcagag acgcatctga ccctctggag aacactgcct 900

cccgctggcc caggtctcct ctccagtccc cctgcgactc cctgtttcct gggctagtct 960

tggaccccac gagagagaat cgttcctcag cctcatggtg aactcgcgcc ctccagcctg 1020

atcccccgct ccctcctccc tgccttctct gctggtaccc agtcctaaaa tattgctgct 1080

tcctcttcct ttgaagcatc atcagtagtc acaccctcac agctggcctg ccctcttgcc 1140

aggatattta tttgtgctat tcactccctt ccctttggat gtaacttctc cgttcagttc 1200

cctccttttc ttgcatgtaa gttgtccccc atcccaaagt attccatcta cttttctatc 1260

gccgtcccct tttgcagccc tctctgggga tggactgggt aaatgttgac agaggccctg 1320

ccccgttcac agatcctggc cctgagccag ccctgtgctc ctccctcccc caacactccc 1380

taccaacccc ctaatcccct actccctcca ccccccctcc actgtaggcc actggatggt 1440

catttgcatc tccgtaaatg tgctctgctc ctcagctgag agagaaaaaa ataaactgta 1500

tttggctgca agaaaaaaaa aaaaaaaaaa aaaa 1534

<210> 10
<211> 207
<212> PRT
<213> Homo sapiens

<300>
<308> NP_000724.1
<309> 2014-05-03
<313> (1)..(207)

<400> 10

Met Gln Ser Gly Thr His Trp Arg Val Leu Gly Leu Cys Leu Leu Ser
1 5 10 15

Val Gly Val Trp Gly Gln Asp Gly Asn Glu Glu Met Gly Gly Ile Thr
20 25 30

Gln Thr Pro Tyr Lys Val Ser Ile Ser Gly Thr Thr Val Ile Leu Thr
35 40 45

Cys Pro Gln Tyr Pro Gly Ser Glu Ile Leu Trp Gln His Asn Asp Lys
50 55 60

Asn Ile Gly Gly Asp Glu Asp Asp Lys Asn Ile Gly Ser Asp Glu Asp
65 70 75 80

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His Leu Ser Leu Lys Glu Phe Ser Glu Leu Glu Gln Ser Gly Tyr Tyr
                85                  90                  95

Val Cys Tyr Pro Arg Gly Ser Lys Pro Glu Asp Ala Asn Phe Tyr Leu
            100                 105                 110

Tyr Leu Arg Ala Arg Val Cys Glu Asn Cys Met Glu Met Asp Val Met
        115                 120                 125

Ser Val Ala Thr Ile Val Ile Val Asp Ile Cys Ile Thr Gly Gly Leu
    130                 135                 140

Leu Leu Leu Val Tyr Tyr Trp Ser Lys Asn Arg Lys Ala Lys Ala Lys
145                 150                 155                 160

Pro Val Thr Arg Gly Ala Gly Ala Gly Gly Arg Gln Arg Gly Gln Asn
                165                 170                 175

Lys Glu Arg Pro Pro Pro Val Pro Asn Pro Asp Tyr Glu Pro Ile Arg
            180                 185                 190

Lys Gly Gln Arg Asp Leu Tyr Ser Gly Leu Asn Gln Arg Arg Ile
        195                 200                 205
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<210> 11
<211> 1436
<212> DNA
<213> Canine

<300>
<308> NM_001003379.1
<309> 2014-02-22
<313> (1)..(1436)

<400> 11

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aaagacagta taatacagca gctcacacag actttcggat tcagaaaaac agttgtgtcg      120

ggccttgggt aaattatgta aggcaagcct cagtttgctc agcggtaaaa cgaggaaagt      180

aataagccac ccgcctccgc cattttgggt agaataaggg tgcatccagt gagagaagga      240

tgcagtcgag gaacctctgg agaattctgg gactctgtct cttatcagtt ggtgcttggg      300

ggcaggacga ggatttcaaa gcttctgatg acttgacaag tatatctcca gagaaacggt      360

ttaaggtctc catctctgga accgaggtag tggtgacatg ccctgatgtt tttggatatg      420

ataatataaa atgggaaaaa aatgataacc ttgtggaagg tgctagtaac agagagctat      480

ctcagaagga gttttcagaa gtggacgaca gtggttatta tgcctgctat gcagattcca      540

taaaggagaa gagctatctc tacctgagag caagagtgtg tgcaaactgc atagaggtga      600

atctgatggc agtggtcaca atcattgtag ctgacatctg ccttactctg gggttgctgc      660

tgatggtgta ttactggagc aagactagaa aggccaatgc caagcctgtg atgagaggaa      720
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caggtgccgg cagcaggccc aggggacaaa acaaggagaa gccaccacct gttcccaatc 780

cagactacga gcccatccgg aaaggccagc aggacctgta ttctggcctg aatcagagag 840

gcatctgacg gctcctgagg acacggcctc ccagggccc aggtcttggt gtctccaggt 900

cctgctactc ccagtaccct gggtaaatct tgaaccccag aagagaatta ttcctctgcc 960

ttctggagaa ctaactccca gcctgcagcc ttatccccag caccctccaa cccgccttct 1020

ctgctggcac ttggtcctgc aatatcacct cctcatcatg gccactcacc gccccccac 1080

cagccagact gccctctggt cggggtattt atttctgtta ccctgacgcc cccaccatca 1140

ccaattcctt cctacccttc agaggtatcc ttgctccctt ccgtacccct ctcccggaca 1200

gaacctgccc ccatccctta ctatcccacc tacctttccg tttttccagc tctctctttg 1260

gtgaccctct gtggggatgg actaggtaac tctggtagag gtcctgcccc atccatgacc 1320

ttggcccaga gccaccctct gccagcaggc ccctggatga tcatttgcat tcttacaaat 1380

gtgctaggct ccttgacagc tagagagaaa ataataaagt gtatttggtt gaaaaa 1436

<210> 12
<211> 202
<212> PRT
<213> Canine

<300>
<308> NP_001003379.1
<309> 2014-02-22
<313> (1)..(202)

<400> 12

Met Gln Ser Arg Asn Leu Trp Arg Ile Leu Gly Leu Cys Leu Leu Ser
1 5 10 15

Val Gly Ala Trp Gly Gln Asp Glu Asp Phe Lys Ala Ser Asp Asp Leu
20 25 30

Thr Ser Ile Ser Pro Glu Lys Arg Phe Lys Val Ser Ile Ser Gly Thr
35 40 45

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

Trp Glu Lys Asn Asp Asn Leu Val Glu Gly Ala Ser Asn Arg Glu Leu
65 70 75 80

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

Tyr Ala Asp Ser Ile Lys Glu Lys Ser Tyr Leu Tyr Leu Arg Ala Arg
100 105 110

Val Cys Ala Asn Cys Ile Glu Val Asn Leu Met Ala Val Val Thr Ile
115 120 125

Ile Val Ala Asp Ile Cys Leu Thr Leu Gly Leu Leu Leu Met Val Tyr
130 135 140

Tyr Trp Ser Lys Thr Arg Lys Ala Asn Ala Lys Pro Val Met Arg Gly
145 150 155 160

Thr Gly Ala Gly Ser Arg Pro Arg Gly Gln Asn Lys Glu Lys Pro Pro
165 170 175

Pro Val Pro Asn Pro Asp Tyr Glu Pro Ile Arg Lys Gly Gln Gln Asp
180 185 190

Leu Tyr Ser Gly Leu Asn Gln Arg Gly Ile
195 200

<210> 13
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Peptide linker (G4S)3

<400> 13
ggcggcggag gatctggcgg aggtggaagt gggggaggcg gatct 45

<210> 14
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Peptide linker (G4S)3

<400> 14

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

<210> 15
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> OKT3 VH

<400> 15

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser
115

<210> 16
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> OKT3 VL

<400> 16

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

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
100 105

<210> 17
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> OKT3 VH C105S

<400> 17

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser
115

<210> 18
<211> 723
<212> DNA
<213> Artificial Sequence

<220>
<223> OKT3 scFv C105S

<400> 18
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tcttgcaagg ccagcggcta caccttcacc cggtacacca tgcactgggt gcgacaggcc 120

cctggcaagg gcctggaatg gatcggctac atcaacccct cccggggcta caccaactac 180

aaccagaagt tcaaggaccg gttcaccatc agccgggaca actccaagaa caccgccttt 240

ctgcagatgg actccctgcg gcctgaggat accggcgtgt actttgcgc ccggtactac 300

gacgaccact actctctgga ctactggggc cagggaaccc ctgtgacagt gtctagcgga 360

gggggaggtt caggtggcgg tggatcaggg ggcggaggct ctgatatcca gatgacccag 420

tccccctcca gcctgtctgc ctctgtggga gacagagtga caattacatg ctccgccagc 480

tccagcgtgt cctacatgaa ttggtatcag cagacccctg gcaaggctcc caagcggtgg 540

atctacgaca cctccaagct ggcctccggc gtgccctcca gattttctgg cagcggctcc 600

ggcacagact ataccttac aatcagctcc ctgcagcccg aagatatcgc cacctactac 660

tgccagcagt ggtcctccaa ccccttcacc tttggccagg ggacaaaact gcagatcacc 720

aga 723

<210> 19
<211> 241
<212> PRT
<213> Artificial Sequence

<220>
<223> OKT3 scFv C105S

<400> 19

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125

Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
    130                 135                 140

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser
145                 150                 155                 160

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala
                165                 170                 175

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
            180                 185                 190

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile
        195                 200                 205

Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp
    210                 215                 220
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Ser Ser Asn Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
225 230 235 240

Arg

<210> 20
<211> 1350
<212> DNA
<213> Artificial Sequence

<220>
<223> exemplary heavy chain N297A

<400> 20
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cctggcaagg gactggaatg ggtggccaga atctacccca ccaacggcta caccagatac 180

gccgactctg tgaagggccg gttcaccatc tccgccgaca cctccaagaa caccgcctac 240

ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgtag tagatgggga 300

ggcgacggct tctacgccat ggactattgg ggccagggca ccctcgtgac cgtgtcctct 360

gcttctacca agggcccctc tgtgtttcct ctggcccccc ccagcaagtc cacctctggt 420

ggaacagccg ccctgggctg cctcgtgaag gactactttc ccgagcccgt gaccgtgtcc 480

tggaactctg gcgctctgac ctctggcgtg cacaccttcc ctgctgtgct gcagtctagc 540

ggcctgtact ccctgtcctc cgtcgtgaca gtgccctcca gctctctggg cacccagacc 600

tacatctgca acgtgaacca caagccctcc aataccaagg tggacaagcg ggtggaaccc 660

aagtcctgcg acaagaccca cacctgtccc ccttgtcctg cccctgaact gctgggcgga 720

ccttccgtgt tcctgttccc cccaaagccc aaggacaccc tgatgatctc ccggacccct 780

gaagtgacct gcgtggtggt ggatgtgtcc cacgaggacc ctgaagtgaa gttcaattgg 840

tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ctagagagga acagtacgcc 900

tccacctacc gggtggtgtc cgtgctgaca gtgctgcacc aggactggct gaacggcaaa 960

gagtacaagt gcaaagtgtc caacaaggcc ctgcctgccc ccatcgaaaa gaccatctcc 1020

aaggccaagg gccagccccg ggaaccccag gtgtacacac tgcccccctag cagggacgag 1080

ctgaccaaga accaggtgtc cctgacctgt ctcgtgaaag gcttctaccc ctccgatatc 1140

gccgtggaat gggagtccaa cggccagcct gagaacaact acaagaccac ccccccctgtg 1200

ctggactccg acggctcatt cttcctgtac agcaagctga ccgtggacaa gtcccggtgg 1260

cagcagggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc 1320

cagaagtccc tgtccctgag ccccggcaaa 1350

<210> 21
<211> 1350
<212> DNA
<213> Artificial Sequence

<220>
<223> exemplary heavy chain K322A

<400> 21
gaggtgcagc tggtggaatc tggcggagga ctggtgcagc ctggcggctc tctgagactg 60

tcttgtgccg cctccggctt caacatcaag gacacctaca tccactgggt gcgacaggcc 120

cctggcaagg gactggaatg ggtggccaga atctacccca ccaacggcta caccagatac 180

gccgactctg tgaagggccg gttcaccatc tccgccgaca cctccaagaa caccgcctac 240

ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgtag tagatgggga 300

ggcgacggct tctacgccat ggactattgg ggccagggca ccctcgtgac cgtgtcctct 360

gcttctacca agggcccctc tgtgtttcct ctggcccccc ccagcaagtc cacctctggt 420

ggaacagccg ccctgggctg cctcgtgaag gactactttc ccgagcccgt gaccgtgtcc 480

tggaactctg gcgctctgac ctctggcgtg cacaccttcc ctgctgtgct gcagtctagc 540

ggcctgtact ccctgtcctc cgtcgtgaca gtgccctcca gctctctggg cacccagacc 600

tacatctgca acgtgaacca caagccctcc aataccaagg tggacaagcg ggtggaaccc 660

aagtcctgcg acaagaccca cacctgtccc ccttgtcctg cccctgaact gctgggcgga 720

ccttccgtgt tcctgttccc cccaaagccc aaggacaccc tgatgatctc ccggaccccc 780

gaagtgacct gcgtggtggt ggatgtgtcc cacgaggacc ctgaagtgaa gttcaattgg 840

tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ctagagagga acagtacaac 900

tccacctacc gggtggtgtc cgtgctgaca gtgctgcacc aggactggct gaacggcaaa 960

gagtacaagt gcgccgtgtc caacaaggcc ctgcctgccc ccatcgaaaa gaccatctcc 1020

aaggccaagg gccagccccg ggaaccccag gtgtacacac tgcccctag cagggacgag 1080

ctgaccaaga accaggtgtc cctgacctgt ctcgtgaaag gcttctaccc ctccgatatc 1140

gccgtggaat gggagtccaa cggccagcct gagaacaact acaagaccac ccccctgtg 1200

ctggactccg acggctcatt cttcctgtac agcaagctga ccgtggacaa gtcccggtgg 1260

cagcagggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc 1320

cagaagtccc tgtccctgag ccccggcaaa 1350

<210> 22
<211> 1350
<212> DNA
<213> Artificial Sequence

<220>
<223> exemplary heavy chain

<400> 22
gaggtgcagc tggtggaatc tggcggagga ctggtgcagc ctggcggctc tctgagactg 60

tcttgtgccg cctccggctt caacatcaag gacacctaca tccactgggt gcgacaggcc 120

cctggcaagg gactggaatg ggtggccaga atctacccca ccaacggcta caccagatac 180

gccgactctg tgaagggccg gttcaccatc tccgccgaca cctccaagaa caccgcctac 240

ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgtag tagatgggga 300

ggcgacggct tctacgccat ggactattgg ggccagggca ccctcgtgac cgtgtcctct 360

gcttctacca agggcccctc tgtgtttcct ctggcccccc ccagcaagtc cacctctggt 420

ggaacagccg ccctgggctg cctcgtgaag gactactttc ccgagcccgt gaccgtgtcc 480

tggaactctg gcgctctgac ctctggcgtg cacaccttcc ctgctgtgct gcagtctagc 540

ggcctgtact ccctgtcctc cgtcgtgaca gtgccctcca gctctctggg cacccagacc 600

tacatctgca acgtgaacca caagccctcc aataccaagg tggacaagcg ggtggaaccc 660

aagtcctgcg acaagaccca cacctgtccc ccttgtcctg cccctgaact gctgggcgga 720

ccttccgtgt tcctgttccc cccaaagccc aaggacaccc tgatgatctc ccggaccccc 780

gaagtgacct gcgtggtggt ggatgtgtcc cacgaggacc ctgaagtgaa gttcaattgg 840

tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ctagagagga acagtacaac 900

tccacctacc gggtggtgtc cgtgctgaca gtgctgcacc aggactggct gaacggcaaa 960

gagtacaagt gcaaagtgtc caacaaggcc ctgcctgccc ccatcgaaaa gaccatctcc 1020

aaggccaagg gccagccccg ggaaccccag gtgtacacac tgcccctag cagggacgag 1080

ctgaccaaga accaggtgtc cctgacctgt ctcgtgaaag gcttctaccc ctccgatatc 1140

gccgtggaat gggagtccaa cggccagcct gagaacaact acaagaccac ccccctgtg 1200

ctggactccg acggctcatt cttcctgtac agcaagctga ccgtggacaa gtcccggtgg 1260

cagcagggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc 1320

cagaagtccc tgtccctgag ccccggcaaa 1350

<210> 23
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> exemplary heavy chain

<400> 23

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30

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

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

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

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

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

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

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

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350
```

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

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

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450

<210> 24
<211> 642
<212> DNA
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain

<400> 24
gacatccaga tgacccagag cccttccagc ctgtccgcct ctgtgggcga cagagtgacc 60

atcacctgtc gggcctccca ggacgtgaac accgccgtgg cttggtatca gcagaagccc 120

ggcaaggccc ccaagctgct gatctactcc gcctccttcc tgtactccgg cgtgccctcc 180

agattctccg gcagcagatc tggcaccgac tttaccctga ccatctccag cctgcagccc 240

gaggacttcg ccacctacta ctgccagcag cactacacca ccccccccac ctttggccag 300

ggcaccaagg tggaaatcaa gcggaccgtg gccgctccct ccgtgttcat cttcccacct 360

tccgacgagc agctgaagtc cggcaccgct tctgtcgtgt gcctgctgaa caacttctac 420

ccccgcgagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag 480

gaatccgtga ccgagcagga ctccaaggac agcacctact ccctgtcctc caccctgacc 540

ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaagtgac ccaccagggc 600

ctgtctagcc ccgtgaccaa gtctttcaac cggggcgagt gc 642

<210> 25
<211> 216
<212> PRT
<213> Artificial Sequence

<220>

<223> HER2-BsAb light chain

<400> 25

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser
    210                 215
```

<210> 26
<211> 1350
<212> DNA
<213> Artificial Sequence

<220>
<223> HER2-BsAb heavy chain

<400> 26
gaggtgcagc tggtggaatc tggcggagga ctggtgcagc ctggcggctc tctgagactg 60

tcttgtgccg cctccggctt caacatcaag gacacctaca tccactgggt gcgacaggcc 120

cctggcaagg gactggaatg ggtggccaga atctacccca ccaacggcta caccagatac 180

gccgactctg tgaagggccg gttcaccatc tccgccgaca cctccaagaa caccgcctac 240

ctgcagatga actccctgcg ggccgaggac accgccgtgt actactgtag tagatgggga 300

ggcgacggct tctacgccat ggactattgg ggccagggca ccctcgtgac cgtgtcctct 360

gcttctacca agggcccctc tgtgtttcct ctggcccccc ccagcaagtc cacctctggt 420

ggaacagccg ccctgggctg cctcgtgaag gactactttc ccgagcccgt gaccgtgtcc 480

tggaactctg gcgctctgac ctctggcgtg cacaccttcc ctgctgtgct gcagtctagc 540

ggcctgtact ccctgtcctc cgtcgtgaca gtgccctcca gctctctggg cacccagacc 600

tacatctgca acgtgaacca caagccctcc aataccaagg tggacaagcg ggtggaaccc 660

aagtcctgcg acaagaccca cacctgtccc ccttgtcctg cccctgaact gctgggcgga 720

ccttccgtgt tcctgttccc cccaaagccc aaggacaccc tgatgatctc ccggaccccc 780

gaagtgacct gcgtggtggt ggatgtgtcc cacgaggacc ctgaagtgaa gttcaattgg 840

tacgtggacg gcgtggaagt gcacaacgcc aagaccaagc ctagagagga acagtacgcc 900

tccacctacc gggtggtgtc cgtgctgaca gtgctgcacc aggactggct gaacggcaaa 960

gagtacaagt gcgccgtgtc caacaaggcc ctgcctgccc ccatcgaaaa gaccatctcc 1020

aaggccaagg gccagccccg ggaaccccag gtgtacacac tgcccccctag cagggacgag 1080

ctgaccaaga accaggtgtc cctgacctgt ctcgtgaaag gcttctaccc ctccgatatc 1140

gccgtggaat gggagtccaa cggccagcct gagaacaact acaagaccac ccccccctgtg 1200

ctggactccg acggctcatt cttcctgtac agcaagctga ccgtggacaa gtcccggtgg 1260

cagcagggca acgtgttctc ctgctccgtg atgcacgagg ccctgcacaa ccactacacc 1320

cagaagtccc tgtccctgag ccccggcaaa 1350

<210> 27
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb heavy chain

<400> 27

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30

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

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

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

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

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

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

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

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320
```

```
Glu Tyr Lys Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

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

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Lys
    450
```

<210> 28
<211> 1416
<212> DNA
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide

<400> 28

```
gacatccaga tgacccagag cccttccagc ctgtccgcct ctgtgggcga cagagtgacc       60

atcacctgtc gggcctccca ggacgtgaac accgccgtgg cttggtatca gcagaagccc      120

ggcaaggccc ccaagctgct gatctactcc gcctccttcc tgtactccgg cgtgccctcc      180

agattctccg gcagcagatc tggcaccgac tttaccctga ccatctccag cctgcagccc      240

gaggacttcg ccacctacta ctgccagcag cactacacca ccccccccac ctttggccag      300

ggcaccaagg tggaaatcaa gcggaccgtg gccgctccct ccgtgttcat cttcccacct      360

tccgacgagc agctgaagtc cggcaccgct tctgtcgtgt gcctgctgaa caacttctac      420

ccccgcgagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag      480

gaatccgtga ccgagcagga ctccaaggac agcacctact ccctgtcctc caccctgacc      540

ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaagtgac ccaccagggc      600

ctgtctagcc ccgtgaccaa gtctttcaac cggggcgagt gcactagtgg cggcggagga      660
```

tctggcggag gtggaagtgg gggaggcgga tctcaggtgc agctggtgca gagtggtggc 720

ggagtggtgc agcctggcag atccctgaga ctgtcttgca aggccagcgg ctacaccttc 780

acccggtaca ccatgcactg ggtgcgacag gcccctggca agggcctgga atggatcggc 840

tacatcaacc cctcccgggg ctacaccaac tacaaccaga agttcaagga ccggttcacc 900

atcagccggg acaactccaa gaacaccgcc tttctgcaga tggactccct gcggcctgag 960

gataccggcg tgtacttttg cgcccggtac tacgacgacc actactctct ggactactgg 1020

ggccagggaa cccctgtgac agtgtctagc ggaggggggag gttcaggtgg cggtggatca 1080

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cagcagaccc ctggcaaggc tcccaagcgg tggatctacg acacctccaa gctggcctcc 1260

ggcgtgccct ccagattttc tggcagcggc tccggcacag actatacctt tacaatcagc 1320

tccctgcagc ccgaagatat cgccacctac tactgccagc agtggtcctc caacccttc 1380

acctttggcc aggggacaaa actgcagatc accaga 1416

<210> 29
<211> 472
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide

<400> 29

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

```
Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225                 230                 235                 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270

Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
        275                 280                 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
    290                 295                 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305                 310                 315                 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
                325                 330                 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
            340                 345                 350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln
        355                 360                 365

Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
    370                 375                 380

Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr
385                 390                 395                 400
```

```
Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser
                405                 410                 415

Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly
            420                 425                 430

Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala
        435                 440                 445

Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Gln
    450                 455                 460

Gly Thr Lys Leu Gln Ile Thr Arg
465                 470
```

<210> 30
<211> 462
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide (5aa scFv linker)

<400> 30

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140
```

```
Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225                 230                 235                 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270

Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
        275                 280                 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
    290                 295                 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305                 310                 315                 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
                325                 330                 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
            340                 345                 350

Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
        355                 360                 365

Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val
    370                 375                 380

Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg
385                 390                 395                 400

Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe
                405                 410                 415
```

```
Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu
            420                 425                 430

Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn
        435                 440                 445

Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
    450                 455                 460
```

<210> 31
<211> 467
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide (10aa scFv linker)

<400> 31

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
```

180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
210 215 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225 230 235 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
245 250 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
260 265 270

Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
275 280 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
290 295 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305 310 315 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
325 330 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
340 345 350

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
355 360 365

Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser
370 375 380

Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly
385 390 395 400

Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly
405 410 415

Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe
420 425 430

Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln
435 440 445

Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Gln

450 455 460

Ile Thr Arg
465

<210> 32
<211> 477
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide (20aa scFv linker)

<400> 32

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

```
Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225                 230                 235                 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270

Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
        275                 280                 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
    290                 295                 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305                 310                 315                 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
                325                 330                 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
            340                 345                 350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        355                 360                 365

Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
    370                 375                 380

Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser
385                 390                 395                 400

Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp
                405                 410                 415

Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
            420                 425                 430

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln
        435                 440                 445

Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro
    450                 455                 460

Phe Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
465                 470                 475
```

<210> 33
<211> 482
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide (25aa scFv linker)

<400> 33

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
```

225 230 235 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
245 250 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
260 265 270

Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
275 280 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
290 295 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305 310 315 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
325 330 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
340 345 350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
355 360 365

Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
370 375 380

Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala
385 390 395 400

Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys
405 410 415

Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val
420 425 430

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr
435 440 445

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
450 455 460

Trp Ser Ser Asn Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile
465 470 475 480

Thr Arg

<210> 34

<211> 487
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide (30aa scFv linker)

<400> 34

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225                 230                 235                 240
```

```
Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270

Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
        275                 280                 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
    290                 295                 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305                 310                 315                 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
                325                 330                 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
            340                 345                 350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        355                 360                 365

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met
    370                 375                 380

Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
385                 390                 395                 400

Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln
                405                 410                 415

Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys
            420                 425                 430

Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
        435                 440                 445

Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr
    450                 455                 460

Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Gln Gly
465                 470                 475                 480

Thr Lys Leu Gln Ile Thr Arg
                485
```

<210> 35
<211> 17
<212> DNA

<213> Artificial Sequence

<220>
<223> T(G4S)3 Linker

<400> 35
tsggggsggg gsggggs 17

<210> 36
<211> 5
<212> DNA
<213> Artificial Sequence

<220>
<223> G4S Linker

<400> 36
ggggs 5

<210> 37
<211> 10
<212> DNA
<213> Artificial Sequence

<220>
<223> (G4S)2 Linker

<400> 37
ggggsggggs 10

<210> 38
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> (G4S)3 Linker

<400> 38
ggggsggggs ggggs 15

<210> 39
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> (G4S)4 Linker

<400> 39
ggggsggggs ggggsggggs 20

<210> 40
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> (G4S)5 Linker

<400> 40
ggggsggggs ggggsggggs ggggs 25

<210> 41
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> (G4S)6 Linker

<400> 41
ggggsggggs ggggsggggs ggggsggggs 30

<210> 42
<211> 462
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide
(5aa scFv linker) + disulfide mut

<400> 42

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

```
Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225                 230                 235                 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270

Gly Lys Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
        275                 280                 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
    290                 295                 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305                 310                 315                 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
                325                 330                 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
            340                 345                 350

Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala
        355                 360                 365

Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val
    370                 375                 380

Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg
385                 390                 395                 400

Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe
                405                 410                 415

Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu
            420                 425                 430

Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn
        435                 440                 445
```

Pro Phe Thr Phe Gly Cys Gly Thr Lys Leu Gln Ile Thr Arg
450 455 460

<210> 43
<211> 467
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide (10aa scFv linker) + disulfide mut

<400> 43

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly

210 215 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225 230 235 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
245 250 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
260 265 270

Gly Lys Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
275 280 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
290 295 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305 310 315 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
325 330 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
340 345 350

Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
355 360 365

Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser
370 375 380

Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly
385 390 395 400

Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly
405 410 415

Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe
420 425 430

Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln
435 440 445

Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Cys Gly Thr Lys Leu Gln
450 455 460

Ile Thr Arg
465

<210> 44

<211> 472
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide (15aa scFv linker) + disulfide mut

<400> 44

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
210 215 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225 230 235 240

```
Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270

Gly Lys Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
        275                 280                 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
    290                 295                 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305                 310                 315                 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
                325                 330                 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
            340                 345                 350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln
        355                 360                 365

Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
    370                 375                 380

Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr
385                 390                 395                 400

Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser
                405                 410                 415

Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly
            420                 425                 430

Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala
        435                 440                 445

Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Cys
    450                 455                 460

Gly Thr Lys Leu Gln Ile Thr Arg
465                 470
```

<210> 45
<211> 477
<212> PRT
<213> Artificial Sequence

<220>

<223> HER2-BsAb light chain fusion polypeptide (20aa scFv linker) + disulfide mut

<400> 45

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225                 230                 235                 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255
```

```
Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270

Gly Lys Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
        275                 280                 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
    290                 295                 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305                 310                 315                 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
                325                 330                 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
            340                 345                 350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        355                 360                 365

Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser
    370                 375                 380

Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser
385                 390                 395                 400

Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp
                405                 410                 415

Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser
            420                 425                 430

Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln
        435                 440                 445

Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro
    450                 455                 460

Phe Thr Phe Gly Cys Gly Thr Lys Leu Gln Ile Thr Arg
465                 470                 475
```

<210> 46
<211> 483
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide (25aa scFv linker) + disulfide mut

<400> 46

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225                 230                 235                 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270
```

```
Gly Lys Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
        275                 280                 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
    290                 295                 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305                 310                 315                 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
                325                 330                 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
            340                 345                 350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
        355                 360                 365

Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser
    370                 375                 380

Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala
385                 390                 395                 400

Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys
                405                 410                 415

Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val
            420                 425                 430

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr
        435                 440                 445

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
    450                 455                 460

Trp Ser Ser Asn Pro Phe Thr Phe Gly Gln Cys Gly Thr Lys Leu Gln
465                 470                 475                 480

Ile Thr Arg
```

<210> 47
<211> 487
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-BsAb light chain fusion polypeptide (30aa scFv linker) + disulfide mut

<400> 47

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
```

```
1               5                   10                  15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225                 230                 235                 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270

Gly Lys Cys Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
```

275 280 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
290 295 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305 310 315 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Ser
325 330 335

Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
340 345 350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
355 360 365

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met
370 375 380

Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
385 390 395 400

Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln
405 410 415

Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys
420 425 430

Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
435 440 445

Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr
450 455 460

Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Cys Gly
465 470 475 480

Thr Lys Leu Gln Ile Thr Arg
485

<210> 48
<211> 231
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) (5 aa scFv linker)

<400> 48

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Asp Ile Gln Met
        115                 120                 125

Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
    130                 135                 140

Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln
145                 150                 155                 160

Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys
                165                 170                 175

Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
            180                 185                 190

Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr
        195                 200                 205

Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Gln Gly
    210                 215                 220

Thr Lys Leu Gln Ile Thr Arg
225                 230
```

<210> 49
<211> 236
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) (10 aa scFv linker)

<400> 49

```
Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
```

1 5 10 15

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
130 135 140

Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr
145 150 155 160

Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile
165 170 175

Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
180 185 190

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
195 200 205

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe
210 215 220

Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
225 230 235

<210> 50
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) (20 aa scFv linker)

<400> 50

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr
    130                 135                 140

Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145                 150                 155                 160

Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln
                165                 170                 175

Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
            180                 185                 190

Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
        195                 200                 205

Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr
    210                 215                 220

Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Gln Gly Thr
225                 230                 235                 240

Lys Leu Gln Ile Thr Arg
                245
```

<210> 51
<211> 251
<212> PRT

<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) (25 aa scFv linker)

<400> 51

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
130 135 140

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
145 150 155 160

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
165 170 175

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
180 185 190

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
195 200 205

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
210 215 220

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
225 230 235 240

Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg

245 250

<210> 52
<211> 256
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) (30 aa scFv linker)

<400> 52

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
145 150 155 160

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

Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro
180 185 190

Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser
195 200 205

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser
210 215 220

Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser
225 230 235 240

Ser Asn Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
245 250 255

<210> 53
<211> 231
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) + disulfide mut (5 aa scFv linker)

<400> 53

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Asp Ile Gln Met
115 120 125

Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr
130 135 140

Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln
145 150 155 160

Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys
165 170 175

Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr
180 185 190

Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr

195 200 205

Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Cys Gly
210 215 220

Thr Lys Leu Gln Ile Thr Arg
225 230

<210> 54
<211> 236
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) + disulfide mut (10 aa scFv linker)

<400> 54

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val
130 135 140

Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr
145 150 155 160

Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile
165 170 175

Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly
180 185 190

Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
195 200 205

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe
210 215 220

Thr Phe Gly Cys Gly Thr Lys Leu Gln Ile Thr Arg
225 230 235

<210> 55
<211> 241
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) + disulfide mut (15 aa scFv linker)

<400> 55

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
130 135 140

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser
145 150 155 160

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala
165 170 175

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro

180 185 190

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile
195 200 205

Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp
210 215 220

Ser Ser Asn Pro Phe Thr Phe Gly Cys Gly Thr Lys Leu Gln Ile Thr
225 230 235 240

Arg

<210> 56
<211> 246
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) + disulfide mut (20 aa scFv linker)

<400> 56

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr
130 135 140

Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
145 150 155 160

```
Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln
                165                 170                 175

Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu
            180                 185                 190

Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
        195                 200                 205

Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr
    210                 215                 220

Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Cys Gly Thr
225                 230                 235                 240

Lys Leu Gln Ile Thr Arg
                245
```

<210> 57
<211> 252
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) + disulfide mut (25 aa scFv linker)

<400> 57

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
                85                  90                  95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
            100                 105                 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
        115                 120                 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
```

130 135 140

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
145 150 155 160

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
165 170 175

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
180 185 190

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
195 200 205

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
210 215 220

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
225 230 235 240

Phe Gly Gln Cys Gly Thr Lys Leu Gln Ile Thr Arg
245 250

<210> 58
<211> 256
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv(C105S) + disulfide mut (30 aa scFv linker)

<400> 58

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
130 135 140

Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu
145 150 155 160

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

Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro
180 185 190

Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser
195 200 205

Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser
210 215 220

Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser
225 230 235 240

Ser Asn Pro Phe Thr Phe Gly Cys Gly Thr Lys Leu Gln Ile Thr Arg
245 250 255

<210> 59
<211> 241
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3scFv + disulfide mut (15 aa scFv linker)

<400> 59

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys

85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
130 135 140

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser
145 150 155 160

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala
165 170 175

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
180 185 190

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile
195 200 205

Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp
210 215 220

Ser Ser Asn Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
225 230 235 240

Arg

<210> 60
<211> 472
<212> PRT
<213> Artificial Sequence

<220>
<223> Her2-BsAb light chain fusion polypeptide

<400> 60

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

```
Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65                  70                  75                  80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly
    210                 215                 220

Gly Ser Gly Gly Gly Gly Ser Gln Val Gln Leu Val Gln Ser Gly Gly
225                 230                 235                 240

Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser
                245                 250                 255

Gly Tyr Thr Phe Thr Arg Tyr Thr Met His Trp Val Arg Gln Ala Pro
            260                 265                 270

Gly Lys Gly Leu Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr
        275                 280                 285

Thr Asn Tyr Asn Gln Lys Phe Lys Asp Arg Phe Thr Ile Ser Arg Asp
    290                 295                 300

Asn Ser Lys Asn Thr Ala Phe Leu Gln Met Asp Ser Leu Arg Pro Glu
305                 310                 315                 320

Asp Thr Gly Val Tyr Phe Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys
                325                 330                 335
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```
Leu Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Gly Gly
            340                 345                 350

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln
        355                 360                 365

Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val
    370                 375                 380

Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr
385                 390                 395                 400

Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr Asp Thr Ser
                405                 410                 415

Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly
            420                 425                 430

Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala
        435                 440                 445

Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr Phe Gly Gln
    450                 455                 460

Gly Thr Lys Leu Gln Ile Thr Arg
465                 470


<210>  61
<211>  214
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  Her2-bsab light chain sequence

<400>  61

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

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
```

85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
130 135 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145 150 155 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
165 170 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
180 185 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
195 200 205

Phe Asn Arg Gly Glu Cys
210

<210> 62
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> her2 bsab heavy chain sequence

<400> 62

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
20 25 30

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

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

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

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

```
Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

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

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

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145                 150                 155                 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
                165                 170                 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
            180                 185                 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
        195                 200                 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
    210                 215                 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225                 230                 235                 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
                245                 250                 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
            260                 265                 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
        275                 280                 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg
    290                 295                 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305                 310                 315                 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
                325                 330                 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
            340                 345                 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
        355                 360                 365
```

```
Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
    370                 375                 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385                 390                 395                 400

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

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
            420                 425                 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
        435                 440                 445

Gly Lys
    450
```

<210> 63
<211> 450
<212> PRT
<213> Artificial Sequence

<220>
<223> Trastuzumab heavy chain K322A

<400> 63

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30

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

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

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

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

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
            100                 105                 110

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

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
```

130 135 140

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
305 310 315 320

Glu Tyr Lys Cys Ala Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp

405 410 415

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450

<210> 64
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3 VH C105S + VH44

<400> 64

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Ser Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser
115

<210> 65
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3 VL + VL100

<400> 65

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly

1 5 10 15

Asp Arg Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala Pro Lys Arg Trp Ile Tyr
35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu
65 70 75 80

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95

Phe Gly Cys Gly Thr Lys Leu Gln Ile Thr Arg
100 105

<210> 66
<211> 241
<212> PRT
<213> Artificial Sequence

<220>
<223> huOKT3 scFv; 15 aa linker

<400> 66

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

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

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

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

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

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Gly Val Tyr Phe Cys
85 90 95

Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Pro Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly
115 120 125

Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro Ser Ser
130 135 140

Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Ser Ala Ser
145 150 155 160

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Thr Pro Gly Lys Ala
165 170 175

Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
180 185 190

Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile
195 200 205

Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp
210 215 220

Ser Ser Asn Pro Phe Thr Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr
225 230 235 240

Arg

<210> 67
<211> 1416
<212> DNA
<213> Artifial Sequence

<220>
<223> DNA encoding sequence of SEQ ID NO:60

<400> 67
gatattcaga tgactcagtc tccctcttcc ctgtccgctt cagtcggcga tcgggtcact 60

attacttgtc gggcttcaca ggatgtcaac acagccgtgg cttggtacca gcagaagccc 120

gggaaagcac ctaagctgct gatctactct gccagtttcc tgtattctgg cgtcccaagt 180

aggttttcag gctcccggag cggaactgac ttcaccctga caatttccag cctgcagccc 240

gaggattttg ctacctacta ttgccagcag cattatacta ccccccccaac attcggccag 300

ggcacaaaag tcgaaatcaa gcggaccgtg gccgcccccct ccgtgttcat cttccccccc 360

tccgacgagc agctgaagtc cggcaccgcc tccgtggtgt gcctgctgaa caacttctac 420

ccccgggagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag 480

gagtccgtga ccgagcagga ctccaaggac tccacctact ccctgtcctc caccctgacc 540

ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaggtgac ccaccagggc 600

ctgtcctccc ccgtgaccaa gtccttcaac cggggcgagt gcactagtgg aggaggaggt 660

agcggaggag gaggttctgg cggaggggt tcccaggtgc agctggtgca gagcggagga 720

ggagtggtgc agccaggaag gagcctgcga ctgtcttgca aggctagtgg ctacaccttc 780

acacgatata ctatgcactg ggtgaggcag gcacctggta aaggcctgga gtggatcggc 840

tacattaacc cctctagggg atacaccaac tataatcaga agttcaaaga caggttcacc 900

atctcacgcg ataactccaa gaataccgcc ttcctgcaga tggactccct gcggcccgaa 960

gatacaggcg tgtatttttg cgctagatac tatgacgatc attactgtct ggactattgg 1020

ggacagggga cccctgtgac agtgtccagc ggtggaggag ggtcaggtgg aggagggagc 1080

ggtggcggag ggtctgacat ccagatgacc cagtccccat ctagtctgag cgcctctgtg 1140

ggcgatagag tgactattac ctgcagtgct tcatccagcg tgagctacat gaactggtat 1200

cagcagacac ccggaaaggc acctaaacgc tggatctacg atactagcaa gctggcctct 1260

ggcgtgccca gtcgattcag tggttcaggc tccggaaccg actatacctt caccatctct 1320

agtctgcagc ctgaggatat tgccacatac tattgtcagc agtggtcatc caatccattc 1380

acttttgggc agggtaccaa actgcagatt acaagg 1416

<210> 68
<211> 1350
<212> DNA
<213> Artificial Sequence

<220>
<223> DNA encoding sequence of SEQ ID NO:62

<400> 68
gaagtgcagc tggtcgagag cggaggaggt ctggtgcagc ccggaggttc cctgagactg 60

tcctgtgccg catctgggtt taatatcaag gacacataca tccactgggt gagacaggca 120

cccggcaaag gactggagtg ggtcgccagg atctacccta ccaacgggta cacaagatat 180

gctgactctg tgaagggccg gttcaccatc tccgccgata ctagcaaaaa caccgcttac 240

ctgcagatga attccctgag ggcagaagat accgctgtct actactgttc aagatggggg 300

ggggatggtt tttacgctat ggattattgg ggccagggca ccctggtgac cgtgtcctcc 360

gcctccacca agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg 420

ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 480

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggccgtcct acagtcctca 540

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 600

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagag agttgagccc 660

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 720

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggacccct 780

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 840

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacgcc 900

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 960

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 1020

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggatgag 1080

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1140

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1200

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 1260

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1320

cagaagagcc tctccctgtc tccgggtaaa 1350

<210> 69
<211> 1410
<212> DNA
<213> Artificial Sequence

<220>
<223> HER2-C825 Light Chain

<400> 69
gatattcaga tgactcagtc tccctcttcc ctgtccgctt cagtcggcga tcgggtcact 60

attacttgtc gggcttcaca ggatgtcaac acagccgtgg cttggtacca gcagaagccc 120

gggaaagcac ctaagctgct gatctactct gccagtttcc tgtattctgg cgtcccaagt 180

aggttttcag gctcccggag cggaactgac ttcaccctga caatttccag cctgcagccc 240

gaggattttg ctacctacta ttgccagcag cattatacta ccccccccaac attcggccag 300

ggcacaaaag tcgaaatcaa gcggaccgtg gccgcccccct ccgtgttcat cttcccccccc 360

tccgacgagc agctgaagtc cggcaccgcc tccgtggtgt gcctgctgaa caacttctac 420

ccccgggagg ccaaggtgca gtggaaggtg gacaacgccc tgcagtccgg caactcccag 480

gagtccgtga ccgagcagga ctccaaggac tccacctact ccctgtcctc caccctgacc 540

ctgtccaagg ccgactacga gaagcacaag gtgtacgcct gcgaggtgac ccaccagggc 600

ctgtcctccc ccgtgaccaa gtccttcaac cggggcgagt gcggtggtgg tggtagcggc 660

ggcggtggaa gcgcatccca tgtgaaactg caggaaagcg gcccaggtct ggtccagcca 720

tcccagtctc tgagcctgac atgcactgtg agcggattct ctctgacaga ctatggggtg 780

cactgggtca gacagagtcc aggaaagggg ctggagtggc tgggcgtcat ctggtcaggc 840

ggagggactg cttataacac cgcactgatc agcagactga atatctaccg cgacaactct 900

aaaaatcagg tgttcctgga gatgaacagt ctgcaggccg aagataccgc tatgtactat 960

tgcgccaggc ggggcagcta cccttataat tactttgacg cttggggttg tggcaccaca 1020

gtgacagtct ccagcggtgg aggagggagt ggtggaggag ggtcaggtgg aggagggtcc 1080

caggcagtgg tcattcagga gtctgccctg actacccccc ctggagaaac cgtgacactg 1140

acttgcggat ctagtacagg ggcagtgact gcctccaact atgcaaattg ggtccaggaa 1200

aagcctgatc actgtttcac tggcctgatc ggtggccata acaatcgacc acccggagtg 1260

ccagctaggt tttcaggttc cctgatcggc gacaaagccg ctctgaccat tgctggcacc 1320

cagacagagg atgaagcaat ctacttttgt gccctgtggt attccgatca ctgggtcatt 1380

ggggggggga cacgtctgac tgtgctgggg 1410

<210> 70

<211> 1350
<212> DNA
<213> Artificial Sequence

<220>
<223> HER2-C825 Heavy Chain

<400> 70
gaagtgcagc tggtcgagag cggaggaggt ctggtgcagc ccggaggttc cctgagactg 60

tcctgtgccg catctgggtt taatatcaag gacacataca tccactgggt gagacaggca 120

cccggcaaag gactggagtg ggtcgccagg atctacccta ccaacgggta cacaagatat 180

gctgactctg tgaagggccg gttcaccatc tccgccgata ctagcaaaaa caccgcttac 240

ctgcagatga attccctgag ggcagaagat accgctgtct actactgttc aagatggggg 300

ggggatggtt tttacgctat ggattattgg ggccagggca ccctggtgac cgtgtcctcc 360

gcctccacca agggcccatc ggtcttcccc ctggcaccct cctccaagag cacctctggg 420

ggcacagcgg ccctgggctg cctggtcaag gactacttcc ccgaaccggt gacggtgtcg 480

tggaactcag gcgccctgac cagcggcgtg cacaccttcc cggccgtcct acagtcctca 540

ggactctact ccctcagcag cgtggtgacc gtgccctcca gcagcttggg cacccagacc 600

tacatctgca acgtgaatca caagcccagc aacaccaagg tggacaagag agttgagccc 660

aaatcttgtg acaaaactca cacatgccca ccgtgcccag cacctgaact cctgggggga 720

ccgtcagtct tcctcttccc cccaaaaccc aaggacaccc tcatgatctc ccggaccccct 780

gaggtcacat gcgtggtggt ggacgtgagc cacgaagacc ctgaggtcaa gttcaactgg 840

tacgtggacg gcgtggaggt gcataatgcc aagacaaagc cgcgggagga gcagtacgcc 900

agcacgtacc gtgtggtcag cgtcctcacc gtcctgcacc aggactggct gaatggcaag 960

gagtacaagt gcaaggtctc caacaaagcc ctcccagccc ccatcgagaa aaccatctcc 1020

aaagccaaag ggcagccccg agaaccacag gtgtacaccc tgcccccatc ccgggatgag 1080

ctgaccaaga accaggtcag cctgacctgc ctggtcaaag gcttctatcc cagcgacatc 1140

gccgtggagt gggagagcaa tgggcagccg gagaacaact acaagaccac gcctcccgtg 1200

ctggactccg acggctcctt cttcctctac agcaagctca ccgtggacaa gagcaggtgg 1260

cagcagggga acgtcttctc atgctccgtg atgcatgagg ctctgcacaa ccactacacg 1320

cagaagagcc tctccctgtc tccgggtaaa 1350

<210> 71
<211> 470
<212> PRT
<213> Artificial Sequence

<220>
<223> HER2-C825 Light Chain sequence

<400> 71

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

```
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Val Asn Thr Ala
            20                  25                  30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
        35                  40                  45

Tyr Ser Ala Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60

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

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
                85                  90                  95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala
            100                 105                 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
        115                 120                 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
    130                 135                 140

Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
145                 150                 155                 160

Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
                165                 170                 175

Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
            180                 185                 190

Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
        195                 200                 205

Phe Asn Arg Gly Glu Cys Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
    210                 215                 220

Ala Ser His Val Lys Leu Gln Glu Ser Gly Pro Gly Leu Val Gln Pro
225                 230                 235                 240

Ser Gln Ser Leu Ser Leu Thr Cys Thr Val Ser Gly Phe Ser Leu Thr
                245                 250                 255

Asp Tyr Gly Val His Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu
            260                 265                 270

Trp Leu Gly Val Ile Trp Ser Gly Gly Gly Thr Ala Tyr Asn Thr Ala
        275                 280                 285
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```
Leu Ile Ser Arg Leu Asn Ile Tyr Arg Asp Asn Ser Lys Asn Gln Val
    290                 295                 300

Phe Leu Glu Met Asn Ser Leu Gln Ala Glu Asp Thr Ala Met Tyr Tyr
305                 310                 315                 320

Cys Ala Arg Arg Gly Ser Tyr Pro Tyr Asn Tyr Phe Asp Ala Trp Gly
                325                 330                 335

Cys Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
            340                 345                 350

Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Ile Gln Glu Ser
        355                 360                 365

Ala Leu Thr Thr Pro Pro Gly Glu Thr Val Thr Leu Thr Cys Gly Ser
    370                 375                 380

Ser Thr Gly Ala Val Thr Ala Ser Asn Tyr Ala Asn Trp Val Gln Glu
385                 390                 395                 400

Lys Pro Asp His Cys Phe Thr Gly Leu Ile Gly Gly His Asn Asn Arg
                405                 410                 415

Pro Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp Lys
            420                 425                 430

Ala Ala Leu Thr Ile Ala Gly Thr Gln Thr Glu Asp Glu Ala Ile Tyr
        435                 440                 445

Phe Cys Ala Leu Trp Tyr Ser Asp His Trp Val Ile Gly Gly Gly Thr
    450                 455                 460

Arg Leu Thr Val Leu Gly
465                 470


<210>  72
<211>  450
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  HER2-C825 Heavy Chain sequence

<400>  72

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

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Asn Ile Lys Asp Thr
            20                  25                  30

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

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

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

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

Ser Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

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

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

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
145 150 155 160

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
165 170 175

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
180 185 190

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
195 200 205

Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp
210 215 220

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
225 230 235 240

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
245 250 255

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
260 265 270

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
275 280 285

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Ala Ser Thr Tyr Arg
290 295 300

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys

305 310 315 320

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
325 330 335

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
340 345 350

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
355 360 365

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
370 375 380

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
385 390 395 400

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

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
420 425 430

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
435 440 445

Gly Lys
450