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(54) Title: ANTI-HER2 ANTIBODIES AND USES THEREOF

(57) Abstract:

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ANTI-HER2 ANTIBODIES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/229,134, filed August 4, 2021, the entire contents of which are incorporated herein by
5 reference.

TECHNICAL FIELD

[0002] The present technology relates generally to the preparation of immunoglobulin-related compositions (*e.g.*, antibodies or antigen binding fragments thereof) that specifically bind HER2 protein and uses of the same. In particular, the present technology relates to the preparation of
10 HER2 binding antibodies and their use in detecting and treating HER2-associated cancer.

BACKGROUND

[0003] The following description of the background of the present technology is provided simply as an aid in understanding the present technology and is not admitted to describe or constitute prior art to the present technology.

15 [0004] Monoclonal antibody (mAb)-based therapy for cancer is one of the most successful strategies for treating patients with both hematological and nonhematological malignancies.

[0005] One of the main clinical accomplishments for mAbs was the discovery of trastuzumab/Herceptin, a humanized monoclonal antibody that binds to the extracellular domain of HER2. In fact, for HER2-positive breast cancer the standard of care treatment includes
20 chemotherapy in combination with trastuzumab. Trastuzumab was designed to inhibit cell growth and proliferation, and kills HER2-positive tumor cells through antibody-dependent cellular cytotoxicity (ADCC). Both the combination of trastuzumab with conventional chemotherapies in breast cancer and gastric cancer and the use of trastuzumab as a single agent have proven to prolong the progression-free survival of patients with amplified HER2.

25 Importantly, standard HER2-targeted therapies are exclusively offered to patients scoring HER2 immunohistochemistry (IHC) 3+ (*i.e.* strongly overexpressed), or SPoT-Light® HER2

Chromogenic IHC (CISH) positive (*i.e.* *ERBB2* gene-amplified). However, a significant group of patients do not respond to this targeted therapy and most of the patients that initially respond acquire resistance in response to trastuzumab treatment. There are multiple mechanisms contributing to trastuzumab resistance that include activation of the HER2 downstream signaling pathways and parallel receptor tyrosine kinase pathways, all of which provide potential targets to combat trastuzumab resistance. A major mechanism of resistance involves the activation of a bypass-signaling pathway making inhibition of HER2-signaling irrelevant to tumor progression. Up to 70% of HER2+ breast cancers become resistant to a single-agent treatment of trastuzumab. Despite the drug resistance, these tumors generally still overexpress HER2.

[0006] Accordingly, there is an urgent need for developing alternative HER2-targeted therapies that overcome the resistance to signaling inhibition and benefit more patients.

SUMMARY OF THE PRESENT TECHNOLOGY

[0007] In one aspect, the present disclosure provides an antibody or an antigen binding fragment thereof, comprising an antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein (a) (i) the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 1, a V_H -CDR2 sequence of SEQ ID NO: 2 or SEQ ID NO: 7 and a V_H -CDR3 sequence of SEQ ID NO: 8; or (ii) the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 1, a V_H -CDR2 sequence of SEQ ID NO: 7 and a V_H -CDR3 sequence of SEQ ID NO: 3 or SEQ ID NO: 8; and/or (b) (i) the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 5, SEQ ID NO: 10, or SEQ ID NO: 11, and a V_L -CDR3 sequence of SEQ ID NO: 6 or SEQ ID NO: 12; or (ii) the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 4 or SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 10, or SEQ ID NO: 11, and a V_L -CDR3 sequence of SEQ ID NO: 6 or SEQ ID NO: 12; or (iii) the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 4 or SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 5, SEQ ID NO: 10, or SEQ ID NO: 11, and a V_L -CDR3 sequence of SEQ ID NO: 12.

[0008] In one aspect, the present disclosure provides an antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain

immunoglobulin variable domain (V_L), wherein: (a) the V_H comprises an amino acid sequence selected from any one of SEQ ID NOs: 13, 15, or 17; and/or (b) the V_L comprises an amino acid sequence selected from any one of SEQ ID NOs: 14, 16, 18, 19, or 20. In some embodiments, the antibody or antigen binding fragment thereof comprises heavy chain immunoglobulin variable domain (V_H) and light chain immunoglobulin variable domain (V_L) amino acid sequences selected from the group consisting of: SEQ ID NOs: 13 and 14, SEQ ID NOs: 15 and 16, SEQ ID NOs: 17 and 14, SEQ ID NOs: 15 and 18, SEQ ID NOs: 15 and 19, and SEQ ID NOs: 15 and 20, respectively.

[0009] In another aspect, the present disclosure provides an antibody comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain and a fourth polypeptide chain, wherein the first and second polypeptide chains are covalently bonded to one another, the second and third polypeptide chains are covalently bonded to one another, and the third and fourth polypeptide chain are covalently bonded to one another, and wherein: (a) each of the first polypeptide chain and the fourth polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a light chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope; (ii) a light chain constant domain of the first immunoglobulin; (iii) a flexible peptide linker comprising the amino acid sequence (GGGS)₃; and (iv) a light chain variable domain of a second immunoglobulin that is linked to a complementary heavy chain variable domain of the second immunoglobulin, or a heavy chain variable domain of a second immunoglobulin that is linked to a complementary light chain variable domain of the second immunoglobulin, wherein the light chain and heavy chain variable domains of the second immunoglobulin are capable of specifically binding to a second epitope, and are linked together via a flexible peptide linker comprising the amino acid sequence (GGGS)₆ to form a single-chain variable fragment; and (b) each of the second polypeptide chain and the third polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a heavy chain variable domain of the first immunoglobulin that is capable of specifically binding to the first epitope; and (ii) a heavy chain constant domain of the first immunoglobulin; and wherein the heavy chain variable domain of the first immunoglobulin or the heavy chain variable domain of the second immunoglobulin comprises any one of SEQ ID NOs: 13, 15, or 17, and/or

the light chain variable domain of the first immunoglobulin or the light chain variable domain of the second immunoglobulin comprises any one of SEQ ID NOs: 14, 16, 18, 19, or 20.

[0010] In another aspect, the present disclosure provides an antibody or antigen binding fragment comprising a heavy chain (HC) and a light chain (LC) selected from the group consisting of SEQ ID NOs: 21 and 22, SEQ ID NOs: 21 and 23, SEQ ID NOs: 21 and 24, SEQ ID NOs: 21 and 25, SEQ ID NOs: 21 and 26, SEQ ID NOs: 21 and 27, SEQ ID NOs: 21 and 28, SEQ ID NOs: 21 and 29, SEQ ID NOs: 21 and 30, SEQ ID NOs: 21 and 31, SEQ ID NOs: 21 and 32, SEQ ID NOs: 21 and 33, SEQ ID NOs: 34 and 33, SEQ ID NOs: 21 and 35, SEQ ID NOs: 36 and 33, SEQ ID NOs: 21 and 37, SEQ ID NOs: 21 and 38, SEQ ID NOs: 21 and 39, SEQ ID NOs: 21 and 40, SEQ ID NOs: 21 and 41, SEQ ID NOs: 21 and 42, SEQ ID NOs: 21 and 43, SEQ ID NOs: 21 and 44, SEQ ID NOs: 21 and 45, SEQ ID NOs: 21 and 46, SEQ ID NOs: 21 and 47, SEQ ID NOs: 21 and 48, SEQ ID NOs: 21 and 49, SEQ ID NOs: 21 and 50, SEQ ID NOs: 21 and 51, SEQ ID NOs: 21 and 52, SEQ ID NOs: 21 and 53, SEQ ID NOs: 21 and 54, SEQ ID NOs: 21 and 55, SEQ ID NOs: 21 and 56, SEQ ID NOs: 21 and 57, SEQ ID NOs: 21 and 58, SEQ ID NOs: 21 and 59, SEQ ID NOs: 21 and 60, SEQ ID NOs: 21 and 61, SEQ ID NOs: 21 and 62, SEQ ID NOs: 21 and 63, SEQ ID NOs: 21 and 64, SEQ ID NOs: 21 and 65, SEQ ID NOs: 21 and 66, SEQ ID NOs: 21 and 67, SEQ ID NOs: 21 and 68, SEQ ID NOs: 21 and 69, SEQ ID NOs: 21 and 70, SEQ ID NOs: 21 and 71, SEQ ID NOs: 21 and 72, and SEQ ID NOs: 21 and 85, respectively.

[0011] Additionally or alternatively, in some embodiments, the antibody or antigen binding fragment further comprises a Fc domain of an isotype selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, and IgE. In certain embodiments, the antibody or antigen binding fragment comprises an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A, L234A, L235A, and K322A. In other embodiments, the antibody or antigen binding fragment comprises an IgG4 constant region comprising a S228P mutation.

[0012] In any and all embodiments of the antigen binding fragment disclosed herein, the antigen binding fragment is selected from the group consisting of Fab, F(ab')₂, Fab', scFv, and Fv.

Additionally or alternatively, in some embodiments, the antibody or antigen binding fragment of the present technology is a monoclonal antibody, a chimeric antibody, a humanized antibody, a bispecific antibody, or multi-specific antibody, and/or lacks α -1,6-fucose modifications.

[0013] In any and all embodiments of the antibody or antigen binding fragment disclosed herein, the multi-specific antibody or antigen binding fragment binds to T cells, B-cells, myeloid cells, plasma cells, or mast-cells. Additionally or alternatively, in some embodiments of the antibody or antigen binding fragment disclosed herein, the multi-specific antibody or antigen binding fragment binds to CD3, CD4, CD8, CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, CD22, CD14, CD15, CD16, CD123, TCR gamma/delta, NKp46, KIR, or a small molecule DOTA hapten.

[0014] Additionally or alternatively, in some embodiments, the multi-specific antibody or antigen binding fragment of the present technology also binds to T cells and/or CD3. In one aspect, the present disclosure provides a T cell that is armed *ex vivo* with a multi-specific antibody or antigen binding fragment of the present technology that also binds to T cells and/or CD3. In another aspect, the present disclosure provides an *ex vivo* method of making a therapeutic T cell, comprising arming a T cell *ex vivo* with a multi-specific antibody or antigen binding fragment of the present technology that is capable of binding to T cells and/or CD3, wherein the T cell is optionally a human T cell, and wherein the binding is noncovalent. In another aspect, the present disclosure provides a method for treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of a T cell that is armed *ex vivo* with a multi-specific antibody or antigen binding fragment of the present technology that also binds to T cells and/or CD3.

[0015] In one aspect, the present disclosure provides a recombinant nucleic acid sequence encoding any of the antibodies or antigen binding fragments described herein. In another aspect, the present disclosure provides a host cell or vector comprising any of the recombinant nucleic acid sequences disclosed herein.

[0016] In another aspect, the present disclosure provides a pharmaceutical composition comprising any of the antibodies or antigen binding fragments described herein and a

pharmaceutically-acceptable carrier, wherein the antibody or antigen binding fragment is optionally conjugated to an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof. In some embodiments, the pharmaceutical composition further comprises an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

[0017] In one aspect, the present disclosure provides a method for treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of any of the antibodies or antigen binding fragments described herein, or any of the pharmaceutical compositions disclosed herein, wherein the antibody or antigen binding fragment specifically binds to HER2. In some embodiments, the cancer is a solid tumor. Examples of cancer include, but are not limited to, breast cancer, gastric cancer, 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, and neuroblastoma.

[0018] Additionally or alternatively, in some embodiments of the method, the antibody or antigen binding fragment is administered to the subject separately, sequentially or simultaneously with an additional therapeutic agent. Examples of additional therapeutic agents include one or more of alkylating agents, platinum agents, taxanes, vinca agents, anti-estrogen drugs, aromatase inhibitors, ovarian suppression agents, VEGF/VEGFR inhibitors, EGF/EGFR inhibitors, PARP inhibitors, cytostatic alkaloids, cytotoxic antibiotics, antimetabolites, endocrine/hormonal agents, bisphosphonate therapy agents, T cells, and immuno-modulating/stimulating antibodies (*e.g.*, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-

PD-L2 antibody, an anti-CTLA-4 antibody, an anti-TIM3 antibody, an anti-4-1BB antibody, an anti-CD73 antibody, an anti-GITR antibody, or an anti-LAG-3 antibody).

[0019] In another aspect, the present disclosure provides a method for detecting cancer in a subject *in vivo* comprising (a) administering to the subject an effective amount of the antibody or antigen binding fragment of the present technology, wherein the antibody or antigen binding fragment is configured to localize to a cancer cell expressing HER2, and is labeled with a radioisotope; and (b) detecting the presence of a tumor in the subject by detecting radioactive levels emitted by the antibody or antigen binding fragment that are higher than a reference value.

In certain embodiments, the cancer is a solid tumor. In some embodiments, the subject is diagnosed with or is suspected of having cancer. Examples of cancer include, but are not limited to, breast cancer, gastric cancer, 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, and neuroblastoma. Radioactive levels emitted by the antibody or antigen binding fragment may be detected using positron emission tomography or single photon emission computed tomography. Additionally or alternatively, in some embodiments, the method further comprises administering to the subject an effective amount of an immunoconjugate comprising the antibody or antigen binding fragment of the present technology conjugated to a radionuclide.

[0020] In any and all embodiments of the methods disclosed herein, the subject is human.

[0021] In yet another aspect, the present disclosure provides a method for detecting HER2 protein expression levels in a biological sample comprising contacting the biological sample with any of the antibodies or antigen binding fragments disclosed herein, and detecting binding to HER2 protein in the biological sample.

[0022] Also disclosed herein are kits for the detection and/or treatment of HER2-associated cancers comprising at least one immunoglobulin-related composition of the present technology (*e.g.*, any antibody or antigen binding fragment described herein), and instructions for use. In

certain embodiments, the immunoglobulin-related composition is coupled to one or more detectable labels. In one embodiment, the one or more detectable labels comprise a radioactive label, a fluorescent label, or a chromogenic label. Additionally or alternatively, in some embodiments, the kit further comprises a secondary antibody that specifically binds to an anti-HER2 immunoglobulin-related composition described herein. In some embodiments, the secondary antibody is coupled to at least one detectable label selected from the group consisting of a radioactive label, a fluorescent label, or a chromogenic label.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] FIG. 1 shows the amino acid sequences of V_H CDR1 (SEQ ID NO: 1), V_H CDR2 (SEQ ID NO: 2 or 7), V_H CDR3 (SEQ ID NO: 3 or 8), V_L CDR1 (SEQ ID NO: 4 or 9), V_L CDR2 (SEQ ID NO: 5, 10 or 11), and V_L CDR3 (SEQ ID NO: 6 or 12) of the anti-HER2 immunoglobulin-related compositions of the present technology. Mutations in the CDR regions of the trastuzumab variants compared to CDR regions (SEQ ID NOs: 1-6) of the parental trastuzumab antibody are underlined.

[0024] FIG. 2 shows the amino acid sequences of the variable heavy immunoglobulin domain (V_H) and the variable light immunoglobulin domain (V_L) of 6 of the anti-HER2 immunoglobulin-related compositions of the present technology: ABP100s.10.1 HER2 (SEQ ID NOs: 13 and 14, respectively), ABP100s.10.2 HER2 (SEQ ID NOs: 15 and 16, respectively), ABP100s.10.3 HER2 (SEQ ID NOs: 17 and 14, respectively), ABP100s.10.4 HER2 (SEQ ID NOs: 15 and 18, respectively), ABP100s.10.5 HER2 (SEQ ID NOs: 15 and 19, respectively), and ABP100s.10.6 HER2 (SEQ ID NO: 15 and 20, respectively). The V_H CDR 1-3 and V_L CDR 1-3 amino acid sequences are underlined. Mutations in the CDR regions of the trastuzumab variants compared to CDR regions (SEQ ID NOs: 1-6) of the parental trastuzumab antibody are double underlined.

[0025] FIG. 3 shows the heavy chain (HC) and light chain (LC) amino acid sequences of 40 exemplary HER2×CD3 bispecific antibodies of the present technology. The V_H CDR 1-3 and V_L CDR 1-3 amino acid sequences are underlined. The linker sequences are italicized. Point

mutations and disulfide mutations are bold and double underlined. Fc substitutions are bold and underlined.

[0026] **FIG. 4** shows an overview of two groups of BsAbs: the first targets both HER2 and the T cell co-receptor CD3, and the second selectively targets and kills HER2 amplified cancer cells.

5 [0027] **FIG. 5A-5B** shows individual mouse tumor responses in mouse xenograft models using BsAbs with varying interdomain spacing formats (**FIG. 5A**) administered intravenously (IV) (10 pmol, twice per week) along with 20 million huATCs and subcutaneous human IL-2 (1000 U) or (**FIG. 5B**) ex-vivo “armed” T-cell (EAT) administration of BsAbs and human IL-2 (1,000 U) (subcutaneously, twice per week). Each line represents a single mouse, and the dashed line
10 represents the group average. *See Santich et al., Sci. Transl. Med.* 12: eaax1315 (2020), which is incorporated herein by reference.

[0028] **FIG. 6** summarizes the process for arming activated T-cells with the anti-HER2 × CD3 bispecific antibodies of the present technology.

[0029] **FIG. 7A** shows CD3⁺ T cells that were stained in duplicate wells with either SP34-
15 hIgG1 or bispecific antibodies for 30 minutes at 4 °C, followed by washing and incubation with anti-human IgG Fc specific PE conjugate (Jackson 1:200) for 30 minutes at 4 °C followed by washing and data collection on the BD FACSCelesta, with data analyzed in FlowJo and GraphPad PRISM. Notably, the constructs used in this experiment differed only in their HER2-binding arm, and all had the same CD3-reactive arm. **FIGs. 7B-7C** show differential killing of
20 HER2-low expressing target cells through lowering the affinity of the HER2 arm. A TDCC assay at an effector:target (E:T) ratio of 5:1 was performed with a dose range for bispecific antibodies, with CD3⁺ T cells used as effector cells (50k/well), while target cells (10k/well) expressed either high relative amounts of HER-2 (**FIG. 7B**, SKBR-3), or low relative amounts of HER2 (**FIG. 7C**, MCF-7). Cells were incubated with antibody in RPMI1640/10% FBS for 28
25 hours at 37 °C /5% CO₂, with luminescence quantified on the SpectraMax iD3 plate reader and data analysis in GraphPad PRISM constructs in this experiment differed only in their HER2-binding arm, and all had the same CD3-reactive arm.

[0030] **FIGs. 8A-8B** shows characterization of activated T cells armed with the anti-HER2 × CD3 parent antibody (trastuzumab × huOKT3). **FIG. 8A** shows qualitative flow cytometry data demonstrating binding of trastuzumab × huOKT3 to activated T cells after arming at various concentrations. **FIG. 8B** shows trastuzumab × huOKT3-mediated killing of cell lines expressing different levels of HER2.

[0031] **FIGs. 9A-9B** show differential killing of HER2-high and HER2-low expressing target cells by TDCC assay. **FIG. 9A** and **FIG. 9B** show the effects of the HER2×CD3 bispecific antibodies of the present technology (ABP100s.5, ABP100s.5.1, ABP100s.10.2, ABP100s.10.4, ABP100s.10.5, ABP100s.10.6) on SK-BR-3 cells and MCF-7 cells, respectively.

[0032] **FIG. 10** shows affinities of the HER2×CD3 bispecific antibodies of the present technology to the CD3 and HER2 targets as disclosed in **FIG. 3**.

[0033] **FIG. 11** shows the heavy chain (HC) and light chain (LC) amino acid sequences of 12 additional exemplary HER2×CD3 bispecific antibodies of the present technology. The V_H CDR 1-3 and V_L CDR 1-3 amino acid sequences are indicated in bold. The linker sequences are indicated in italics.

[0034] **FIG. 12** shows affinities of the HER2×CD3 bispecific antibodies of the present technology to the CD3 and HER2 targets as disclosed in **FIG. 11**.

[0035] **FIGs. 13A-13E** show the results of CD3/TCR NFAT T cell activation reporter assays. A Jurkat CD3/TCR NFAT T cell activation reporter assay (Promega) was used to assess bispecific antibody (0.00004 - 40nM) activation of the CD3/TCR complex following incubation with Her2-high (**FIG. 13A**: SK-BR-3, **FIG. 13B**: HCC1954) and Her2-low (**FIG. 13C**: MCF-7, **FIG. 13D**: HT55) target cell lines or no target cells (**FIG. 13E**). The expression of reporter activity was detected and quantified as Relative Luminescence Units (RLU), which were plotted after a 7-hour incubation period.

[0036] **FIGs. 14A-14D** show the results of T cell dependent cellular cytotoxicity (TDCC) on Her2-high and Her2-low target cell lines with human CD3+ T cells. Bispecific antibodies were incubated with CD3+ T cells and target cells (Effector : Target ratio 5:1) for 40 hours at 37°C.

Using a sensitive ATP quantification method (Cell Titer Glo 2.0), %Cytotoxicity was quantified in comparison to [Effector plus target]-only wells for Her2-high target cells (**FIG. 14A**: SKBR-3, **FIG. 14B**: HCC1954) and Her2-low cell lines (**FIG. 14C**: MCF-7, **FIG. 14D**: HT55).

[0037] FIGs. 15A-15H show the results of *in-vitro* multiplex cytokine detection assay in Her2-high and Her2-low target cell lines with human PBMCs. Bispecific antibodies (range: 30, 0.3, 0.003, 0.00003 nM) were incubated with human PBMCs and target cells (Effector (100,000 cells): Target (10,000 cells), E:T ratio 10:1) for 24 hours at 37°C. Cytokine release on SKBR-3 (Her2-high) target cells (**FIGs. 15A-15D**) and MCF-7 (Her2-low) target cells (**FIGs. 15E-15H**) were quantified by diluting supernatants 1:4 for use in a multiplex bead-based assay for TNF- α (**FIG. 15A, FIG. 15E**), IL-6 (**FIG. 15B, FIG. 15F**), IL-2 (**FIG. 15C, FIG. 15G**), IFN- γ (**FIG. 15D, FIG. 15H**), and are presented here in picograms/mL as quantified by Luminex xMAP software.

[0038] FIGs. 16A-16E show the results of flow cytometric analysis of bispecific antibody binding to activated T cells and Her-2 expressing target cells. Bispecific antibodies were incubated with Her2-high (**FIG. 16A**: SKBR-3, **FIG. 16B**: SKOV-3) or Her2-low (**FIG. 16C**: MCF-7, **FIG. 16D**: HT55) target cells or activated T cells (**FIG. 16E**). 100,000 cells/well were incubated with primary bispecific antibodies followed by incubation with anti-human IgG PE secondary antibody. Results were quantified as Median Fluorescence Intensity (MFI) of single live cells in FlowJo software.

[0039] FIGs. 17A-17B show Biacore (SPR) affinity data for the Fab formats (attached to a human IgG1 scaffold) of the anti-HER2 $\times$ CD3 BsAbs of the present technology. The anti-HER2 $\times$ CD3 BsAbs of the present technology cross-react with both human and non-human primate (Cyno) HER2 and CD3 antigens.

DETAILED DESCRIPTION

[0040] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present methods are described below in various levels of detail in order to provide a substantial understanding of the present technology.

[0041] The present disclosure generally provides immunoglobulin-related compositions (*e.g.*, antibodies or antigen binding fragments thereof), which can specifically bind to HER2 polypeptides. The immunoglobulin-related compositions of the present technology are useful in methods for detecting or treating HER2-associated cancers in a subject in need thereof.

5 Accordingly, the various aspects of the present methods relate to the preparation, characterization, and manipulation of anti-HER2 antibodies. The immunoglobulin-related compositions of the present technology are useful alone or in combination with additional therapeutic agents for treating cancer. In some embodiments, the immunoglobulin-related composition is a monoclonal antibody, a humanized antibody, a chimeric antibody, a bispecific
10 antibody, or a multi-specific antibody.

[0042] In practicing the present methods, many conventional techniques in molecular biology, protein biochemistry, cell biology, immunology, microbiology and recombinant DNA are used. *See, e.g.*, Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition; the series Ausubel *et al.* eds. (2007) *Current Protocols in Molecular Biology*; the series
15 *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson *et al.* (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson *et al.* (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds. (1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic
20 Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987)
25 *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); and Herzenberg *et al.* eds (1996) *Weir's Handbook of Experimental Immunology*. Methods to detect and measure levels of polypeptide gene expression products (*i.e.*, gene translation level) are well-known in the art and include the use of polypeptide detection methods such as antibody detection

and quantification techniques. (*See also*, Strachan & Read, *Human Molecular Genetics*, Second Edition. (John Wiley and Sons, Inc., NY, 1999)).

[0043] The present disclosure provides HER2 bispecific antibodies with reduced affinity that take advantage of avidity interactions to selectively bind to and kill cells with a high density of Her2 (such as cells of cancerous tissues) and not bind and spare Her2-low density cells. Without wishing to be bound by theory, it is believed that lowering CD3 affinity in HER2×CD3 bispecific antibodies would reduce T cell activation and cytokine production, leading to lower adverse events such as cytokine release syndrome in the clinic.

Definitions

[0044] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. For example, reference to “a cell” includes a combination of two or more cells, and the like.

Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, analytical chemistry and nucleic acid chemistry and hybridization described below are those well-known and commonly employed in the art.

[0045] As used herein, the term “about” in reference to a number is generally taken to include numbers that fall within a range of 1%, 5%, or 10% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value).

[0046] As used herein, the “administration” of an agent or drug to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function.

Administration can be carried out by any suitable route, including but not limited to, orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intrathecally, intratumorally or topically. Administration includes self-administration and the administration by another.

[0047] As used herein, the term “antibody” collectively refers to immunoglobulins or immunoglobulin-like molecules including by way of example and without limitation, IgA, IgD, IgE, IgG and IgM, combinations thereof, and similar molecules produced during an immune response in any vertebrate, for example, in mammals such as humans, goats, rabbits and mice, as well as non-mammalian species, such as shark immunoglobulins. As used herein, “antibodies” (includes intact immunoglobulins) and “antigen binding fragments” specifically bind to a molecule of interest (or a group of highly similar molecules of interest) to the substantial exclusion of binding to other molecules (for example, antibodies and antibody fragments that have a binding constant for the molecule of interest that is at least 10^3 M^{-1} greater, at least 10^4 M^{-1} greater or at least 10^5 M^{-1} greater than a binding constant for other molecules in a biological sample). The term “antibody” also includes genetically engineered forms such as chimeric antibodies (for example, humanized murine antibodies), heteroconjugate antibodies (such as, bispecific antibodies). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

[0048] More particularly, antibody refers to a polypeptide ligand comprising at least a light chain immunoglobulin variable region or heavy chain immunoglobulin variable region which specifically recognizes and binds an epitope of an antigen. Antibodies are composed of a heavy and a light chain, each of which has a variable region, termed the variable heavy (V_H) region and the variable light (V_L) region. Together, the V_H region and the V_L region are responsible for binding the antigen recognized by the antibody. Typically, an immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each heavy and light chain contains a constant region and a variable region, (the regions are also known as “domains”). In combination, the heavy and the light chain variable regions specifically bind the antigen. Light and heavy chain variable regions contain a “framework” region interrupted by three hypervariable regions, also called “complementarity-determining regions” or “CDRs”. The extent of the framework region and CDRs have been defined (see, Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and

Human Services, 1991, which is hereby incorporated by reference). The Kabat database is now maintained online. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions.

[0049] The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. An antibody that binds HER2 protein will have a specific V_H region and the V_L region sequence, and thus specific CDR sequences. Antibodies with different specificities (*i.e.* different combining sites for different antigens) have different CDRs. Although it is the CDRs that vary from antibody to antibody, only a limited number of amino acid positions within the CDRs are directly involved in antigen binding. These positions within the CDRs are called specificity determining residues (SDRs). “Immunoglobulin-related compositions” as used herein, refers to antibodies (including monoclonal antibodies, polyclonal antibodies, humanized antibodies, chimeric antibodies, recombinant antibodies, multi-specific antibodies, bispecific antibodies, *etc.*,) as well as antibody fragments. An antibody or antigen binding fragment thereof specifically binds to an antigen.

[0050] As used herein, the term “antibody-related polypeptide” means antigen-binding antibody fragments, including single-chain antibodies, that can comprise the variable region(s) alone, or in combination, with all or part of the following polypeptide elements: hinge region, CH_1 , CH_2 , and CH_3 domains of an antibody molecule. Also included in the technology are any combinations of variable region(s) and hinge region, CH_1 , CH_2 , and CH_3 domains. Antibody-related molecules useful in the present methods, *e.g.*, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain

Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Examples include: (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a F(ab')₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, *Nature* 341: 544-546, 1989), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). As such “antibody fragments” or “antigen binding fragments” can comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments or antigen binding fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multi-specific antibodies formed from antibody fragments.

[0051] “Bispecific antibody” or “BsAb,” as used herein, refers to an antibody that can bind simultaneously to two targets that have a distinct structure, *e.g.*, two different target antigens, two different epitopes on the same target antigen, or a hapten and a target antigen or epitope on a target antigen. A variety of different bispecific antibody structures are known in the art. In some embodiments, each antigen binding moiety in a bispecific antibody includes V_H and/or V_L regions; in some such embodiments, the V_H and/or V_L regions are those found in a particular monoclonal antibody. In some embodiments, the bispecific antibody contains two antigen binding moieties, each including V_H and/or V_L regions from different monoclonal antibodies. In some embodiments, the bispecific antibody contains two antigen binding moieties, wherein one of the two antigen binding moieties includes an immunoglobulin molecule having V_H and/or V_L regions that contain CDRs from a first monoclonal antibody, and the other antigen binding moiety includes an antibody fragment (*e.g.*, Fab, F(ab'), F(ab')₂, Fd, Fv, dAB, scFv, *etc.*) having V_H and/or V_L regions that contain CDRs from a second monoclonal antibody.

[0052] As used herein, the term “antibody-dependent cell-mediated cytotoxicity” or “ADCC”, refers to a mechanism of cell-mediated immune defense whereby an effector cell of the immune

system actively lyses a target cell, such as a tumor cell, whose membrane-surface antigens have been bound by antibodies, such as anti-HER2 antibodies.

[0053] As used herein, an “antigen” refers to a molecule to which an antibody (or antigen binding fragment thereof) can selectively bind. The target antigen may be a protein, carbohydrate, nucleic acid, lipid, hapten, or other naturally occurring or synthetic compound. In some embodiments, the target antigen may be a polypeptide (*e.g.*, a HER2 polypeptide). An antigen may also be administered to an animal to generate an immune response in the animal.

[0054] The term “antigen binding fragment” refers to a fragment of the whole immunoglobulin structure which possesses a part of a polypeptide responsible for binding to antigen. Examples of the antigen binding fragment useful in the present technology include scFv, (scFv)₂, scFvFc, Fab, Fab' and F(ab')₂, but are not limited thereto. Any of the above-noted antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for binding specificity and neutralization activity in the same manner as are intact antibodies.

[0055] As used herein, “binding affinity” means the strength of the total noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen or antigenic peptide). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by standard methods known in the art, including those described herein. A low-affinity complex contains an antibody that generally tends to dissociate readily from the antigen, whereas a high-affinity complex contains an antibody that generally tends to remain bound to the antigen for a longer duration.

[0056] As used herein, the term “biological sample” means sample material derived from living cells. Biological samples may include tissues, cells, protein or membrane extracts of cells, and biological fluids (*e.g.*, ascites fluid or cerebrospinal fluid (CSF)) isolated from a subject, as well as tissues, cells and fluids present within a subject. Biological samples of the present technology include, but are not limited to, samples taken from breast tissue, renal tissue, the uterine cervix, the endometrium, the head or neck, the gallbladder, parotid tissue, the prostate, the brain, the pituitary gland, kidney tissue, muscle, the esophagus, the stomach, the small intestine, the colon,

the liver, the spleen, the pancreas, thyroid tissue, heart tissue, lung tissue, the bladder, adipose tissue, lymph node tissue, the uterus, ovarian tissue, adrenal tissue, testis tissue, the tonsils, thymus, blood, hair, buccal, skin, serum, plasma, CSF, semen, prostate fluid, seminal fluid, urine, feces, sweat, saliva, sputum, mucus, bone marrow, lymph, and tears. Biological samples
5 can also be obtained from biopsies of internal organs or from cancers. Biological samples can be obtained from subjects for diagnosis or research or can be obtained from non-diseased individuals, as controls or for basic research. Samples may be obtained by standard methods including, *e.g.*, venous puncture and surgical biopsy. In certain embodiments, the biological sample is a tissue sample obtained by needle biopsy.

10 **[0057]** As used herein, the term “CDR grafting” means replacing at least one CDR of an “acceptor” antibody with a CDR “graft” from a “donor” antibody possessing a desirable antigen specificity.

[0058] As used herein, the term “chimeric antibody” means an antibody in which the Fc constant region of a monoclonal antibody from one species (*e.g.*, a mouse Fc constant region) is replaced,
15 using recombinant DNA techniques, with an Fc constant region from an antibody of another species (*e.g.*, a human Fc constant region). *See generally*, Robinson *et al.*, PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 0125,023;
20 Better *et al.*, *Science* 240: 1041-1043, 1988; Liu *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 3439-3443, 1987; Liu *et al.*, *J. Immunol* 139: 3521-3526, 1987; Sun *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 214-218, 1987; Nishimura *et al.*, *Cancer Res* 47: 999-1005, 1987; Wood *et al.*, *Nature* 314: 446-449, 1985; and Shaw *et al.*, *J. Natl. Cancer Inst.* 80: 1553-1559, 1988.

[0059] As used herein, the term “complement-dependent cytotoxicity” or “CDC” generally
25 refers to an effector function of IgG and IgM antibodies, which trigger classical complement pathway when bound to a surface antigen, inducing formation of a membrane attack complex and target cell lysis.

[0060] As used herein, the term “conjugated” refers to the association of two molecules by any method known to those in the art. Suitable types of associations include chemical bonds and physical bonds. Chemical bonds include, for example, covalent bonds and coordinate bonds. Physical bonds include, for instance, hydrogen bonds, dipolar interactions, van der Waal forces, electrostatic interactions, hydrophobic interactions and aromatic stacking.

[0061] As used herein, the term “consensus FR” means a framework (FR) antibody region in a consensus immunoglobulin sequence. The FR regions of an antibody do not contact the antigen.

[0062] As used herein, a "control" is an alternative sample used in an experiment for comparison purpose. A control can be "positive" or "negative." For example, where the purpose of the experiment is to determine a correlation of the efficacy of a therapeutic agent for the treatment for a particular type of disease, a positive control (a compound or composition known to exhibit the desired therapeutic effect) and a negative control (a subject or a sample that does not receive the therapy or receives a placebo) are typically employed.

[0063] As used herein, the term “diabodies” refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain ($V_H V_L$). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen binding sites. Diabodies are described more fully in, *e.g.*, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc Natl Acad Sci USA*, 90: 6444-6448 (1993).

[0064] As used herein, the term “effective amount” refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, *e.g.*, an amount which results in the prevention of, or a decrease in a disease or condition described herein or one or more signs or symptoms associated with a disease or condition described herein. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will vary depending on the composition, the degree, type, and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and

other factors. The compositions can also be administered in combination with one or more additional therapeutic compounds. In the methods described herein, the therapeutic compositions may be administered to a subject having one or more signs or symptoms of a disease or condition described herein. As used herein, a "therapeutically effective amount" of a composition refers to composition levels in which the physiological effects of a disease or condition are ameliorated or eliminated. A therapeutically effective amount can be given in one or more administrations.

[0065] As used herein, the term "effector cell" means an immune cell which is involved in the effector phase of an immune response, as opposed to the cognitive and activation phases of an immune response. Exemplary immune cells include a cell of a myeloid or lymphoid origin, *e.g.*, lymphocytes (*e.g.*, B cells and T cells including cytolytic T cells (CTLs)), killer cells, natural killer cells, macrophages, monocytes, eosinophils, neutrophils, polymorphonuclear cells, granulocytes, mast cells, and basophils. Effector cells express specific Fc receptors and carry out specific immune functions. An effector cell can induce antibody-dependent cell-mediated cytotoxicity (ADCC), *e.g.*, a neutrophil capable of inducing ADCC. For example, monocytes, macrophages, neutrophils, eosinophils, and lymphocytes which express Fc α R are involved in specific killing of target cells and presenting antigens to other components of the immune system, or binding to cells that present antigens.

[0066] As used herein, the term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. In some embodiments, an "epitope" of the HER2 protein is a region of the protein to which the anti-HER2 antibodies of the present technology specifically bind. In some embodiments, the epitope is a conformational epitope or a non-conformational epitope. To screen for anti-HER2 antibodies which bind to an epitope, a routine cross-blocking assay such as that described in *Antibodies, A Laboratory Manual*, Cold Spring

Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. This assay can be used to determine if an anti-HER2 antibody binds the same site or epitope as an anti-HER2 antibody of the present technology. Alternatively, or additionally, epitope mapping can be performed by methods known in the art. For example, the antibody sequence can be
5 mutagenized such as by alanine scanning, to identify contact residues. In a different method, peptides corresponding to different regions of HER2 protein can be used in competition assays with the test antibodies or with a test antibody and an antibody with a characterized or known epitope.

[0067] As used herein, “expression” includes one or more of the following: transcription of the
10 gene into precursor mRNA; splicing and other processing of the precursor mRNA to produce mature mRNA; mRNA stability; translation of the mature mRNA into protein (including codon usage and tRNA availability); and glycosylation and/or other modifications of the translation product, if required for proper expression and function.

[0068] As used herein, the term “gene” means a segment of DNA that contains all the
15 information for the regulated biosynthesis of an RNA product, including promoters, exons, introns, and other untranslated regions that control expression.

[0069] As used herein, “homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When
20 a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of
25 “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art. In some embodiments, default parameters are used for alignment. One alignment program is

BLAST, using default parameters. In particular, programs are BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by =HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS

5 translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the National Center for Biotechnology Information. Biologically equivalent polynucleotides are those having the specified percent homology and encoding a polypeptide having the same or similar biological activity. Two sequences are deemed “unrelated” or “non-homologous” if they share less than 40% identity, or less than 25% identity, with each other.

10 **[0070]** As used herein, “humanized” forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some embodiments, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance such as binding affinity. Generally, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (*e.g.*, Fab, Fab', F(ab')₂, or Fv), in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus FR sequence although the FR regions may include one or more amino acid substitutions that improve binding affinity. The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Reichmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See *e.g.*, Ahmed & Cheung, *FEBS Letters* 588(2):288-297 (2014).

[0071] As used herein, the term “hypervariable region” refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a “complementarity determining region” or “CDR” (e.g., around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the V_H (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a “hypervariable loop” (e.g., residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the V_L, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the V_H (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

[0072] As used herein, the terms “identical” or percent “identity”, when used in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region (e.g., nucleotide sequence encoding an antibody described herein or amino acid sequence of an antibody described herein)), when compared and aligned for maximum correspondence over a comparison window or designated region as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (e.g., NCBI web site). Such sequences are then said to be “substantially identical.” This term also refers to, or can be applied to, the complement of a test sequence. The term also includes sequences that have deletions and/or additions, as well as those that have substitutions. In some embodiments, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or 50-100 amino acids or nucleotides in length.

[0073] As used herein, the term “intact antibody” or “intact immunoglobulin” means an antibody that has at least two heavy (H) chain polypeptides and two light (L) chain polypeptides interconnected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH₁, CH₂ and CH₃. Each light chain is

comprised of a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR₁, CDR₁, FR₂, CDR₂, FR₃, CDR₃, FR₄. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies can mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

[0074] As used herein, the term “linker” refers to a functional group (*e.g.*, chemical or polypeptide) that covalently attaches two or more polypeptides or nucleic acids so that they are connected to one another. As used herein, a “peptide linker” refers to one or more amino acids used to couple two proteins together (*e.g.*, to couple V_H and V_L domains). In certain embodiments, the linker comprises amino acids sequence of (GGGGS)_n, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more. In certain embodiments, the linker comprises amino acids having the sequence of GGGGSGGGGSGGGGS (SEQ ID NO: 73) or GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 74).

[0075] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. For example, a monoclonal antibody can be an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including, *e.g.*, but not limited to, hybridoma, recombinant, and phage display technologies. For example, the monoclonal antibodies to be used in accordance with the present methods may be made by the hybridoma method first described by Kohler *et al.*, *Nature* 256:495 (1975), or may be made by recombinant DNA methods (*See, e.g.*, U.S. Patent No. 4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson *et al.*, *Nature* 352:624-628 (1991) and Marks *et al.*, *J. Mol. Biol.* 222:581-597 (1991), for example.

[0076] As used herein, the term “nucleic acid” or “polynucleotide” means any RNA or DNA, which may be unmodified or modified RNA or DNA. Polynucleotides include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, RNA that is mixture of single- and double-stranded regions, and hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons.

[0077] As used herein, the term “pharmaceutically acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal compounds, isotonic and absorption delaying compounds, and the like, compatible with pharmaceutical administration. Pharmaceutically-acceptable carriers and their formulations are known to one skilled in the art and are described, for example, in Remington's Pharmaceutical Sciences (20th edition, ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, Pa.).

[0078] As used herein, the term “polyclonal antibody” means a preparation of antibodies derived from at least two (2) different antibody-producing cell lines. The use of this term includes

preparations of at least two (2) antibodies that contain antibodies that specifically bind to different epitopes or regions of an antigen.

[0079] As used herein, the terms “polypeptide,” “peptide” and “protein” are used interchangeably herein to mean a polymer comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres. Polypeptide refers to both short chains, commonly referred to as peptides, glycopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. Polypeptides include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature.

[0080] As used herein, the term “recombinant” when used with reference, *e.g.*, to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the material is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0081] As used herein, the term “separate” therapeutic use refers to an administration of at least two active ingredients at the same time or at substantially the same time by different routes.

[0082] As used herein, the term “sequential” therapeutic use refers to administration of at least two active ingredients at different times, the administration route being identical or different. More particularly, sequential use refers to the whole administration of one of the active ingredients before administration of the other or others commences. It is thus possible to administer one of the active ingredients over several minutes, hours, or days before administering the other active ingredient or ingredients. There is no simultaneous treatment in this case.

[0083] As used herein, the term “simultaneous” therapeutic use refers to the administration of at least two active ingredients by the same route and at the same time or at substantially the same time.

[0084] As used herein, the terms “single-chain antibodies” or “single-chain Fv (scFv)” refer to an antibody fusion molecule of the two domains of the Fv fragment, V_L and V_H. Single-chain antibody molecules may comprise a polymer with a number of individual molecules, for example, dimer, trimer or other polymers. Furthermore, although the two domains of the Fv fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single-chain Fv (scFv)). Bird *et al.* (1988) *Science* 242:423-426 and Huston *et al.* (1988) *Proc Natl Acad Sci* 85:5879-5883. Such single-chain antibodies can be prepared by recombinant techniques or enzymatic or chemical cleavage of intact antibodies.

[0085] As used herein, “specifically binds” refers to a molecule (*e.g.*, an antibody or antigen binding fragment thereof) which recognizes and binds another molecule (*e.g.*, an antigen), but that does not substantially recognize and bind other molecules. The terms “specific binding,” “specifically binds to,” or is “specific for” a particular molecule (*e.g.*, a polypeptide, or an epitope on a polypeptide), as used herein, can be exhibited, for example, by a molecule having a K_D for the molecule to which it binds to of about 10⁻⁴ M, 10⁻⁵ M, 10⁻⁶ M, 10⁻⁷ M, 10⁻⁸ M, 10⁻⁹ M, 10⁻¹⁰ M, 10⁻¹¹ M, or 10⁻¹² M. The term “specifically binds” may also refer to binding where a molecule (*e.g.*, an antibody or antigen binding fragment thereof) binds to a particular polypeptide (*e.g.*, a HER2 polypeptide), or an epitope on a particular polypeptide, without substantially binding to any other polypeptide, or polypeptide epitope.

[0086] As used herein, the terms “subject”, “patient”, or “individual” can be an individual organism, a vertebrate, a mammal, or a human. In some embodiments, the subject, patient or individual is a human.

[0087] As used herein, the term “therapeutic agent” is intended to mean a compound that, when present in an effective amount, produces a desired therapeutic effect on a subject in need thereof.

[0088] “Treating” or “treatment” as used herein covers the treatment of a disease or disorder described herein, in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, *i.e.*, arresting its development; (ii) relieving a disease or disorder, *i.e.*, causing regression of the disorder; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. In some embodiments, treatment means that the symptoms associated with the disease are, *e.g.*, alleviated, reduced, cured, or placed in a state of remission.

[0089] It is also to be appreciated that the various modes of treatment of disorders as described herein are intended to mean “substantial,” which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic disease or a single, or few time administrations for the treatment of an acute condition.

[0090] Amino acid sequence modification(s) of the anti-HER2 antibodies described herein are contemplated. Such modifications may be performed to improve the binding affinity and/or other biological properties of the antibody, for example, to render the encoded amino acid glycosylated, or to destroy the antibody’s ability to bind to C1q, Fc receptor, or to activate the complement system. Amino acid sequence variants of an anti-HER2 antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, by peptide synthesis, or by chemical modifications. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to obtain the antibody of interest, as long as the obtained antibody possesses the desired properties. The modification also includes the change of the pattern of glycosylation of the protein. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated.

[0091] Conservative amino acid substitutions are amino acid substitutions that change a given amino acid to a different amino acid with similar biochemical properties (*e.g.*, charge, hydrophobicity and size). “Conservative substitutions” are shown in the Table below.

Table 1. Amino Acid Substitutions		
Original Residue	Exemplary Substitutions	Conservative Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

[0092] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Specifically, several hypervariable region sites (e.g., 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with similar or superior properties in one or more relevant assays may be selected for further development.

HER2

[0093] HER2 (GenBank: NP 004439.2 (SEQ ID NO: 84)) 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).

Immunoglobulin-related Compositions of the Present Technology

[0094] The present technology describes methods and compositions for the generation and use of anti-HER2 immunoglobulin-related compositions (*e.g.*, anti-HER2 antibodies or antigen binding fragments thereof). The antibodies and antigen binding fragments of the present technology selectively bind to HER2 polypeptides. The anti-HER2 immunoglobulin-related compositions of the present disclosure may be useful in the diagnosis, or treatment of HER2-associated cancers. Anti-HER2 immunoglobulin-related compositions within the scope of the present technology include, *e.g.*, but are not limited to, monoclonal, chimeric, humanized, bispecific antibodies and diabodies that specifically bind the target polypeptide, a homolog, derivative or a fragment thereof. The present disclosure also provides antigen binding fragments of any of the anti-HER2 antibodies disclosed herein, wherein the antigen binding fragment is selected from the group consisting of Fab, F(ab')₂, Fab', scF_v, and F_v. The amino acid sequences of the anti-HER2 immunoglobulin-related compositions of the present technology are described in **Figures 1-3**.

Region	Sequence Fragment
CDR-H1	GFNIKDTYIH (SEQ ID NO: 1)
CDR-H2	RIYPTNGYTRYADSVKG (SEQ ID NO: 2) R <u>G</u> YPTNGYTRYADSVKG (SEQ ID NO: 7)
CDR-H3	WGGDGFYAMDY (SEQ ID NO: 3) W <u>G</u> DDGFYAMDY (SEQ ID NO: 8)
CDR-L1	RASQDVNTAVA (SEQ ID NO: 4) RASQDV <u>P</u> TAVA (SEQ ID NO: 9)
CDR-L2	SASFLYS (SEQ ID NO: 5) SAS <u>D</u> LYS (SEQ ID NO: 10) <u>D</u> ASFLYS (SEQ ID NO: 11)
CDR-L3	QQHYTTPPT (SEQ ID NO: 6) QQHYT <u>A</u> PPT (SEQ ID NO: 12):

[0095] In one aspect, the present disclosure provides an antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein (a) (i) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 1, a V_H-CDR2 sequence of SEQ ID NO: 2 or SEQ ID NO: 7 and a V_H-CDR3

sequence of SEQ ID NO: 8; or (ii) the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 1, a V_H -CDR2 sequence of SEQ ID NO: 7 and a V_H -CDR3 sequence of SEQ ID NO: 3 or SEQ ID NO: 8; and/or (b) (i) the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 5, SEQ ID NO: 10, or SEQ ID NO: 11, and a V_L -CDR3 sequence of
 5 SEQ ID NO: 6 or SEQ ID NO: 12; or (ii) the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 4 or SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 10, or SEQ ID NO: 11, and a V_L -CDR3 sequence of SEQ ID NO: 6 or SEQ ID NO: 12; or (iii) the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 4 or SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 5, SEQ ID NO: 10, or SEQ ID NO: 11, and a V_L -CDR3 sequence of SEQ ID NO: 12.

10 **[0096]** In one aspect, the present disclosure provides an antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein: (a) the V_H comprises an amino acid sequence selected from any one of SEQ ID NOs: 13, 15, or 17; and/or (b) the V_L comprises an amino acid sequence selected from any one of SEQ ID NOs: 14, 16, 18, 19, or 20. In some
 15 embodiments, the antibody or antigen binding fragment thereof comprises a V_H and a V_L selected from the group consisting of SEQ ID NOs: 13 and 14, SEQ ID NOs: 15 and 16, SEQ ID NOs: 17 and 14, SEQ ID NOs: 15 and 18, SEQ ID NOs: 15 and 19, and SEQ ID NOs: 15 and 20, respectively.

[0097] In any of the above embodiments, the antibody further comprises a Fc domain of any
 20 isotype, *e.g.*, but are not limited to, IgG (including IgG1, IgG2, IgG3, and IgG4), IgA (including IgA₁ and IgA₂), IgD, IgE, or IgM, and IgY. Non-limiting examples of constant region sequences include:

[0098] Human IgD constant region, Uniprot: P01880 (SEQ ID NO: 75)

25 APTKAPDVFPIISGCRHPKDNSPVVLACLITGYHPTSVTVTWYMGTSQSPQRTFPEIQRR
 DSYMTSSQLSTPLQQWRQGEYKCVVQHTASKSKKEIFRWPESPKAQASSVPTAQPQA
 EGS LAKATTAPATTRNTGRGEEKKKEKEEKEEERETKTPECPSHTQPLGVYLLTPAV
 QDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLERHSNGSQSHSRLT
 LPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQAPVKLSLNLASSDPPEAASWLLC
 EVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFWAWSVLRVPAPPSPQPATYT
 30 CVVSHEDSRTLNASRSLEVS YVTDHGPMK

[0099] Human IgG1 constant region, Uniprot: P01857 (SEQ ID NO: 76)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
5 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
DELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00100] Human IgG2 constant region, Uniprot: P01859 (SEQ ID NO: 77)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
10 GLYSLSSVVTVPSSNFGTQTYTCNVNHHKPSNTKVDKTKVERKCCVECPPCPAPPVAGPSV
FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
FRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEM
TKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00101] Human IgG3 constant region, Uniprot: P01860 (SEQ ID NO: 78)

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYTCNVNHHKPSNTKVDKRVELKTPLGDTTHTCPRCPEPKSC
DTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQ
20 DWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMH
EALHNRFTQKSLSLSPGK

[00102] Human IgM constant region, Uniprot: P01871 (SEQ ID NO: 79)

GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITLSWKYKNNSDISSTRGFPSVLR
25 GGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSFVPP
RDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSVTTDQVQAEAKESGPTTYKV
TSTLTIKESDWLGQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKS
TKLTCLVTDLTITYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDDWNNGE
RFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPAD
30 VFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEWNTGETYTCVAHEA
LPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY

[00103] Human IgG4 constant region, Uniprot: P01861 (SEQ ID NO: 80)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYTCNVNHHKPSNTKVDKRVESKYGPCCPSCPAPEFLGGPSV
35 FLFPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEM

TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSRLTVDKSRW
QEGNVFSCSVMHEALHNHYTQKSLSLGLK

[00104] Human IgA1 constant region, Uniprot: P01876 (SEQ ID NO: 81)

ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVARNFPPSQDAS
5 GDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPVPCVPSTPPTPSPSTPPTSPS
CCHPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAVQGPPERDLC
GCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPPPSEEL
ALNELVTLTCLARGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILR
VAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSVVMAEVDGTCY

[00105] Human IgA2 constant region, Uniprot: P01877 (SEQ ID NO: 82)

ASPTSPKVFPLSLDSTPQDGNVVVACLVQGFFPQEPLSVTWSESGQNVARNFPPSQDAS
GDLYTTSSQLTLPATQCPDGKSVTCHVKHYTNPSQDVTVPVPCVPPPPCCHPRLSLHRPA
LEDLLLGSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGPPERDLCGCYSVSSVLPGC
AQPWNHGETFTCTAAHPELKTPLTANITKSGNTFRPEVHLLPPPSEELALNELVTLTCLA
15 RGFSPKDVLRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKKGDT
FSCMVGHEALPLAFTQKTIDRMAGKPTHVNVSVVMAEVDGTCY

[00106] Human Ig kappa constant region, Uniprot: P01834 (SEQ ID NO: 83)

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[00107] In some embodiments, the immunoglobulin-related compositions of the present
technology comprise a heavy chain constant region that is at least 80%, at least 85%, at least
90%, at least 95%, at least 99%, or is 100% identical to SEQ ID NOs: 75-82. Additionally or
alternatively, in some embodiments, the immunoglobulin-related compositions of the present
technology comprise a light chain constant region that is at least 80%, at least 85%, at least 90%,
25 at least 95%, at least 99%, or is 100% identical to SEQ ID NO: 83.

[00108] Additionally or alternatively, in some embodiments, the antibody or antigen binding
fragment binds to the extracellular region of a HER2 polypeptide. In certain embodiments, the
epitope is a conformational epitope or non-conformational epitope.

[00109] In some embodiments, the heavy chain (HC) and light chain (LC) immunoglobulin
30 variable domain sequences are components of the same polypeptide chain. In other
embodiments, the HC and LC immunoglobulin variable domain sequences are components of
different polypeptide chains. In certain embodiments, the antibody is a full-length antibody.

[00110] In some embodiments, the immunoglobulin-related compositions of the present technology bind specifically to at least one HER2 polypeptide. In some embodiments, the immunoglobulin-related compositions of the present technology bind at least one HER2 polypeptide with a dissociation constant (K_D) of about 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. In certain embodiments, the immunoglobulin-related compositions are monoclonal antibodies, chimeric antibodies, humanized antibodies, bispecific antibodies, or multi-specific antibodies. In some embodiments, the antibodies comprise a human antibody framework region.

[00111] In certain embodiments, the immunoglobulin-related compositions contain an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A, K322A, L234A and L235A. Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions contain an IgG4 constant region comprising a S228P mutation.

[00112] In one aspect, the present disclosure provides a multi-specific (*e.g.*, bispecific) antibody comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain and a fourth polypeptide chain, wherein the first and second polypeptide chains are covalently bonded to one another, the second and third polypeptide chains are covalently bonded to one another, and the third and fourth polypeptide chain are covalently bonded to one another, and wherein: (a) each of the first polypeptide chain and the fourth polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a light chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope; (ii) a light chain constant domain of the first immunoglobulin; (iii) a flexible peptide linker comprising the amino acid sequence (GGGS)₃; and (iv) a light chain variable domain of a second immunoglobulin that is linked to a complementary heavy chain variable domain of the second immunoglobulin, or a heavy chain variable domain of a second immunoglobulin that is linked to a complementary light chain variable domain of the second immunoglobulin, wherein the light chain and heavy chain variable domains of the second immunoglobulin are capable of specifically binding to a second epitope, and are linked together via a flexible peptide linker comprising the amino acid

sequence (GGGGS)₆ to form a single-chain variable fragment; and (b) each of the second polypeptide chain and the third polypeptide chain comprises in the N-terminal to C-terminal direction: (i) a heavy chain variable domain of the first immunoglobulin that is capable of specifically binding to the first epitope; and (ii) a heavy chain constant domain of the first immunoglobulin; and wherein the heavy chain variable domain of the first immunoglobulin or the heavy chain variable domain of the second immunoglobulin comprises any one of SEQ ID NOs: 13, 15, or 17, and/or the light chain variable domain of the first immunoglobulin or the light chain variable domain of the second immunoglobulin comprises any one of SEQ ID NOs: 14, 16, 18, 19, or 20.

[00113] In one aspect, the immunoglobulin-related compositions of the present technology comprise a heavy chain (HC) and a light chain (LC) selected from the group consisting of SEQ ID NOs: 21 and 22, SEQ ID NOs: 21 and 23, SEQ ID NOs: 21 and 24, SEQ ID NOs: 21 and 25, SEQ ID NOs: 21 and 26, SEQ ID NOs: 21 and 27, SEQ ID NOs: 21 and 28, SEQ ID NOs: 21 and 29, SEQ ID NOs: 21 and 30, SEQ ID NOs: 21 and 31, SEQ ID NOs: 21 and 32, SEQ ID NOs: 21 and 33, SEQ ID NOs: 34 and 33, SEQ ID NOs: 21 and 35, SEQ ID NOs: 36 and 33, SEQ ID NOs: 21 and 37, SEQ ID NOs: 21 and 38, SEQ ID NOs: 21 and 39, SEQ ID NOs: 21 and 40, SEQ ID NOs: 21 and 41, SEQ ID NOs: 21 and 42, SEQ ID NOs: 21 and 43, SEQ ID NOs: 21 and 44, SEQ ID NOs: 21 and 45, SEQ ID NOs: 21 and 46, SEQ ID NOs: 21 and 47, SEQ ID NOs: 21 and 48, SEQ ID NOs: 21 and 49, SEQ ID NOs: 21 and 50, SEQ ID NOs: 21 and 51, SEQ ID NOs: 21 and 52, SEQ ID NOs: 21 and 53, SEQ ID NOs: 21 and 54, SEQ ID NOs: 21 and 55, SEQ ID NOs: 21 and 56, SEQ ID NOs: 21 and 57, SEQ ID NOs: 21 and 58, SEQ ID NOs: 21 and 59, SEQ ID NOs: 21 and 60, SEQ ID NOs: 21 and 61, SEQ ID NOs: 21 and 62, SEQ ID NOs: 21 and 63, SEQ ID NOs: 21 and 64, SEQ ID NOs: 21 and 65, SEQ ID NOs: 21 and 66, SEQ ID NOs: 21 and 67, SEQ ID NOs: 21 and 68, SEQ ID NOs: 21 and 69, SEQ ID NOs: 21 and 70, SEQ ID NOs: 21 and 71, SEQ ID NOs: 21 and 72, and SEQ ID NOs: 21 and 85, respectively.

[00114] In some aspects, the anti-HER2 immunoglobulin-related compositions described herein contain structural modifications to facilitate rapid binding and cell uptake and/or slow

release. In some aspects, the anti-HER2 immunoglobulin-related composition of the present technology (*e.g.*, an antibody) may contain a deletion in the CH2 constant heavy chain region to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a Fab fragment is used to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a F(ab)₂ fragment is used to facilitate rapid binding and cell uptake and/or slow release.

[00115] In one aspect, the present technology provides a nucleic acid sequence encoding any of the immunoglobulin-related compositions described herein. Also disclosed herein are recombinant nucleic acid sequences encoding any of the antibodies described herein.

[00116] In another aspect, the present technology provides a host cell expressing any nucleic acid sequence encoding any of the immunoglobulin-related compositions described herein.

[00117] The immunoglobulin-related compositions of the present technology (*e.g.*, an anti-HER2 antibody) can be monospecific, bispecific, trispecific or of greater multi-specificity. Multi-specific antibodies can be specific for different epitopes of one or more HER2 polypeptides as well as for heterologous compositions such as a heterologous polypeptide or solid support material. *See, e.g.*, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt *et al.*, *J. Immunol.* 147: 60-69 (1991); U.S. Pat. Nos. 5,573,920, 4,474,893, 5,601,819, 4,714,681, 4,925,648; 6,106,835; Kostelny *et al.*, *J. Immunol.* 148: 1547-1553 (1992). In some embodiments, the immunoglobulin-related compositions are chimeric. In certain embodiments, the immunoglobulin-related compositions are humanized.

[00118] The immunoglobulin-related compositions of the present technology can further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, the immunoglobulin-related compositions of the present technology can be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. *See, e.g.*, WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 0 396 387.

[00119] In any of the above embodiments of the immunoglobulin-related compositions of the present technology, the antibody or antigen binding fragment may be optionally conjugated to an

agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof. For a chemical bond or physical bond, a functional group on the immunoglobulin-related composition typically associates with a functional group on the agent. Alternatively, a functional group on the agent associates with a functional group on the immunoglobulin-related composition.

[00120] The functional groups on the agent and immunoglobulin-related composition can associate directly. For example, a functional group (*e.g.*, a sulfhydryl group) on an agent can associate with a functional group (*e.g.*, sulfhydryl group) on an immunoglobulin-related composition to form a disulfide. Alternatively, the functional groups can associate through a cross-linking agent (*i.e.*, linker). Some examples of cross-linking agents are described below. The cross-linker can be attached to either the agent or the immunoglobulin-related composition. The number of agents or immunoglobulin-related compositions in a conjugate is also limited by the number of functional groups present on the other. For example, the maximum number of agents associated with a conjugate depends on the number of functional groups present on the immunoglobulin-related composition. Alternatively, the maximum number of immunoglobulin-related compositions associated with an agent depends on the number of functional groups present on the agent.

[00121] In yet another embodiment, the conjugate comprises one immunoglobulin-related composition associated to one agent. In one embodiment, a conjugate comprises at least one agent chemically bonded (*e.g.*, conjugated) to at least one immunoglobulin-related composition. The agent can be chemically bonded to an immunoglobulin-related composition by any method known to those in the art. For example, a functional group on the agent may be directly attached to a functional group on the immunoglobulin-related composition. Some examples of suitable functional groups include, for example, amino, carboxyl, sulfhydryl, maleimide, isocyanate, isothiocyanate and hydroxyl.

[00122] The agent may also be chemically bonded to the immunoglobulin-related composition by means of cross-linking agents, such as dialdehydes, carbodiimides, dimaleimides, and the

like. Cross-linking agents can, for example, be obtained from Pierce Biotechnology, Inc., Rockford, Ill. The Pierce Biotechnology, Inc. web-site can provide assistance. Additional cross-linking agents include the platinum cross-linking agents described in U.S. Pat. Nos. 5,580,990; 5,985,566; and 6,133,038 of Kreatech Biotechnology, B.V., Amsterdam, The Netherlands.

5 **[00123]** Alternatively, the functional group on the agent and immunoglobulin-related composition can be the same. Homobifunctional cross-linkers are typically used to cross-link identical functional groups. Examples of homobifunctional cross-linkers include EGS (*i.e.*, ethylene glycol bis[succinimidylsuccinate]), DSS (*i.e.*, disuccinimidyl suberate), DMA (*i.e.*, dimethyl adipimidate.2HCl), DTSSP (*i.e.*, 3,3'-dithiobis[sulfosuccinimidylpropionate]), DPDPB
10 (*i.e.*, 1,4-di-[3'-(2'-pyridyldithio)-propionamido]butane), and BMH (*i.e.*, bis-maleimidohexane). Such homobifunctional cross-linkers are also available from Pierce Biotechnology, Inc.

[00124] In other instances, it may be beneficial to cleave the agent from the immunoglobulin-related composition. The web-site of Pierce Biotechnology, Inc. described above can also provide assistance to one skilled in the art in choosing suitable cross-linkers which can be
15 cleaved by, for example, enzymes in the cell. Thus the agent can be separated from the immunoglobulin-related composition. Examples of cleavable linkers include SMPT (*i.e.*, 4-succinimidylloxycarbonyl-methyl-a-[2-pyridyldithio]toluene), Sulfo-LC-SPDP (*i.e.*, sulfosuccinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), LC-SPDP (*i.e.*, succinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), Sulfo-LC-SPDP (*i.e.*, sulfosuccinimidyl 6-(3-
20 [2-pyridyldithio]-propionamido)hexanoate), SPDP (*i.e.*, N-succinimidyl 3-[2-pyridyldithio]-propionamidohexanoate), and AEDP (*i.e.*, 3-[(2-aminoethyl)dithio]propionic acid HCl).

[00125] In another embodiment, a conjugate comprises at least one agent physically bonded with at least one immunoglobulin-related composition. Any method known to those in the art can be employed to physically bond the agents with the immunoglobulin-related compositions.

25 For example, the immunoglobulin-related compositions and agents can be mixed together by any method known to those in the art. The order of mixing is not important. For instance, agents can be physically mixed with immunoglobulin-related compositions by any method known to those in the art. For example, the immunoglobulin-related compositions and agents can be placed in a

container and agitated, by for example, shaking the container, to mix the immunoglobulin-related compositions and agents.

[00126] The immunoglobulin-related compositions can be modified by any method known to those in the art. For instance, the immunoglobulin-related composition may be modified by means of cross-linking agents or functional groups, as described above.

A. Methods of Preparing Anti-HER2 Antibodies of the Present Technology

[00127] *General Overview.* Initially, a target polypeptide is chosen to which an antibody of the present technology can be raised. For example, an antibody may be raised against the full-length HER2 protein, or to a portion of the extracellular domain of the HER2 protein.

Techniques for generating antibodies directed to such target polypeptides are well known to those skilled in the art. Examples of such techniques include, for example, but are not limited to, those involving display libraries, xeno or human mice, hybridomas, and the like. Target polypeptides within the scope of the present technology include any polypeptide derived from HER2 protein containing the extracellular domain which is capable of eliciting an immune response.

[00128] It should be understood that recombinantly engineered antibodies and antibody fragments, *e.g.*, antibody-related polypeptides, which are directed to HER2 protein and fragments thereof are suitable for use in accordance with the present disclosure.

[00129] Anti-HER2 antibodies that can be subjected to the techniques set forth herein include monoclonal and polyclonal antibodies, and antibody fragments such as Fab, Fab', F(ab')₂, Fd, scFv, diabodies, antibody light chains, antibody heavy chains and/or antibody fragments. Methods useful for the high yield production of antibody Fv-containing polypeptides, *e.g.*, Fab' and F(ab')₂ antibody fragments have been described. *See* U.S. Pat. No. 5,648,237.

[00130] Generally, an antibody is obtained from an originating species. More particularly, the nucleic acid or amino acid sequence of the variable portion of the light chain, heavy chain or both, of an originating species antibody having specificity for a target polypeptide antigen is obtained. An originating species is any species which was useful to generate the antibody of the

present technology or library of antibodies, *e.g.*, rat, mouse, rabbit, chicken, monkey, human, and the like.

[00131] Phage or phagemid display technologies are useful techniques to derive the antibodies of the present technology. Techniques for generating and cloning monoclonal antibodies are well known to those skilled in the art. Expression of sequences encoding antibodies of the present technology, can be carried out in *E. coli*.

[00132] Due to the degeneracy of nucleic acid coding sequences, other sequences which encode substantially the same amino acid sequences as those of the naturally occurring proteins may be used in the practice of the present technology. These include, but are not limited to, nucleic acid sequences including all or portions of the nucleic acid sequences encoding the above polypeptides, which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. It is appreciated that the nucleotide sequence of an immunoglobulin according to the present technology tolerates sequence homology variations of up to 25% as calculated by standard methods ("Current Methods in Sequence Comparison and Analysis," *Macromolecule Sequencing and Synthesis, Selected Methods and Applications*, pp. 127-149, 1998, Alan R. Liss, Inc.) so long as such a variant forms an operative antibody which recognizes HER2 proteins. For example, one or more amino acid residues within a polypeptide sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the present technology are proteins or fragments or derivatives thereof which are differentially modified during or after translation, *e.g.*, by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligands, *etc.*

Additionally, an immunoglobulin encoding nucleic acid sequence can be mutated *in vitro* or *in vivo* to create and/or destroy translation, initiation, and/or termination sequences or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to *in vitro* site directed mutagenesis, *J. Biol. Chem.* 253:6551, use of Tab linkers (Pharmacia), and the like.

[00133] *Preparation of Polyclonal Antisera and Immunogens.* Methods of generating antibodies or antibody fragments of the present technology typically include immunizing a subject (generally a non-human subject such as a mouse or rabbit) with a purified HER2 protein or fragment thereof, or with a cell expressing the HER2 protein or fragment thereof. An appropriate immunogenic preparation can contain, *e.g.*, a recombinantly-expressed HER2 protein or a chemically-synthesized HER2 peptide. The extracellular domain of the HER2 protein, or a portion or fragment thereof, can be used as an immunogen to generate an anti-HER2 antibody that binds to the HER2 protein, or a portion or fragment thereof using standard techniques for polyclonal and monoclonal antibody preparation.

[00134] In some embodiments, the antigenic HER2 peptide comprises at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 amino acid residues. Longer antigenic peptides are sometimes desirable over shorter antigenic peptides, depending on use and according to methods well known to those skilled in the art. Multimers of a given epitope are sometimes more effective than a monomer.

[00135] If needed, the immunogenicity of the HER2 protein (or fragment thereof) can be increased by fusion or conjugation to a carrier protein such as keyhole limpet hemocyanin (KLH) or ovalbumin (OVA). Many such carrier proteins are known in the art. One can also combine the HER2 protein with a conventional adjuvant such as Freund's complete or incomplete adjuvant to increase the subject's immune reaction to the polypeptide. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, *etc.*), human

adjuvants such as Bacille Calmette-Guerin and *Corynebacterium parvum*, or similar immunostimulatory compounds. These techniques are standard in the art.

[00136] Alternatively, nanoparticles, for example, virus-like particles (VLPs), can be used to present antigens, *e.g.*, HER2, to a host animal. Virus-like particles are multiprotein structures that mimic the organization and conformation of authentic native viruses without being infectious, since they do not carry any viral genetic material (Urakami A, *et al*, *Clin Vaccine Immunol* 24: e00090-17 (2017)). When introduced to a host immune system, VLPs can evoke effective immune responses, making them attractive carriers of foreign antigens. An important advantage of a VLP-based antigen presenting platform is that it can display antigens in a dense, repetitive manner. Thus, antigen-bearing VLPs are able to induce strong B-cell responses by effectively enabling the cross-linking of B cell receptors (BCRs). VLPs may be genetically manipulated to fine their properties, *e.g.*, immunogenicity. These techniques are standard in the art.

[00137] The isolation of sufficient purified protein or polypeptide to which an antibody is to be raised may be time consuming and sometimes technically challenging. Additional challenges associated with conventional protein-based immunization include concerns over safety, stability, scalability and consistency of the protein antigen. Nucleic acid (DNA and RNA) based immunizations have emerged as promising alternatives. DNA vaccines are usually based on bacterial plasmids that encode the polypeptide sequence of candidate antigen, *e.g.*, HER2. With a robust eukaryotic promoter, the encoded antigen can be expressed to yield enough levels of transgene expression once the host is inoculated with the plasmids (Galvin T.A., *et al.*, *Vaccine* 2000, 18:2566-2583). Modern DNA vaccine generation relies on DNA synthesis, possibly one-step cloning into the plasmid vector and subsequent isolation of the plasmid, which takes significantly less time and cost to manufacture. The resulting plasmid DNA is also highly stable at room temperature, avoiding cold transportation and leading to substantially extended shelf-life. These techniques are standard in the art.

[00138] Alternatively, nucleic acid sequences encoding the antigen of interest, *e.g.*, HER2, can be synthetically introduced into a mRNA molecule. The mRNA is then delivered into a host

animal, whose cells would recognize and translate the mRNA sequence to the polypeptide sequence of the candidate antigen, *e.g.*, HER2, thus triggering the immune response to the foreign antigen. An attractive feature of mRNA antigen or mRNA vaccine is that mRNA is a non-infectious, non-integrating platform. There is no potential risk of infection or insertional mutagenesis associated with DNA vaccines. In addition, mRNA is degraded by normal cellular processes and has a controllable *in vivo* half-life through modification of design and delivery methods (Kariko, K., *et al.*, *Mol Ther* 16: 1833–1840 (2008); Kauffman, K. J., *et al.*, *J Control Release* 240, 227–234 (2016); Guan, S. & Rosenecker, J., *Gene Ther* 24, 133–143 (2017); Thess, A., *et al.*, *Mol Ther* 23, 1456–1464 (2015)). These techniques are standard in the art.

[00139] In describing the present technology, immune responses may be described as either “primary” or “secondary” immune responses. A primary immune response, which is also described as a “protective” immune response, refers to an immune response produced in an individual as a result of some initial exposure (*e.g.*, the initial “immunization” or “priming”) to a particular antigen, *e.g.*, HER2 protein. In some embodiments, the immunization can occur as a result of vaccinating the individual with a vaccine containing the antigen. For example, the vaccine can be a HER2 vaccine comprising one or more HER2 protein-derived antigens. A primary immune response can become weakened or attenuated over time and can even disappear or at least become so attenuated that it cannot be detected. Accordingly, the present technology also relates to a “secondary” immune response, which is also described here as a “memory immune response.” The term secondary immune response refers to an immune response elicited in an individual after a primary immune response has already been produced.

[00140] Thus, a secondary immune response can be elicited, *e.g.*, to enhance an existing immune response that has become weakened or attenuated (*e.g.*, boosting), or to recreate a previous immune response that has either disappeared or can no longer be detected. The secondary or memory immune response can be either a humoral (antibody) response or a cellular response. A secondary or memory humoral response occurs upon stimulation of memory B cells that were generated at the first presentation of the antigen. Delayed type hypersensitivity (DTH) reactions are a type of cellular secondary or memory immune response that are mediated by

CD4⁺ T cells. A first exposure to an antigen primes the immune system and additional exposure(s) results in a DTH.

[00141] Following appropriate immunization, the anti-HER2 antibody can be prepared from the subject's serum. If desired, the antibody molecules directed against the HER2 protein can be
5 isolated from the mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as polypeptide A chromatography to obtain the IgG fraction.

[00142] *Monoclonal Antibody*. In one embodiment of the present technology, the antibody is an anti-HER2 monoclonal antibody. For example, in some embodiments, the anti-HER2 monoclonal antibody may be a human or a mouse anti-HER2 monoclonal antibody. For
10 preparation of monoclonal antibodies directed towards the HER2 protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture can be utilized. Such techniques include, but are not limited to, the hybridoma technique (*See, e.g.*, Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (*See, e.g.*, Kozbor, *et al.*,
15 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (*See, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies can be utilized in the practice of the present technology and can be produced by using human hybridomas (*See, e.g.*, Cote, *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80: 2026-2030) or by transforming human B-cells
20 with Epstein Barr Virus *in vitro* (*See, e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). For example, a population of nucleic acids that encode regions of antibodies can be isolated. PCR utilizing primers derived from sequences encoding conserved regions of antibodies is used to amplify sequences encoding portions of antibodies from the population and then DNAs encoding
25 antibodies or fragments thereof, such as variable domains, are reconstructed from the amplified sequences. Such amplified sequences also can be fused to DNAs encoding other proteins – *e.g.*, a bacteriophage coat, or a bacterial cell surface protein – for expression and display of the fusion polypeptides on phage or bacteria. Amplified sequences can then be expressed and further

selected or isolated based, *e.g.*, on the affinity of the expressed antibody or fragment thereof for an antigen or epitope present on the HER2 protein. Alternatively, hybridomas expressing anti-HER2 monoclonal antibodies can be prepared by immunizing a subject and then isolating hybridomas from the subject's spleen using routine methods. *See, e.g.*, Milstein *et al.*, (Galfre and Milstein, *Methods Enzymol* (1981) 73: 3-46). Screening the hybridomas using standard methods will produce monoclonal antibodies of varying specificity (*i.e.*, for different epitopes) and affinity. A selected monoclonal antibody with the desired properties, *e.g.*, HER2 binding, can be used as expressed by the hybridoma, it can be bound to a molecule such as polyethylene glycol (PEG) to alter its properties, or a cDNA encoding it can be isolated, sequenced and manipulated in various ways. Synthetic dendromeric trees can be added to reactive amino acid side chains, *e.g.*, lysine, to enhance the immunogenic properties of HER2 protein. Also, CPG-dinucleotide techniques can be used to enhance the immunogenic properties of the HER2 protein. Other manipulations include substituting or deleting particular amino acyl residues that contribute to instability of the antibody during storage or after administration to a subject, and affinity maturation techniques to improve affinity of the antibody of the HER2 protein.

[00143] *Hybridoma Technique.* In some embodiments, the antibody of the present technology is an anti-HER2 monoclonal antibody produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell.

Hybridoma techniques include those known in the art and taught in Harlow *et al.*, *Antibodies: A Laboratory Manual* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 349 (1988); Hammerling *et al.*, *Monoclonal Antibodies And T-Cell Hybridomas*, 563-681 (1981). Other methods for producing hybridomas and monoclonal antibodies are well known to those of skill in the art.

[00144] *Phage Display Technique.* As noted above, the antibodies of the present technology can be produced through the application of recombinant DNA and phage display technology. For example, anti-HER2 antibodies, can be prepared using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the

surface of a phage particle which carries polynucleotide sequences encoding them. Phages with a desired binding property are selected from a repertoire or combinatorial antibody library (*e.g.*, human or murine) by selecting directly with an antigen, typically an antigen bound or captured to a solid surface or bead. Phages used in these methods are typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains that are recombinantly fused to either the phage gene III or gene VIII protein. In addition, methods can be adapted for the construction of Fab expression libraries (*See, e.g.*, Huse, *et al.*, *Science* 246: 1275-1281, 1989) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a HER2 polypeptide, *e.g.*, a polypeptide or derivatives, fragments, analogs or homologs thereof. Other examples of phage display methods that can be used to make the antibodies of the present technology include those disclosed in Huston *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, 85: 5879-5883, 1988; Chaudhary *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, 87: 1066-1070, 1990; Brinkman *et al.*, *J. Immunol. Methods* 182: 41-50, 1995; Ames *et al.*, *J. Immunol. Methods* 184: 177-186, 1995; Kettleborough *et al.*, *Eur. J. Immunol.* 24: 952-958, 1994; Persic *et al.*, *Gene* 187: 9-18, 1997; Burton *et al.*, *Advances in Immunology* 57: 191-280, 1994; PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; WO 96/06213; WO 92/01047 (Medical Research Council *et al.*); WO 97/08320 (Morphosys); WO 92/01047 (CAT/MRC); WO 91/17271 (Affymax); and U.S. Pat. Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727 and 5,733,743. Methods useful for displaying polypeptides on the surface of bacteriophage particles by attaching the polypeptides *via* disulfide bonds have been described by Lohning, U.S. Pat. No. 6,753,136. As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax *et al.*, *BioTechniques* 12: 864-869, 1992; and Sawai *et al.*, *AJRI* 34: 26-34, 1995; and Better *et al.*, *Science* 240: 1041-1043, 1988.

[00145] Generally, hybrid antibodies or hybrid antibody fragments that are cloned into a display vector can be selected against the appropriate antigen in order to identify variants that maintain good binding activity, because the antibody or antibody fragment will be present on the surface of the phage or phagemid particle. *See, e.g.,* Barbas III *et al.*, *Phage Display, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001).
5 However, other vector formats could be used for this process, such as cloning the antibody fragment library into a lytic phage vector (modified T7 or Lambda Zap systems) for selection and/or screening.

[00146] *Expression of Recombinant Anti-HER2 Antibodies.* As noted above, the antibodies of
10 the present technology can be produced through the application of recombinant DNA technology. Recombinant polynucleotide constructs encoding an anti-HER2 antibody of the present technology typically include an expression control sequence operably-linked to the coding sequences of anti-HER2 antibody chains, including naturally-associated or heterologous promoter regions. As such, another aspect of the technology includes vectors containing one or
15 more nucleic acid sequences encoding an anti-HER2 antibody of the present technology. For recombinant expression of one or more of the polypeptides of the present technology, the nucleic acid containing all or a portion of the nucleotide sequence encoding the anti-HER2 antibody is inserted into an appropriate cloning vector, or an expression vector (*i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted polypeptide coding
20 sequence) by recombinant DNA techniques well known in the art and as detailed below. Methods for producing diverse populations of vectors have been described by Lerner *et al.*, U.S. Pat. Nos. 6,291,160 and 6,680,192.

[00147] In general, expression vectors useful in recombinant DNA techniques are often in the form of plasmids. In the present disclosure, “plasmid” and “vector” can be used interchangeably
25 as the plasmid is the most commonly used form of vector. However, the present technology is intended to include such other forms of expression vectors that are not technically plasmids, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions. Such viral vectors permit infection of a subject and

expression of a construct in that subject. In some embodiments, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences encoding the anti-HER2 antibody, and the collection and purification of the anti-HER2 antibody, *e.g.*, cross-reacting anti-HER2 antibodies. *See generally*, U.S. 2002/0199213. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers, *e.g.*, ampicillin-resistance or hygromycin-resistance, to permit detection of those cells transformed with the desired DNA sequences. Vectors can also encode signal peptide, *e.g.*, pectate lyase, useful to direct the secretion of extracellular antibody fragments. *See* U.S. Pat. No. 5,576,195.

[00148] The recombinant expression vectors of the present technology comprise a nucleic acid encoding a protein with HER2 binding properties in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression that is operably-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, “operably-linked” is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, *e.g.*, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, *etc.* Typical regulatory sequences useful as promoters of recombinant polypeptide expression (*e.g.*, anti-HER2 antibody), include, *e.g.*, but

are not limited to, promoters of 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization. In one embodiment, a polynucleotide encoding an anti-HER2 antibody of the present technology is operably-linked to an *ara B* promoter and expressible in a host cell. *See* U.S. Pat. 5,028,530. The expression vectors of the present technology can be introduced into host cells to thereby produce polypeptides or peptides, including fusion polypeptides, encoded by nucleic acids as described herein (*e.g.*, anti-HER2 antibody, *etc.*).

[00149] Another aspect of the present technology pertains to anti-HER2 antibody-expressing host cells, which contain a nucleic acid encoding one or more anti-HER2 antibodies. The recombinant expression vectors of the present technology can be designed for expression of an anti-HER2 antibody in prokaryotic or eukaryotic cells. For example, an anti-HER2 antibody can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors), fungal cells, *e.g.*, yeast, yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, *e.g.*, using T7 promoter regulatory sequences and T7 polymerase. Methods useful for the preparation and screening of polypeptides having a predetermined property, *e.g.*, anti-HER2 antibody, *via* expression of stochastically generated polynucleotide sequences has been previously described. *See* U.S. Pat. Nos. 5,763,192; 5,723,323; 5,814,476; 5,817,483; 5,824,514; 5,976,862; 6,492,107; 6,569,641.

[00150] Expression of polypeptides in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant polypeptide; (ii) to increase the solubility of the recombinant polypeptide; and (iii) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion

expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion polypeptide. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding polypeptide, or polypeptide A, respectively, to the target recombinant polypeptide.

[00151] Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69: 301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89). Methods for targeted assembly of distinct active peptide or protein domains to yield multifunctional polypeptides *via* polypeptide fusion has been described by Pack *et al.*, U.S. Pat. Nos. 6,294,353; 6,692,935. One strategy to maximize recombinant polypeptide expression, *e.g.*, an anti-HER2 antibody, in *E. coli* is to express the polypeptide in host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide. *See, e.g.*, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the expression host, *e.g.*, *E. coli* (*See, e.g.*, Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the present technology can be carried out by standard DNA synthesis techniques.

[00152] In another embodiment, the anti-HER2 antibody expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, *Cell* 30: 933-943, 1982), pJRY88 (Schultz *et al.*, *Gene* 54: 113-123, 1987), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.). Alternatively, an anti-HER2 antibody can be expressed in insect cells using baculovirus expression vectors.

Baculovirus vectors available for expression of polypeptides, *e.g.*, anti-HER2 antibody, in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, *Mol. Cell. Biol.* 3: 2156-2165, 1983) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

[00153] In yet another embodiment, a nucleic acid encoding an anti-HER2 antibody of the present technology is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include, *e.g.*, but are not limited to, pCDM8 (Seed, *Nature* 329: 840, 1987) and pMT2PC (Kaufman, *et al.*, *EMBO J.* 6: 187-195, 1987). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells that are useful for expression of the anti-HER2 antibody of the present technology, see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

[00154] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid in a particular cell type (*e.g.*, tissue-specific regulatory elements). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, *Genes Dev.* 1: 268-277, 1987), lymphoid-specific promoters (Calame and Eaton, *Adv. Immunol.* 43: 235-275, 1988), promoters of T cell receptors (Winoto and Baltimore, *EMBO J.* 8: 729-733, 1989) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, *Cell* 33: 741-748, 1983.), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, *Proc. Natl. Acad. Sci. USA* 86: 5473-5477, 1989), pancreas-specific promoters (Edlund, *et al.*, 1985. *Science* 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, *Science* 249: 374-379, 1990) and the α -fetoprotein promoter (Campes and Tilghman, *Genes Dev.* 3: 537-546, 1989).

[00155] Another aspect of the present methods pertains to host cells into which a recombinant expression vector of the present technology has been introduced. The terms “host cell” and “recombinant host cell” are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell.

5 Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00156] A host cell can be any prokaryotic or eukaryotic cell. For example, an anti-HER2 antibody can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian
10 cells. Mammalian cells are a suitable host for expressing nucleotide segments encoding immunoglobulins or fragments thereof. See Winnacker, *From Genes To Clones*, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include Chinese hamster ovary (CHO) cell lines, various COS cell lines, HeLa cells, L cells and myeloma cell lines. In some
15 embodiments, the cells are non-human. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Queen *et al.*, *Immunol. Rev.* 89: 49, 1986. Illustrative expression control sequences are promoters derived from endogenous genes,
20 cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. Co *et al.*, *J Immunol.* 148: 1149, 1992. Other suitable host cells are known to those skilled in the art.

[00157] Vector DNA can be introduced into prokaryotic or eukaryotic cells *via* conventional transformation or transfection techniques. As used herein, the terms “transformation” and “transfection” are intended to refer to a variety of art-recognized techniques for introducing
25 foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, biolistics or viral-based transfection. Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection (See

generally, Sambrook *et al.*, *Molecular Cloning*). Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals. The vectors
5 containing the DNA segments of interest can be transferred into the host cell by well-known methods, depending on the type of cellular host.

[00158] 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
10 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,
15 pMAM and pMAMneo (Clontech). The TOPO cloning system (Invitrogen, Calsbad, CA, Carlsbad, CA) can also be used in accordance with the manufacturer's recommendations.

[00159] 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 antibody-coding sequence, and signals required for the termination
20 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,
25 HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). Cellular elements can also be used (*e.g.*, the human actin promoter). Non-limiting examples of mammalian expression vectors include, vectors such as pIRESneo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clonetechn Labs, Palo Alto, Calif.), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro

(+/-) (Invitrogen, Calsbad, CA), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Non-limiting examples of mammalian host cells that can be used in combination with such mammalian vectors include human Hela 293, HEK 293, H9 and Jurkat cells, mouse 3T3, NIH3T3 and C127 cells, 5 Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

[00160] 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 10 (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.*, 15 Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994).

[00161] 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 an anti-HER2 antibody or antigen binding 20 fragment described herein. Accordingly, provided herein are cells comprising a polynucleotide encoding an anti-HER2 antibody or antigen binding fragment thereof operably linked to a regulatory expression element (*e.g.*, 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 operably linked to a promoter can be co-expressed in the cell for 25 expression of the entire anti-HER2 antibody or antigen binding fragment. In certain embodiments, a cell comprises a vector comprising a polynucleotide encoding both the heavy chain and the light chain of an anti-HER2 antibody or antigen binding fragment described herein that are 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 operably linked to a promoter. In certain embodiments, a first cell comprises a first vector comprising a polynucleotide encoding a heavy chain of an anti-HER2 antibody or antigen binding fragment described herein, and a second cell comprises a second vector comprising a polynucleotide encoding a light chain of an anti-HER2 antibody or antigen binding fragment described herein. In certain embodiments, provided herein is a mixture of cells comprising said first cell and said second cell. 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. aeruginosa*, *S. cerevisiae*, *N. crassa*, insect cells (e.g., Sf9, Ea4) and the like.

[00162] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding the anti-HER2 antibody or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

[00163] A host cell that includes an anti-HER2 antibody of the present technology, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) recombinant anti-HER2 antibody. In one embodiment, the method comprises culturing the host cell (into which a recombinant expression vector encoding the anti-HER2 antibody has been introduced) in a suitable medium such that the anti-HER2 antibody is produced. In another embodiment, the method further comprises the step of isolating the anti-HER2 antibody from the medium or the host cell. Once expressed, collections of the anti-HER2 antibody, *e.g.*, the anti-HER2 antibodies or the anti-HER2 antibody-related polypeptides are purified from culture media and host cells.

The anti-HER2 antibody can be purified according to standard procedures of the art, including HPLC purification, column chromatography, gel electrophoresis and the like. In one embodiment, the anti-HER2 antibody is produced in a host organism by the method of Boss *et al.*, U.S. Pat. No. 4,816,397. Usually, anti-HER2 antibody chains are expressed with signal sequences and are thus released to the culture media. However, if the anti-HER2 antibody chains are not naturally secreted by host cells, the anti-HER2 antibody chains can be released by treatment with mild detergent. Purification of recombinant polypeptides is well known in the art and includes ammonium sulfate precipitation, affinity chromatography purification technique, column chromatography, ion exchange purification technique, gel electrophoresis and the like (See generally Scopes, Protein Purification (Springer-Verlag, N.Y., 1982).

[00164] Polynucleotides encoding anti-HER2 antibodies, *e.g.*, the anti-HER2 antibody coding sequences, can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal. See, *e.g.*, U.S. Pat. Nos. 5,741,957, 5,304,489, and 5,849,992. Suitable transgenes include coding sequences for light and/or heavy chains in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or β -lactoglobulin. For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

[00165] *Single-Chain Antibodies.* In one embodiment, the anti-HER2 antibody of the present technology is a single-chain anti-HER2 antibody. According to the present technology, techniques can be adapted for the production of single-chain antibodies specific to a HER2 protein (See, *e.g.*, U.S. Pat. No. 4,946,778). Examples of techniques which can be used to produce single-chain Fvs and antibodies of the present technology include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston *et al.*, *Methods in Enzymology*, 203: 46-88, 1991; Shu, L. *et al.*, *Proc. Natl. Acad. Sci. USA*, 90: 7995-7999, 1993; and Skerra *et al.*, *Science* 240: 1038-1040, 1988.

[00166] *Chimeric and Humanized Antibodies.* In one embodiment, the anti-HER2 antibody of the present technology is a chimeric anti-HER2 antibody. In one embodiment, the anti-HER2

antibody of the present technology is a humanized anti-HER2 antibody. In one embodiment of the present technology, the donor and acceptor antibodies are monoclonal antibodies from different species. For example, the acceptor antibody is a human antibody (to minimize its antigenicity in a human), in which case the resulting CDR-grafted antibody is termed a
5 “humanized” antibody.

[00167] Recombinant anti-HER2 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, can be made using standard recombinant DNA techniques, and are within the scope of the present technology. For some uses, including *in vivo* use of the anti-HER2 antibody of the present technology in humans as
10 well as use of these agents in *in vitro* detection assays, it is possible to use chimeric or humanized anti-HER2 antibodies. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art. Such useful methods include, *e.g.*, but are not limited to, methods described in International Application No. PCT/US86/02269; U.S. Pat. No. 5,225,539; European Patent No. 184187; European Patent No. 171496; European
15 Patent No. 173494; PCT International Publication No. WO 86/01533; U.S. Pat. Nos. 4,816,567; 5,225,539; European Patent No. 125023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987. *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314: 446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.*
20 80: 1553-1559; Morrison (1985) *Science* 229: 1202-1207; Oi, *et al.* (1986) *BioTechniques* 4: 214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; Morrison, *Science* 229: 1202, 1985; Oi *et al.*, *BioTechniques* 4: 214, 1986; Gillies *et al.*, *J. Immunol. Methods*, 125: 191-202, 1989; U.S. Pat. No. 5,807,715; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. For example, antibodies can be humanized using a variety of
25 techniques including CDR-grafting (EP 0 239 400; WO 91/09967; U.S. Pat. No. 5,530,101; 5,585,089; 5,859,205; 6,248,516; EP460167), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E. A., *Molecular Immunology*, 28: 489-498, 1991; Studnicka *et al.*, *Protein Engineering* 7: 805-814, 1994; Roguska *et al.*, *PNAS* 91: 969-973, 1994), and chain shuffling (U.S. Pat. No. 5,565,332). In one embodiment, a cDNA encoding a murine anti-HER2

monoclonal antibody is digested with a restriction enzyme selected specifically to remove the sequence encoding the Fc constant region, and the equivalent portion of a cDNA encoding a human Fc constant region is substituted (*See Robinson et al.*, PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988) *Science* 240: 1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 3439-3443; Liu *et al.* (1987) *J Immunol* 139: 3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura *et al.* (1987) *Cancer Res* 47: 999-1005; Wood *et al.* (1985) *Nature* 314: 446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80: 1553-1559; U.S. Pat. No. 6,180,370; U.S. Pat. Nos. 6,300,064; 6,696,248; 6,706,484; 6,828,422.

[00168] In one embodiment, the present technology provides the construction of humanized anti-HER2 antibodies that are unlikely to induce a human anti-mouse antibody (hereinafter referred to as “HAMA”) response, while still having an effective antibody effector function. As used herein, the terms “human” and “humanized”, in relation to antibodies, relate to any antibody which is expected to elicit a therapeutically tolerable weak immunogenic response in a human subject. In one embodiment, the present technology provides for a humanized anti-HER2 antibodies, heavy and light chain immunoglobulins.

[00169] *CDR Antibodies.* In some embodiments, the anti-HER2 antibody of the present technology is an anti-HER2 CDR antibody. Generally the donor and acceptor antibodies used to generate the anti-HER2 CDR antibody are monoclonal antibodies from different species; typically the acceptor antibody is a human antibody (to minimize its antigenicity in a human), in which case the resulting CDR-grafted antibody is termed a “humanized” antibody. The graft may be of a single CDR (or even a portion of a single CDR) within a single V_H or V_L of the acceptor antibody, or can be of multiple CDRs (or portions thereof) within one or both of the V_H and V_L. Frequently, all three CDRs in all variable domains of the acceptor antibody will be replaced with the corresponding donor CDRs, though one needs to replace only as many as necessary to permit adequate binding of the resulting CDR-grafted antibody to HER2 protein.

Methods for generating CDR-grafted and humanized antibodies are taught by Queen *et al.* U.S. Pat. No. 5,585,089; U.S. Pat. No. 5,693,761; U.S. Pat. No. 5,693,762; and Winter U.S. 5,225,539; and EP 0682040. Methods useful to prepare V_H and V_L polypeptides are taught by Winter *et al.*, U.S. Pat. Nos. 4,816,397; 6,291,158; 6,291,159; 6,291,161; 6,545,142; 5 EP 0368684; EP0451216; and EP0120694.

[00170] After selecting suitable framework region candidates from the same family and/or the same family member, either or both the heavy and light chain variable regions are produced by grafting the CDRs from the originating species into the hybrid framework regions. Assembly of hybrid antibodies or hybrid antibody fragments having hybrid variable chain regions with regard 10 to either of the above aspects can be accomplished using conventional methods known to those skilled in the art. For example, DNA sequences encoding the hybrid variable domains described herein (*i.e.*, frameworks based on the target species and CDRs from the originating species) can be produced by oligonucleotide synthesis and/or PCR. The nucleic acid encoding CDR regions can also be isolated from the originating species antibodies using suitable restriction enzymes 15 and ligated into the target species framework by ligating with suitable ligation enzymes. Alternatively, the framework regions of the variable chains of the originating species antibody can be changed by site-directed mutagenesis.

[00171] Since the hybrids are constructed from choices among multiple candidates corresponding to each framework region, there exist many combinations of sequences which are 20 amenable to construction in accordance with the principles described herein. Accordingly, libraries of hybrids can be assembled having members with different combinations of individual framework regions. Such libraries can be electronic database collections of sequences or physical collections of hybrids.

[00172] This process typically does not alter the acceptor antibody's FRs flanking the grafted 25 CDRs. However, one skilled in the art can sometimes improve antigen binding affinity of the resulting anti-HER2 CDR-grafted antibody by replacing certain residues of a given FR to make the FR more similar to the corresponding FR of the donor antibody. Suitable locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of

interacting with a CDR (*See, e.g.*, US 5,585,089, especially columns 12-16). Or one skilled in the art can start with the donor FR and modify it to be more similar to the acceptor FR or a human consensus FR. Techniques for making these modifications are known in the art. Particularly if the resulting FR fits a human consensus FR for that position, or is at least 90% or more identical to such a consensus FR, doing so may not increase the antigenicity of the resulting modified anti-HER2 CDR-grafted antibody significantly compared to the same antibody with a fully human FR.

[00173] *Bispecific Antibodies (BsAbs)*. A bispecific antibody is an antibody that can bind simultaneously to two targets that have a distinct structure, *e.g.*, two different target antigens, two different epitopes on the same target antigen, or a hapten and a target antigen or epitope on a target antigen. BsAbs can be made, for example, by combining heavy chains and/or light chains that recognize different epitopes of the same or different antigen. In some embodiments, by molecular function, a bispecific binding agent binds one antigen (or epitope) on one of its two binding arms (one VH/VL pair), and binds a different antigen (or epitope) on its second arm (a different VH/VL pair). By this definition, a bispecific binding agent has two distinct antigen binding arms (in both specificity and CDR sequences), and is monovalent for each antigen to which it binds.

[00174] Multi-specific antibodies, such as bispecific antibodies (BsAb) and bispecific antibody fragments (BsFab) have at least one arm that specifically binds to, for example, HER2 and at least one other arm that specifically binds to a second target antigen. In some embodiments, the second target antigen is an antigen or epitope of a B-cell, a T-cell, a myeloid cell, a plasma cell, or a mast-cell. Additionally or alternatively, in certain embodiments, the second target antigen is selected from the group consisting of CD3, CD4, CD8, CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, CD22, CD14, CD15, CD16, CD123, TCR gamma/delta, NKp46 and KIR. Exemplary V_H and V_L sequences that bind to a second target antigen (*e.g.*, CD3) are shown in **FIG. 3** (included in the LC sequences). In certain embodiments, the BsAbs are capable of binding to tumor cells that express HER2 antigen on the cell surface. In some embodiments, the BsAbs have been engineered to facilitate killing of

tumor cells by directing (or recruiting) cytotoxic T cells to a tumor site. Other exemplary BsAbs include those with a first antigen binding site specific for HER2 and a second antigen binding site specific for a small molecule hapten (*e.g.*, DTP A, IMP288, DOTA, DOTA-Bn, DOTA-desferrioxamine, other DOTA-chelates described herein, Biotin, fluorescein, or those disclosed in Goodwin, D A. *et al.*, 1994, *Cancer Res.* 54(22):5937-5946).

[00175] A variety of bispecific fusion proteins can be produced using molecular engineering. For example, BsAbs have been constructed that either utilize the full immunoglobulin framework (*e.g.*, IgG), single chain variable fragment (scFv), or combinations thereof. In some embodiments, the bispecific fusion protein is divalent, comprising, for example, a scFv with a single binding site for one antigen and a Fab fragment with a single binding site for a second antigen. In some embodiments, the bispecific fusion protein is divalent, comprising, for example, an scFv with a single binding site for one antigen and another scFv fragment with a single binding site for a second antigen. In other embodiments, the bispecific fusion protein is tetravalent, comprising, for example, an immunoglobulin (*e.g.*, IgG) with two binding sites for one antigen and two identical scFvs for a second antigen. BsAbs composed of two scFv units in tandem have been shown to be a clinically successful bispecific antibody format. In some embodiments, BsAbs comprise two single chain variable fragments (scFvs) in tandem have been designed such that an scFv that binds a tumor antigen (*e.g.*, HER2) is linked with an scFv that engages T cells (*e.g.*, by binding CD3). In this way, T cells are recruited to a tumor site such that they can mediate cytotoxic killing of the tumor cells. See *e.g.*, Dreier *et al.*, *J. Immunol.* 170:4397-4402 (2003); Bargou *et al.*, *Science* 321 :974- 977 (2008)). In some embodiments, BsAbs of the present technology comprise two single chain variable fragments (scFvs) in tandem have been designed such that an scFv that binds a tumor antigen (*e.g.*, HER2) is linked with an scFv that engages a small molecule DOTA hapten.

[00176] Recent methods for producing BsAbs include engineered recombinant monoclonal antibodies which have additional cysteine residues so that they crosslink more strongly than the more common immunoglobulin isotypes. See, *e.g.*, FitzGerald *et al.*, *Protein Eng.* 10(10):1221-1225 (1997). Another approach is to engineer recombinant fusion proteins linking two or more

different single-chain antibody or antibody fragment segments with the needed dual specificities. See, *e.g.*, Coloma *et al.*, *Nature Biotech.* 15:159-163 (1997). A variety of bispecific fusion proteins can be produced using molecular engineering.

[00177] Bispecific fusion proteins linking two or more different single-chain antibodies or antibody fragments are produced in a similar manner. Recombinant methods can be used to produce a variety of fusion proteins. In some certain embodiments, a BsAb according to the present technology comprises an immunoglobulin, which immunoglobulin comprises a heavy chain and a light chain, and an scFv. In some certain embodiments, the scFv is linked to the C-terminal end of the heavy chain of any HER2 immunoglobulin disclosed herein. In some certain
10 embodiments, scFvs are linked to the C-terminal end of the light chain of any HER2 immunoglobulin disclosed herein. In various embodiments, scFvs are linked to heavy or light chains *via* a linker sequence. Appropriate linker sequences necessary for the in-frame connection of the heavy chain Fd to the scFv are introduced into the V_L and V_κ domains through PCR reactions. The DNA fragment encoding the scFv is then ligated into a staging
15 vector containing a DNA sequence encoding the CH1 domain. The resulting scFv-CH1 construct is excised and ligated into a vector containing a DNA sequence encoding the V_H region of a HER2 antibody. The resulting vector can be used to transfect an appropriate host cell, such as a mammalian cell for the expression of the bispecific fusion protein.

[00178] In some embodiments, a linker is at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,
20 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more amino acids in length. In some embodiments, a linker is characterized in that it tends not to adopt a rigid three-dimensional structure, but rather provides flexibility to the polypeptide (*e.g.*, first and/or second antigen binding sites). In some embodiments, a linker is employed in a BsAb described herein based on specific properties imparted to the BsAb such as,
25 for example, an increase in stability. In some embodiments, a BsAb of the present technology comprises a G₄S linker. In some certain embodiments, a BsAb of the present technology comprises a (G₄S)_n linker, wherein n is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more.

[00179] *Fc Modifications.* In some embodiments, the anti-HER2 antibodies of the present technology comprise a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region (or the parental Fc region), such that said molecule has an altered affinity for an Fc receptor (*e.g.*, an FcγR), provided that said variant Fc region does not have a substitution at positions that make a direct contact with Fc receptor based on crystallographic and structural analysis of Fc-Fc receptor interactions such as those disclosed by Sondermann *et al.*, *Nature*, 406:267-273 (2000). Examples of positions within the Fc region that make a direct contact with an Fc receptor such as an FcγR, include amino acids 234-239 (hinge region), amino acids 265-269 (B/C loop), amino acids 297-299 (C7E loop), and amino acids 327-332 (F/G) loop.

[00180] In some embodiments, an anti-HER2 antibody of the present technology has an altered affinity for activating and/or inhibitory receptors, having a variant Fc region with one or more amino acid modifications, wherein said one or more amino acid modification is a N297 substitution with alanine, or a K322 substitution with alanine. Additionally or alternatively, in some embodiments, the Fc regions of the HER2 antibodies disclosed herein comprise two amino acid substitutions, Leu234Ala and Leu235Ala (so called LALA mutations) to eliminate FcγRIIa binding. The LALA mutations are commonly used to alleviate the cytokine induction from T cells, thus reducing toxicity of the antibodies (Wines BD, *et al.*, *J Immunol* 164:5313–5318 (2000)).

[00181] *Glycosylation Modifications.* In some embodiments, anti-HER2 antibodies of the present technology have an Fc region with variant glycosylation as compared to a parent Fc region. In some embodiments, variant glycosylation includes the absence of fucose; in some embodiments, variant glycosylation results from expression in GnT1-deficient CHO cells.

[00182] In some embodiments, the antibodies of the present technology, may have a modified glycosylation site relative to an appropriate reference antibody that binds to an antigen of interest (*e.g.*, HER2), without altering the functionality of the antibody, *e.g.*, binding activity to the antigen. 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.

[00183] 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. For example, an Fc-glycoform (hHER2-IgGln) that lacks certain oligosaccharides including fucose and terminal N- acetylglucosamine may be produced in special CHO cells and exhibit enhanced ADCC effector function.

[00184] In some embodiments, the carbohydrate content of an immunoglobulin-related composition disclosed herein is modified by adding or deleting a glycosylation site. Methods for modifying the carbohydrate content of antibodies are well known in the art and are included within the present technology, see, *e.g.*, U.S. Patent No. 6,218,149; EP 0359096B1; U.S. Patent Publication No. US 2002/0028486; International Patent Application Publication WO 03/035835; U.S. Patent Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are incorporated herein by reference in their entirety. In some embodiments, the carbohydrate content of an antibody (or relevant portion or component thereof) is modified by deleting one or more endogenous carbohydrate moieties of the antibody. In some certain embodiments, the present technology includes deleting the glycosylation site of the Fc region of an antibody, by modifying position 297 from asparagine to alanine.

[00185] Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Engineered glycoforms may be generated by any method known to one skilled in the art, for example by using engineered or variant expression strains, by co-expression with one or more enzymes, for example N-acetylglucosaminyltransferase III (GnTIII), by expressing a molecule comprising an Fc region in various organisms or cell lines from various organisms, or by modifying carbohydrate(s) after the molecule comprising Fc region has been expressed. Methods for generating engineered glycoforms are known in the art, and include but are not limited to those described in Umana *et*

al., 1999, *Nat. Biotechnol.* 17: 176-180; Davies *et al.*, 2001, *Biotechnol. Bioeng.* 74:288-294; Shields *et al.*, 2002, *J. Biol. Chem.* 277:26733-26740; Shinkawa *et al.*, 2003, *J. Biol. Chem.* 278:3466-3473; U.S. Patent No. 6,602,684; U.S. Patent Application Serial No. 10/277,370; U.S. Patent Application Serial No. 10/113,929; International Patent Application Publications WO 00/61739A1 ; WO 01/292246A1; WO 02/311140A1; WO 02/30954A1; POTILLEGENT™ technology (Biowa, Inc. Princeton, N.J.); GLYCOMAB™ glycosylation engineering technology (GLYCART biotechnology AG, Zurich, Switzerland); each of which is incorporated herein by reference in its entirety. See, *e.g.*, International Patent Application Publication WO 00/061739; U.S. Patent Application Publication No. 2003/0115614; Okazaki *et al.*, 2004, *JMB*, 336: 1239-49.

[00186] *Fusion Proteins.* In one embodiment, the anti-HER2 antibody of the present technology is a fusion protein. The anti-HER2 antibodies of the present technology, when fused to a second protein, can be used as an antigenic tag. Examples of domains that can be fused to polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but can occur through linker sequences. Moreover, fusion proteins of the present technology can also be engineered to improve characteristics of the anti-HER2 antibodies. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of the anti-HER2 antibody to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties can be added to an anti-HER2 antibody to facilitate purification. Such regions can be removed prior to final preparation of the anti-HER2 antibody. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art. The anti-HER2 antibody of the present technology can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In select embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, Calif), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 821-824, 1989, for instance, hexa-histidine provides for convenient purification of the fusion protein.

Another peptide tag useful for purification, the “HA” tag, corresponds to an epitope derived from the influenza hemagglutinin protein. Wilson *et al.*, *Cell* 37: 767, 1984.

[00187] Thus, any of these above fusion proteins can be engineered using the polynucleotides or the polypeptides of the present technology. Also, in some embodiments, the fusion proteins
5 described herein show an increased half-life *in vivo*.

[00188] Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can be more efficient in binding and neutralizing other molecules compared to the monomeric secreted protein or protein fragment alone. Fountoulakis *et al.*, *J. Biochem.* 270: 3958-3964, 1995.

[00189] Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins
10 comprising various portions of constant region of immunoglobulin molecules together with another human protein or a fragment thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, *e.g.*, improved pharmacokinetic properties. See EP-A 0232 262. Alternatively, deleting or modifying the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion
15 can hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, *e.g.*, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. Bennett *et al.*, *J. Molecular Recognition* 8: 52-58, 1995; Johanson *et al.*, *J. Biol. Chem.*, 270: 9459-9471, 1995.

[00190] *Labeled Anti-HER2 antibodies.* In one embodiment, the anti-HER2 antibody of the
20 present technology is coupled with a label moiety, *i.e.*, detectable group. The particular label or detectable group conjugated to the anti-HER2 antibody is not a critical aspect of the technology, so long as it does not significantly interfere with the specific binding of the anti-HER2 antibody of the present technology to the HER2 protein. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in
25 the field of immunoassays and imaging. In general, almost any label useful in such methods can be applied to the present technology. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Labels useful in the practice of the present technology include magnetic beads (*e.g.*,

Dynabeads™), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.*, ^3H , ^{14}C , ^{35}S , ^{125}I , ^{121}I , ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$), other imaging agents such as microbubbles (for ultrasound imaging), ^{18}F , ^{11}C , ^{15}O , ^{89}Zr (for Positron emission tomography), $^{99\text{m}}\text{Tc}$, ^{111}In (for Single photon emission tomography), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and calorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, and the like) beads. Patents that describe the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241, each incorporated herein by reference in their entirety and for all purposes. *See also* Handbook of Fluorescent Probes and Research Chemicals (6th Ed., Molecular Probes, Inc., Eugene OR.).

[00191] The label can be coupled directly or indirectly to the desired component of an assay according to methods well known in the art. As indicated above, a wide variety of labels can be used, with the choice of label depending on factors such as required sensitivity, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[00192] Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (*e.g.*, biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (*e.g.*, streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, *e.g.*, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally-occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody, *e.g.*, an anti-HER2 antibody.

[00193] The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds useful as labeling moieties, include, but are not limited to, *e.g.*, fluorescein and its derivatives, rhodamine and its derivatives, dansyl,

umbelliferone, and the like. Chemiluminescent compounds useful as labeling moieties, include, but are not limited to, *e.g.*, luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. For a review of various labeling or signal-producing systems which can be used, see U.S. Pat. No. 4,391,904.

5 [00194] Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it can be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence can be detected visually, by means of photographic
10 film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels can be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels can be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various
15 conjugated beads appear the color of the bead.

[00195] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies, *e.g.*, the anti-HER2 antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of
20 the target antibody is detected by simple visual inspection.

B. Identifying and Characterizing the Anti-HER2 Antibodies of the Present Technology

[00196] *Methods for identifying and/or screening the anti-HER2 antibodies of the present technology.* Methods useful to identify and screen antibodies against HER2 polypeptides for those that possess the desired specificity to HER2 protein (*e.g.*, those that bind to the
25 extracellular domain of HER2 protein, such as polypeptides comprising the amino acid sequence of GenBank: NP 004439.2 (SEQ ID NO: 84) include any immunologically-mediated techniques known within the art. Components of an immune response can be detected *in vitro* by various methods that are well known to those of ordinary skill in the art. For example, (1) cytotoxic T

lymphocytes can be incubated with radioactively labeled target cells and the lysis of these target cells detected by the release of radioactivity; (2) helper T lymphocytes can be incubated with antigens and antigen presenting cells and the synthesis and secretion of cytokines measured by standard methods (Windhagen A *et al.*, *Immunity*, 2: 373-80, 1995); (3) antigen presenting cells
5 can be incubated with whole protein antigen and the presentation of that antigen on MHC detected by either T lymphocyte activation assays or biophysical methods (Harding *et al.*, *Proc. Natl. Acad. Sci.*, 86: 4230-4, 1989); (4) mast cells can be incubated with reagents that cross-link their Fc-epsilon receptors and histamine release measured by enzyme immunoassay (Siraganian *et al.*, *TIPS*, 4: 432-437, 1983); and (5) enzyme-linked immunosorbent assay (ELISA).

10 **[00197]** Similarly, products of an immune response in either a model organism (*e.g.*, mouse) or a human subject can also be detected by various methods that are well known to those of ordinary skill in the art. For example, (1) the production of antibodies in response to vaccination can be readily detected by standard methods currently used in clinical laboratories, *e.g.*, an ELISA; (2) the migration of immune cells to sites of inflammation can be detected by scratching
15 the surface of skin and placing a sterile container to capture the migrating cells over scratch site (Peters *et al.*, *Blood*, 72: 1310-5, 1988); (3) the proliferation of peripheral blood mononuclear cells (PBMCs) in response to mitogens or mixed lymphocyte reaction can be measured using ³H-thymidine; (4) the phagocytic capacity of granulocytes, macrophages, and other phagocytes in PBMCs can be measured by placing PBMCs in wells together with labeled particles (Peters *et al.*, *Blood*, 72: 1310-5, 1988); and (5) the differentiation of immune system cells can be
20 measured by labeling PBMCs with antibodies to CD molecules such as CD4 and CD8 and measuring the fraction of the PBMCs expressing these markers.

[00198] In one embodiment, anti-HER2 antibodies of the present technology are selected using display of HER2 peptides on the surface of replicable genetic packages. *See, e.g.*, U.S.
25 Pat. Nos. 5,514,548; 5,837,500; 5,871,907; 5,885,793; 5,969,108; 6,225,447; 6,291,650; 6,492,160; EP 585 287; EP 605522; EP 616640; EP 1024191; EP 589 877; EP 774 511; EP 844 306. Methods useful for producing/selecting a filamentous bacteriophage particle containing a

phagemid genome encoding for a binding molecule with a desired specificity has been described. See, e.g., EP 774 511; US 5871907; US 5969108; US 6225447; US 6291650; US 6492160.

[00199] In some embodiments, anti-HER2 antibodies of the present technology are selected using display of HER2 peptides on the surface of a yeast host cell. Methods useful for the isolation of scFv polypeptides by yeast surface display have been described by Kiecke *et al.*, *Protein Eng.* 1997 Nov; 10(11): 1303-10.

[00200] In some embodiments, anti-HER2 antibodies of the present technology are selected using ribosome display. Methods useful for identifying ligands in peptide libraries using ribosome display have been described by Mattheakis *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 9022-26, 1994; and Hanes *et al.*, *Proc. Natl. Acad. Sci. USA* 94: 4937-42, 1997.

[00201] In certain embodiments, anti-HER2 antibodies of the present technology are selected using tRNA display of HER2 peptides. Methods useful for *in vitro* selection of ligands using tRNA display have been described by Merryman *et al.*, *Chem. Biol.*, 9: 741-46, 2002.

[00202] In one embodiment, anti-HER2 antibodies of the present technology are selected using RNA display. Methods useful for selecting peptides and proteins using RNA display libraries have been described by Roberts *et al.* *Proc. Natl. Acad. Sci. USA*, 94: 12297-302, 1997; and Nemoto *et al.*, *FEBS Lett.*, 414: 405-8, 1997. Methods useful for selecting peptides and proteins using unnatural RNA display libraries have been described by Frankel *et al.*, *Curr. Opin. Struct. Biol.*, 13: 506-12, 2003.

[00203] In some embodiments, anti-HER2 antibodies of the present technology are expressed in the periplasm of gram negative bacteria and mixed with labeled HER2 protein. See WO 02/34886. In clones expressing recombinant polypeptides with affinity for HER2 protein, the concentration of the labeled HER2 protein bound to the anti-HER2 antibodies is increased and allows the cells to be isolated from the rest of the library as described in Harvey *et al.*, *Proc. Natl. Acad. Sci.* 22: 9193-98 2004 and U.S. Pat. Publication No. 2004/0058403.

[00204] After selection of the desired anti-HER2 antibodies, it is contemplated that said antibodies can be produced in large volume by any technique known to those skilled in the art,

e.g., prokaryotic or eukaryotic cell expression and the like. The anti-HER2 antibodies which are, *e.g.*, but not limited to, anti-HER2 hybrid antibodies or fragments can be produced by using conventional techniques to construct an expression vector that encodes an antibody heavy chain in which the CDRs and, if necessary, a minimal portion of the variable region framework, that are required to retain original species antibody binding specificity (as engineered according to the techniques described herein) are derived from the originating species antibody and the remainder of the antibody is derived from a target species immunoglobulin which can be manipulated as described herein, thereby producing a vector for the expression of a hybrid antibody heavy chain.

[00205] *Measurement of HER2 Binding.* In some embodiments, a HER2 binding assay refers to an assay format wherein HER2 protein and an anti-HER2 antibody are mixed under conditions suitable for binding between the HER2 protein and the anti-HER2 antibody and assessing the amount of binding between the HER2 protein and the anti-HER2 antibody. The amount of binding is compared with a suitable control, which can be the amount of binding in the absence of the HER2 protein, the amount of the binding in the presence of a non-specific immunoglobulin composition, or both. The amount of binding can be assessed by any suitable method. Binding assay methods include, *e.g.*, ELISA, radioimmunoassays, scintillation proximity assays, fluorescence energy transfer assays, liquid chromatography, membrane filtration assays, and the like. Biophysical assays for the direct measurement of HER2 protein binding to anti-HER2 antibody are, *e.g.*, nuclear magnetic resonance, fluorescence, fluorescence polarization, surface plasmon resonance (BIAcore chips) and the like. Specific binding is determined by standard assays known in the art, *e.g.*, radioligand binding assays, ELISA, FRET, immunoprecipitation, SPR, NMR (2D-NMR), mass spectroscopy and the like. If the specific binding of a candidate anti-HER2 antibody is at least 1 percent greater than the binding observed in the absence of the candidate anti-HER2 antibody, the candidate anti-HER2 antibody is useful as an anti-HER2 antibody of the present technology.

Uses of the Anti-HER2 Antibodies of the Present Technology

[00206] *General.* The anti-HER2 antibodies of the present technology are useful in methods known in the art relating to the localization and/or quantitation of HER2 protein (*e.g.*, for use in measuring levels of the HER2 protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the polypeptide, and the like). Antibodies of the present technology are useful to isolate a HER2 protein by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-HER2 antibody of the present technology can facilitate the purification of natural immunoreactive HER2 proteins from biological samples, *e.g.*, mammalian sera or cells as well as recombinantly-produced immunoreactive HER2 proteins expressed in a host system. Moreover, anti-HER2 antibodies can be used to detect an immunoreactive HER2 protein (*e.g.*, in plasma, a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the immunoreactive polypeptide. The anti-HER2 antibodies of the present technology can be used diagnostically to monitor immunoreactive HER2 protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. As noted above, the detection can be facilitated by coupling (*i.e.*, physically linking) the anti-HER2 antibodies of the present technology to a detectable substance.

[00207] *Detection of HER2 protein.* An exemplary method for detecting the presence or absence of an immunoreactive HER2 protein in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with an anti-HER2 antibody of the present technology capable of detecting an immunoreactive HER2 protein such that the presence of an immunoreactive HER2 protein is detected in the biological sample. Detection may be accomplished by means of a detectable label attached to the antibody.

[00208] The term “labeled” with regard to the anti-HER2 antibody is intended to encompass direct labeling of the antibody by coupling (*i.e.*, physically linking) a detectable substance to the antibody, as well as indirect labeling of the antibody by reactivity with another compound that is directly labeled, such as a secondary antibody. Examples of indirect labeling include detection

of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin.

[00209] In some embodiments, the anti-HER2 antibodies disclosed herein are conjugated to one or more detectable labels. For such uses, anti-HER2 antibodies may be detectably labeled
 5 by covalent or non-covalent attachment of a chromogenic, enzymatic, radioisotopic, isotopic, fluorescent, toxic, chemiluminescent, nuclear magnetic resonance contrast agent or other label.

[00210] Examples of suitable chromogenic labels include diaminobenzidine and 4-hydroxyazo-benzene-2-carboxylic acid. Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, Δ -5-steroid isomerase, yeast-alcohol dehydrogenase, α -
 10 glycerol phosphate dehydrogenase, triose phosphate isomerase, peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β -galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

[00211] Examples of suitable radioisotopic labels include ^3H , ^{111}In , ^{125}I , ^{131}I , ^{32}P , ^{35}S , ^{14}C , ^{51}Cr , ^{57}Co , ^{58}Co , ^{59}Fe , ^{75}Se , ^{152}Eu , ^{90}Y , ^{67}Cu , ^{217}Bi , ^{211}At , ^{212}Pb , ^{47}Sc , ^{109}Pd , *etc.* ^{111}In is an
 15 exemplary isotope where *in vivo* imaging is used since it avoids the problem of dehalogenation of the ^{125}I or ^{131}I -labeled HER2-binding antibodies by the liver. In addition, this isotope has a more favorable gamma emission energy for imaging (Perkins *et al*, *Eur. J. Nucl. Med.* 70:296-301 (1985); Carasquillo *et al.*, *J. Nucl. Med.* 25:281-287 (1987)). For example, ^{111}In coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA exhibits little uptake in non-
 20 tumorous tissues, particularly the liver, and enhances specificity of tumor localization (Esteban *et al.*, *J. Nucl. Med.* 28:861-870 (1987)). Examples of suitable non-radioactive isotopic labels include ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Tr , and ^{56}Fe .

[00212] Examples of suitable fluorescent labels include an ^{152}Eu label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an
 25 allophycocyanin label, a Green Fluorescent Protein (GFP) label, an o-phthaldehyde label, and a fluorescamine label. Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin.

[00213] 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. Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

5 [00214] The detection method of the present technology can be used to detect an immunoreactive HER2 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of an immunoreactive HER2 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, radioimmunoassay, and immunofluorescence. Furthermore, *in vivo* techniques for detection of an immunoreactive HER2
10 protein include introducing into a subject a labeled anti-HER2 antibody. For example, the anti-HER2 antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques. In one embodiment, the biological sample contains HER2 protein molecules from the test subject.

[00215] *Immunoassay and Imaging.* An anti-HER2 antibody of the present technology can be
15 used to assay immunoreactive HER2 protein levels in a biological sample (*e.g.*, human plasma) using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. Jalkanen, M. *et al.*, *J. Cell. Biol.* 101: 976-985, 1985; Jalkanen, M. *et al.*, *J. Cell. Biol.* 105: 3087-3096, 1987. Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked
20 immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes or other radioactive agent, such as iodine (^{125}I , ^{121}I , ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium ($^{99\text{m}}\text{Tc}$), and fluorescent labels, such as fluorescein, rhodamine, and green fluorescent protein (GFP), as well as biotin.

25 [00216] In addition to assaying immunoreactive HER2 protein levels in a biological sample, anti-HER2 antibodies of the present technology may be used for *in vivo* imaging of HER2. Antibodies useful for this method include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit

detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which can be incorporated into the anti-HER2 antibodies by labeling of nutrients for the relevant scFv clone.

[00217] An anti-HER2 antibody which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (*e.g.*, ^{131}I , ^{112}In , $^{99\text{m}}\text{Tc}$), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (*e.g.*, parenterally, subcutaneously, or intraperitoneally) into the subject. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled anti-HER2 antibody will then accumulate at the location of cells which contain the specific target polypeptide. For example, labeled anti-HER2 antibodies of the present technology will accumulate within the subject in cells and tissues in which the HER2 protein has localized.

[00218] Thus, the present technology provides a diagnostic method of a medical condition, which involves: (a) assaying the expression of immunoreactive HER2 protein by measuring binding of an anti-HER2 antibody of the present technology in cells or body fluid of an individual; (b) comparing the amount of immunoreactive HER2 protein present in the sample with a standard reference, wherein an increase or decrease in immunoreactive HER2 protein levels compared to the standard is indicative of a medical condition.

[00219] *Affinity Purification.* The anti-HER2 antibodies of the present technology may be used to purify immunoreactive HER2 protein from a sample. In some embodiments, the antibodies are immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir *et al.*, "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby *et al.*, *Meth. Enzym.* 34 Academic Press, N.Y. (1974)).

[00220] The simplest method to bind the antigen to the antibody-support matrix is to collect the beads in a column and pass the antigen solution down the column. The efficiency of this method depends on the contact time between the immobilized antibody and the antigen, which can be extended by using low flow rates. The immobilized antibody captures the antigen as it flows past. Alternatively, an antigen can be contacted with the antibody-support matrix by mixing the antigen solution with the support (*e.g.*, beads) and rotating or rocking the slurry, allowing maximum contact between the antigen and the immobilized antibody. After the binding reaction has been completed, the slurry is passed into a column for collection of the beads. The beads are washed using a suitable washing buffer and then the pure or substantially pure antigen is eluted.

[00221] An antibody or polypeptide of interest can be conjugated to a solid support, such as a bead. In addition, a first solid support such as a bead can also be conjugated, if desired, to a second solid support, which can be a second bead or other support, by any suitable means, including those disclosed herein for conjugation of a polypeptide to a support. Accordingly, any of the conjugation methods and means disclosed herein with reference to conjugation of a polypeptide to a solid support can also be applied for conjugation of a first support to a second support, where the first and second solid support can be the same or different.

[00222] Appropriate linkers, which can be cross-linking agents, for use for conjugating a polypeptide to a solid support include a variety of agents that can react with a functional group present on a surface of the support, or with the polypeptide, or both. Reagents useful as cross-linking agents include homo-bi-functional and, in particular, hetero-bi-functional reagents. Useful bi-functional cross-linking agents include, but are not limited to, *N*-SIAB, dimaleimide, DTNB, *N*-SATA, *N*-SPDP, SMCC and 6-HYNIC. A cross-linking agent can be selected to provide a selectively cleavable bond between a polypeptide and the solid support. For example, a photolabile cross-linker, such as 3-amino-(2-nitrophenyl)propionic acid can be employed as a means for cleaving a polypeptide from a solid support. (Brown *et al.*, *Mol. Divers*, pp. 4-12 (1995); Rothschild *et al.*, *Nucl. Acids Res.*, 24:351-66 (1996); and US. Pat. No. 5,643,722).

Other cross-linking reagents are well-known in the art. (*See, e.g., Wong (1991), supra; and Hermanson (1996), supra*).

[00223] An antibody or polypeptide can be immobilized on a solid support, such as a bead, through a covalent amide bond formed between a carboxyl group functionalized bead and the amino terminus of the polypeptide or, conversely, through a covalent amide bond formed between an amino group functionalized bead and the carboxyl terminus of the polypeptide. In addition, a bi-functional trityl linker can be attached to the support, *e.g.,* to the 4-nitrophenyl active ester on a resin, such as a Wang resin, through an amino group or a carboxyl group on the resin *via* an amino resin. Using a bi-functional trityl approach, the solid support can require treatment with a volatile acid, such as formic acid or trifluoroacetic acid to ensure that the polypeptide is cleaved and can be removed. In such a case, the polypeptide can be deposited as a beadless patch at the bottom of a well of a solid support or on the flat surface of a solid support. After addition of a matrix solution, the polypeptide can be desorbed into a MS.

[00224] Hydrophobic trityl linkers can also be exploited as acid-labile linkers by using a volatile acid or an appropriate matrix solution, *e.g.,* a matrix solution containing 3-HPA, to cleave an amino linked trityl group from the polypeptide. Acid lability can also be changed. For example, trityl, monomethoxytrityl, dimethoxytrityl or trimethoxytrityl can be changed to the appropriate *p*-substituted, or more acid-labile tritylamine derivatives, of the polypeptide, *i.e.,* trityl ether and tritylamine bonds can be made to the polypeptide. Accordingly, a polypeptide can be removed from a hydrophobic linker, *e.g.,* by disrupting the hydrophobic attraction or by cleaving tritylether or tritylamine bonds under acidic conditions, including, if desired, under typical MS conditions, where a matrix, such as 3-HPA acts as an acid.

[00225] Orthogonally cleavable linkers can also be useful for binding a first solid support, *e.g.,* a bead to a second solid support, or for binding a polypeptide of interest to a solid support. Using such linkers, a first solid support, *e.g.,* a bead, can be selectively cleaved from a second solid support, without cleaving the polypeptide from the support; the polypeptide then can be cleaved from the bead at a later time. For example, a disulfide linker, which can be cleaved using a reducing agent, such as DTT, can be employed to bind a bead to a second solid support,

and an acid cleavable bi-functional trityl group could be used to immobilize a polypeptide to the support. As desired, the linkage of the polypeptide to the solid support can be cleaved first, *e.g.*, leaving the linkage between the first and second support intact. Trityl linkers can provide a covalent or hydrophobic conjugation and, regardless of the nature of the conjugation, the trityl group is readily cleaved in acidic conditions.

[00226] For example, a bead can be bound to a second support through a linking group which can be selected to have a length and a chemical nature such that high density binding of the beads to the solid support, or high density binding of the polypeptides to the beads, is promoted. Such a linking group can have, *e.g.*, “tree-like” structure, thereby providing a multiplicity of functional groups per attachment site on a solid support. Examples of such linking group; include polylysine, polyglutamic acid, penta-erythrole and *tris*-hydroxy-aminomethane.

[00227] *Noncovalent Binding Association.* An antibody or polypeptide can be conjugated to a solid support, or a first solid support can also be conjugated to a second solid support, through a noncovalent interaction. For example, a magnetic bead made of a ferromagnetic material, which is capable of being magnetized, can be attracted to a magnetic solid support, and can be released from the support by removal of the magnetic field. Alternatively, the solid support can be provided with an ionic or hydrophobic moiety, which can allow the interaction of an ionic or hydrophobic moiety, respectively, with a polypeptide, *e.g.*, a polypeptide containing an attached trityl group or with a second solid support having hydrophobic character.

[00228] A solid support can also be provided with a member of a specific binding pair and, therefore, can be conjugated to a polypeptide or a second solid support containing a complementary binding moiety. For example, a bead coated with avidin or with streptavidin can be bound to a polypeptide having a biotin moiety incorporated therein, or to a second solid support coated with biotin or derivative of biotin, such as iminobiotin.

[00229] It should be recognized that any of the binding members disclosed herein or otherwise known in the art can be reversed. Thus, biotin, *e.g.*, can be incorporated into either a polypeptide or a solid support and, conversely, avidin or other biotin binding moiety would be incorporated into the support or the polypeptide, respectively. Other specific binding pairs

contemplated for use herein include, but are not limited to, hormones and their receptors, enzyme, and their substrates, a nucleotide sequence and its complementary sequence, an antibody and the antigen to which it interacts specifically, and other such pairs known to those skilled in the art.

5 A. Diagnostic Uses of Anti-HER2 Antibodies of the Present Technology

[00230] *General.* The anti-HER2 antibodies of the present technology are useful in diagnostic methods. As such, the present technology provides methods using the antibodies in the diagnosis of HER2 activity in a subject. Anti-HER2 antibodies of the present technology may be selected such that they have any level of epitope binding specificity and very high binding affinity to a
10 HER2 protein. In general, the higher the binding affinity of an antibody the more stringent wash conditions can be performed in an immunoassay to remove nonspecifically bound material without removing target polypeptide. Accordingly, anti-HER2 antibodies of the present technology useful in diagnostic assays usually have binding affinities of about 10^8 M^{-1} , 10^9 M^{-1} , 10^{10} M^{-1} , 10^{11} M^{-1} or 10^{12} M^{-1} . Further, it is desirable that anti-HER2 antibodies used as
15 diagnostic reagents have a sufficient kinetic on-rate to reach equilibrium under standard conditions in at least 12 h, at least five (5) h, or at least one (1) hour.

[00231] Anti-HER2 antibodies can be used to detect an immunoreactive HER2 protein in a variety of standard assay formats. Such formats include immunoprecipitation, Western blotting, ELISA, radioimmunoassay, and immunometric assays. See Harlow & Lane, *Antibodies, A*
20 *Laboratory Manual* (Cold Spring Harbor Publications, New York, 1988); U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,879,262; 4,034,074; 3,791,932; 3,817,837; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876. Biological samples can be obtained from any tissue or body fluid of a subject. In certain embodiments, the subject is at an early stage of cancer. In one
25 embodiment, the early stage of cancer is determined by the level or expression pattern of HER2 protein in a sample obtained from the subject. In certain embodiments, the sample is selected from the group consisting of urine, blood, serum, plasma, saliva, amniotic fluid, cerebrospinal fluid (CSF), and biopsied body tissue.

[00232] Immunometric or sandwich assays are one format for the diagnostic methods of the present technology. See U.S. Pat. No. 4,376,110, 4,486,530, 5,914,241, and 5,965,375. Such assays use one antibody, *e.g.*, an anti-HER2 antibody or a population of anti-HER2 antibodies immobilized to a solid phase, and another anti-HER2 antibody or a population of anti-HER2 antibodies in solution. Typically, the solution anti-HER2 antibody or population of anti-HER2 antibodies is labeled. If an antibody population is used, the population can contain antibodies binding to different epitope specificities within the target polypeptide. Accordingly, the same population can be used for both solid phase and solution antibody. If anti-HER2 monoclonal antibodies are used, first and second HER2 monoclonal antibodies having different binding specificities are used for the solid and solution phase. Solid phase (also referred to as “capture”) and solution (also referred to as “detection”) antibodies can be contacted with target antigen in either order or simultaneously. If the solid phase antibody is contacted first, the assay is referred to as being a forward assay. Conversely, if the solution antibody is contacted first, the assay is referred to as being a reverse assay. If the target is contacted with both antibodies simultaneously, the assay is referred to as a simultaneous assay. After contacting the HER2 protein with the anti-HER2 antibody, a sample is incubated for a period that usually varies from about 10 min to about 24 hr and is usually about 1 hr. A wash step is then performed to remove components of the sample not specifically bound to the anti-HER2 antibody being used as a diagnostic reagent. When solid phase and solution antibodies are bound in separate steps, a wash can be performed after either or both binding steps. After washing, binding is quantified, typically by detecting a label linked to the solid phase through binding of labeled solution antibody. Usually for a given pair of antibodies or populations of antibodies and given reaction conditions, a calibration curve is prepared from samples containing known concentrations of target antigen. Concentrations of the immunoreactive HER2 protein in samples being tested are then read by interpolation from the calibration curve (*i.e.*, standard curve). Analyte can be measured either from the amount of labeled solution antibody bound at equilibrium or by kinetic measurements of bound labeled solution antibody at a series of time points before equilibrium is reached. The slope of such a curve is a measure of the concentration of the HER2 protein in a sample.

[00233] Suitable supports for use in the above methods include, *e.g.*, nitrocellulose membranes, nylon membranes, and derivatized nylon membranes, and also particles, such as agarose, a dextran-based gel, dipsticks, particulates, microspheres, magnetic particles, test tubes, microtiter wells, SEPHADEX™ (Amersham Pharmacia Biotech, Piscataway N.J.), and the like.

5 Immobilization can be by absorption or by covalent attachment. Optionally, anti-HER2 antibodies can be joined to a linker molecule, such as biotin for attachment to a surface bound linker, such as avidin.

[00234] In some embodiments, the present disclosure provides an anti-HER2 antibody of the present technology conjugated to a diagnostic agent. The diagnostic agent may comprise a
10 radioactive or non-radioactive label, a contrast agent (such as for magnetic resonance imaging, computed tomography or ultrasound), and the radioactive label can be a gamma-, beta-, alpha-, Auger electron-, or positron-emitting isotope. A diagnostic agent is a molecule which is administered conjugated to an antibody moiety, *i.e.*, antibody or antibody fragment, or subfragment, and is useful in diagnosing or detecting a disease by locating the cells containing
15 the antigen.

[00235] 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
20 enhancing agent and is incorporated in its entirety by reference. In some embodiments, 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
25 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, *e.g.*, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and like groups

known to be useful for this purpose. Chelates may be coupled to the antibodies of the present technology 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 methods and reagents for conjugating chelates to antibodies are disclosed in U.S. Pat. No. 4,824,659. 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 with the HER2 antibodies of the present technology. Macrocyclic chelates such as NOTA (1,4,7-triaza-cyclononane-N,N',N''-triacetic acid), DOTA, and TETA (p-bromoacetamido-benzyl-tetraethylaminetetraacetic acid) are of use with a variety of metals and radiometals, such as radionuclides of gallium, yttrium and copper, respectively. Such metal-chelate complexes can be stabilized by tailoring the ring size to the metal of interest. Examples of other DOTA chelates include (i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH₂; (ii) Ac-Lys(HSG)-D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH₂; (iii) DOTA-D-Asp-D-Lys(HSG)-D-Asp-D-Lys(HSG)-NH₂; (iv) DOTA-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (v) DOTA-D-Tyr-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (vi) DOTA-D-Ala-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (vii) DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH₂; (viii) Ac-D-Phe-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-NH₂; (ix) Ac-D-Phe-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH₂; (x) Ac-D-Phe-D-Lys(Bz-DTPA)-D-Tyr-D-Lys(Bz-DTPA)-NH₂; (xi) Ac-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH₂; (xii) DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH₂; (xiii) (Tscg-Cys)-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(DOTA)-NH₂; (xiv) Tscg-D-Cys-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (xv) (Tscg-Cys)-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH₂; (xvi) Ac-D-Cys-D-Lys(DOTA)-D-Tyr-D-Ala-D-Lys(DOTA)-D-Cys-NH₂; (xvii) Ac-D-Cys-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH₂; (xviii) Ac-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-D-Lys(Tscg-Cys)-NH₂; and (xix) Ac-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-D-Lys(Tscg-Cys)-NH₂.

[00236] Other ring-type chelates such as macrocyclic polyethers, which are of interest for stably binding nuclides, such as ²²³Ra for RAIT are also contemplated.

B. Therapeutic Use of Anti-HER2 Antibodies of the Present Technology

[00237] In one aspect, the immunoglobulin-related compositions (*e.g.*, antibodies or antigen binding fragments thereof) of the present technology are useful for the treatment of HER2-associated cancers. Examples of HER2-associated cancers include, but are not limited to, breast cancer, gastric cancer, 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. In some embodiments, the HER2-associated cancer is a solid tumor. Such treatment can be used in patients identified as having pathologically high levels of the HER2 (*e.g.*, those diagnosed by the methods described herein) or in patients diagnosed with a disease known to be associated with such pathological levels.

[00238] The compositions of the present technology may be employed in conjunction with other therapeutic agents useful in the treatment of HER2-associated cancers. For example, the antibodies or antigen binding fragments of the present technology may be separately, sequentially or simultaneously administered with at least one additional therapeutic agent selected from the group consisting of alkylating agents, platinum agents, taxanes, vinca agents, anti-estrogen drugs, aromatase inhibitors, ovarian suppression agents, VEGF/VEGFR inhibitors, EGF/EGFR inhibitors, PARP inhibitors, cytostatic alkaloids, cytotoxic antibiotics, antimetabolites, endocrine/hormonal agents, T cells, bisphosphonate therapy agents and targeted biological therapy agents (*e.g.*, therapeutic peptides described in US 6306832, WO 2012007137, WO 2005000889, WO 2010096603 *etc.*). In some embodiments, the at least one additional therapeutic agent is a chemotherapeutic agent. Specific chemotherapeutic agents include, but are not limited to, cyclophosphamide, fluorouracil (or 5-fluorouracil or 5-FU), methotrexate, edatrexate (10-ethyl-10-deaza-aminopterin), thiotepa, carboplatin, cisplatin, taxanes, paclitaxel, protein-bound paclitaxel, docetaxel, vinorelbine, tamoxifen, raloxifene, toremifene, fulvestrant, gemcitabine, irinotecan, ixabepilone, temozolamide, topotecan, vincristine, vinblastine, eribulin, mutamycin, capecitabine, anastrozole, exemestane, letrozole, leuprolide, abarelix, buserlin,

goserelin, megestrol acetate, risedronate, pamidronate, ibandronate, alendronate, denosumab, zoledronate, trastuzumab, tykerb, anthracyclines (*e.g.*, daunorubicin and doxorubicin), bevacizumab, oxaliplatin, melphalan, etoposide, mechlorethamine, bleomycin, microtubule poisons, annonaceous acetogenins, or combinations thereof.

5 [00239] Additionally or alternatively, in some embodiments, the antibodies or antigen binding fragments of the present technology may be separately, sequentially or simultaneously administered with at least one additional immuno-modulating/stimulating antibody including but not limited to anti-PD-1 antibody, anti-PD-L1 antibody, anti-PD-L2 antibody, anti-CTLA-4 antibody, anti-TIM3 antibody, anti-4-1BB antibody, anti-CD73 antibody, anti-GITR antibody,
10 and anti-LAG-3 antibody.

[00240] The compositions of the present technology may optionally be administered as a single bolus to a subject in need thereof. Alternatively, the dosing regimen may comprise multiple administrations performed at various times after the appearance of tumors.

[00241] Administration can be carried out by any suitable route, including orally, intranasally,
15 parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intracranially, intratumorally, intrathecally, or topically. Administration includes self-administration and the administration by another. It is also to be appreciated that the various modes of treatment of medical conditions as described are intended to mean “substantial”, which includes total but also less than total treatment, and wherein some biologically or medically
20 relevant result is achieved.

[00242] In some embodiments, the antibodies of the present technology comprise pharmaceutical formulations which may be administered to subjects in need thereof in one or more doses. Dosage regimens can be adjusted to provide the desired response (*e.g.*, a therapeutic response).

25 [00243] Typically, an effective amount of the antibody compositions of the present technology, sufficient for achieving a therapeutic effect, range from about 0.000001 mg per kilogram body weight per day to about 10,000 mg per kilogram body weight per day. Typically, the dosage ranges are from about 0.0001 mg per kilogram body weight per day to about 100 mg

per kilogram body weight per day. For administration of anti-HER2 antibodies, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg every week, every two weeks or every three weeks, of the subject body weight. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight every week, every two weeks or every three weeks or within the range of 1-10 mg/kg every week, every two weeks or every three weeks. In one embodiment, a single dosage of antibody ranges from 0.1-10,000 micrograms per kg body weight. In one embodiment, antibody concentrations in a carrier range from 0.2 to 2000 micrograms per delivered milliliter. An exemplary treatment regime entails administration once per every two weeks or once a month or once every 3 to 6 months. Anti-HER2 antibodies may be administered on multiple occasions. Intervals between single dosages can be hourly, daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the antibody in the subject. In some methods, dosage is adjusted to achieve a serum antibody concentration in the subject of from about 75 µg/mL to about 125 µg/mL, 100 µg/mL to about 150 µg/mL, from about 125 µg/mL to about 175 µg/mL, or from about 150 µg/mL to about 200 µg/mL. Alternatively, anti-HER2 antibodies can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the subject. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, or until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[00244] In another aspect, the present disclosure provides a method for detecting cancer in a subject *in vivo* comprising (a) administering to the subject an effective amount of an antibody (or antigen binding fragment thereof) of the present technology, wherein the antibody is configured to localize to a cancer cell expressing HER2 and is labeled with a radioisotope; and (b) detecting the presence of a tumor in the subject by detecting radioactive levels emitted by the antibody that are higher than a reference value. In some embodiments, the reference value is expressed as

injected dose per gram (%ID/g). The reference value may be calculated by measuring the radioactive levels present in non-tumor (normal) tissues, and computing the average radioactive levels present in non-tumor (normal) tissues $\pm$ standard deviation. In some embodiments, the ratio of radioactive levels between a tumor and normal tissue is about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1 or 100:1.

[00245] In some embodiments, the subject is diagnosed with or is suspected of having cancer. Radioactive levels emitted by the antibody may be detected using positron emission tomography or single photon emission computed tomography.

[00246] Additionally or alternatively, in some embodiments, the method further comprises administering to the subject an effective amount of an immunoconjugate comprising an antibody of the present technology conjugated to a radionuclide. In some embodiments, the radionuclide is an alpha particle-emitting isotope, a beta particle-emitting isotope, an Auger-emitter, or any combination thereof. Examples of beta particle-emitting isotopes include ^{86}Y , ^{90}Y , ^{89}Sr , ^{165}Dy , ^{186}Re , ^{188}Re , ^{177}Lu , and ^{67}Cu . Examples of alpha particle-emitting isotopes include ^{213}Bi , ^{211}At , ^{225}Ac , ^{152}Dy , ^{212}Bi , ^{223}Ra , ^{219}Rn , ^{215}Po , ^{211}Bi , ^{221}Fr , ^{217}At , and ^{255}Fm . Examples of Auger-emitters include ^{111}In , ^{67}Ga , ^{51}Cr , ^{58}Co , $^{99\text{m}}\text{Tc}$, $^{103\text{m}}\text{Rh}$, $^{195\text{m}}\text{Pt}$, ^{119}Sb , ^{161}Ho , $^{189\text{m}}\text{Os}$, ^{192}Ir , ^{201}Tl , and ^{203}Pb . In some embodiments of the method, nonspecific FcR-dependent binding in normal tissues is eliminated or reduced (*e.g.*, *via* N297A mutation in Fc region, which results in aglycosylation). The therapeutic effectiveness of such an immunoconjugate may be determined by computing the area under the curve (AUC) tumor: AUC normal tissue ratio. In some embodiments, the immunoconjugate has a AUC tumor: AUC normal tissue ratio of about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1 or 100:1.

[00247] *Toxicity.* Optimally, an effective amount (*e.g.*, dose) of an anti-HER2 antibody described herein will provide therapeutic benefit without causing substantial toxicity to the subject. Toxicity of the anti-HER2 antibody described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD₅₀ (the dose lethal to 50% of the population) or the LD₁₀₀ (the dose lethal to 100% of the

population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the anti-HER2 antibody described herein lies within a range of circulating concentrations that include the effective dose with little
5 or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the subject's condition. *See, e.g., Fingl et al., In: The Pharmacological Basis of Therapeutics, Ch. 1 (1975).*

[00248] *Formulations of Pharmaceutical Compositions.* According to the methods of the
10 present technology, the anti-HER2 antibody can be incorporated into pharmaceutical compositions suitable for administration. The pharmaceutical compositions generally comprise recombinant or substantially purified antibody and a pharmaceutically-acceptable carrier in a form suitable for administration to a subject. Pharmaceutically-acceptable carriers are determined in part by the particular composition being administered, as well as by the particular
15 method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions for administering the antibody compositions (*See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA 18th ed., 1990*). The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food
20 and Drug Administration. The pharmaceutical composition may further comprise an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

[00249] The terms "pharmaceutically-acceptable," "physiologically-tolerable," and
25 grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a subject without the production of undesirable physiological effects to a degree that would prohibit administration of the composition. For example, "pharmaceutically-acceptable

excipient” means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous. “Pharmaceutically-acceptable salts and esters”

5 means salts and esters that are pharmaceutically-acceptable and have the desired pharmacological properties. Such salts include salts that can be formed where acidic protons present in the composition are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, *e.g.*, sodium and potassium, magnesium, calcium, and aluminum. Suitable organic salts include those formed with organic
10 bases such as the amine bases, *e.g.*, ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (*e.g.*, hydrochloric and hydrobromic acids) and organic acids (*e.g.*, acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic acids such as methanesulfonic acid and benzenesulfonic acid). Pharmaceutically-acceptable esters include
15 esters formed from carboxy, sulfonyloxy, and phosphonoxy groups present in the anti-HER2 antibody, *e.g.*, C₁₋₆ alkyl esters. When there are two acidic groups present, a pharmaceutically-acceptable salt or ester can be a mono-acid-mono-salt or ester or a di-salt or ester; and similarly where there are more than two acidic groups present, some or all of such groups can be salified or esterified. An anti-HER2 antibody named in this technology can be present in unsalified or
20 unesterified form, or in salified and/or esterified form, and the naming of such anti-HER2 antibody is intended to include both the original (unsalified and unesterified) compound and its pharmaceutically-acceptable salts and esters. Also, certain embodiments of the present technology can be present in more than one stereoisomeric form, and the naming of such anti-HER2 antibody is intended to include all single stereoisomers and all mixtures (whether racemic
25 or otherwise) of such stereoisomers. A person of ordinary skill in the art, would have no difficulty determining the appropriate timing, sequence and dosages of administration for particular drugs and compositions of the present technology.

[00250] Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-

aqueous vehicles such as fixed oils may also be used. The use of such media and compounds for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or compound is incompatible with the anti-HER2 antibody, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00251] A pharmaceutical composition of the present technology is formulated to be compatible with its intended route of administration. The anti-HER2 antibody compositions of the present technology can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intradermal, transdermal, rectal, intracranial, intrathecal, intraperitoneal, intranasal; or intramuscular routes, or as inhalants. The anti-HER2 antibody can optionally be administered in combination with other agents that are at least partly effective in treating various HER2-associated cancers.

[00252] Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00253] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of

microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, *e.g.*, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, *e.g.*, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal compounds, *e.g.*, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be desirable to include isotonic compounds, *e.g.*, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition a compound which delays absorption, *e.g.*, aluminum monostearate and gelatin.

[00254] Sterile injectable solutions can be prepared by incorporating an anti-HER2 antibody of the present technology in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the anti-HER2 antibody into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The antibodies of the present technology can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

[00255] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the anti-HER2 antibody can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding compounds, and/or adjuvant

materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating compound such as alginic acid, Primogel, or corn starch; a lubricant
5 such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening compound such as sucrose or saccharin; or a flavoring compound such as peppermint, methyl salicylate, or orange flavoring.

[00256] For administration by inhalation, the anti-HER2 antibody is delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*,
10 a gas such as carbon dioxide, or a nebulizer.

[00257] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, *e.g.*, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal
15 administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the anti-HER2 antibody is formulated into ointments, salves, gels, or creams as generally known in the art.

[00258] The anti-HER2 antibody can also be prepared as pharmaceutical compositions in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other
20 glycerides) or retention enemas for rectal delivery.

[00259] In one embodiment, the anti-HER2 antibody is prepared with carriers that will protect the anti-HER2 antibody against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides,
25 polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can

also be used as pharmaceutically-acceptable carriers. These can be prepared according to methods known to those skilled in the art, *e.g.*, as described in U.S. Pat. No. 4,522,811.

[00260] *T Cells Bound to HER2 Multi-specific Binding Molecules Disclosed Herein.* Without being bound by any theory, it is believed that when the anti-CD3 multi-specific binding molecules provided herein (*e.g.*, HER2 $\times$ CD3) are bound to T cells, by, for example, procedures such as those described herein, an anti-CD3 scFv of the multi-specific 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 multi-specific 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.

[00261] Thus, the present disclosure also provides T cells which are bound to a multi-specific binding molecule of the present technology. In specific embodiments, the T cells are bound to the multi-specific binding molecule noncovalently. In specific embodiments, the T cells 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.

[00262] In specific embodiments, the T cells which are bound to multi-specific binding molecules of the invention are used in accordance with the therapeutic methods described herein. In specific embodiments, the T cells which are bound to multi-specific binding molecules of the present disclosure are used as part of a combination therapy as described below.

[00263] In specific embodiments involving combination therapy with infusion of T cells, provided herein is a pharmaceutical composition comprising (a) a multi-specific binding molecule described herein; (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 either bound or not bound to the multi-specific binding molecule. In specific embodiments, the binding of the T cells to the multi-specific binding molecule is noncovalently. In specific embodiments, the T cells are human T

cells. Methods that can be used to bind multi-specific 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.

[00264] In a specific embodiment, the administering of a multi-specific binding molecule provided herein, polynucleotide, vector, or cell encoding the multi-specific binding molecule, or a pharmaceutical composition comprising the multi-specific 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 multi-specific binding molecule as described herein. In specific embodiments, the binding of the T cells to molecules identical to the multi-specific binding molecule is noncovalently. In specific embodiments, the T cells are human T cells.

C. Kits

[00265] The present technology provides kits for the detection of HER2 and/or treatment of HER2-associated cancers, comprising at least one immunoglobulin-related composition of the present technology (*e.g.*, any antibody or antigen binding fragment described herein), or a functional variant (*e.g.*, substitutional variant) thereof. Optionally, the above described components of the kits of the present technology are packed in suitable containers and labeled for diagnosis and/or treatment of HER2-associated cancers. The above-mentioned components may be stored in unit or multi-dose containers, for example, sealed ampoules, vials, bottles, syringes, and test tubes, as an aqueous, preferably sterile, solution or as a lyophilized, preferably sterile, formulation for reconstitution. The kit may further comprise a second container which holds a diluent suitable for diluting the pharmaceutical composition towards a higher volume. Suitable diluents include, but are not limited to, the pharmaceutically acceptable excipient of the pharmaceutical composition and a saline solution. Furthermore, the kit may comprise instructions for diluting the pharmaceutical composition and/or instructions for administering the

pharmaceutical composition, whether diluted or not. The containers may be formed from a variety of materials such as glass or plastic and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper which may be pierced by a hypodermic injection needle). The kit may further comprise more containers comprising a pharmaceutically acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, culture medium for one or more of the suitable hosts. The kits may optionally include instructions customarily included in commercial packages of therapeutic or diagnostic products, that contain information about, for example, the indications, usage, dosage, manufacture, administration, contraindications and/or warnings concerning the use of such therapeutic or diagnostic products.

[00266] The kits are useful for detecting the presence of an immunoreactive HER2 protein in a biological sample, *e.g.*, any body fluid including, but not limited to, *e.g.*, serum, plasma, lymph, cystic fluid, urine, stool, cerebrospinal fluid, ascitic fluid or blood and including biopsy samples of body tissue. For example, the kit can comprise: one or more humanized, chimeric, bispecific, or multi-specific anti-HER2 antibodies of the present technology (or antigen binding fragments thereof) capable of binding a HER2 protein in a biological sample; means for determining the amount of the HER2 protein in the sample; and means for comparing the amount of the immunoreactive HER2 protein in the sample with a standard. One or more of the anti-HER2 antibodies may be labeled. The kit components, (*e.g.*, reagents) can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect the immunoreactive HER2 protein.

[00267] For antibody-based kits, the kit can comprise, *e.g.*, 1) a first antibody, *e.g.* a humanized, chimeric, bispecific, or multi-specific HER2 antibody of the present technology (or an antigen binding fragment thereof), attached to a solid support, which binds to a HER2 protein; and, optionally; 2) a second, different antibody which binds to either the HER2 protein or to the first antibody, and is conjugated to a detectable label.

[00268] The kit can also comprise, *e.g.*, a buffering agent, a preservative or a protein-stabilizing agent. The kit can further comprise components necessary for detecting the detectable-label, *e.g.*, an enzyme or a substrate. The kit can also contain a control sample or a series of control samples, which can be assayed and compared to the test sample. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit. The kits of the present technology may contain a written product on or in the kit container. The written product describes how to use the reagents contained in the kit, *e.g.*, for detection of a HER2 protein *in vitro* or *in vivo*, or for treatment of HER2-associated cancers in a subject in need thereof. In certain embodiments, the use of the reagents can be according to the methods of the present technology.

EXAMPLES

[00269] The present technology is further illustrated by the following Examples, which should not be construed as limiting in any way. The following Examples demonstrate the preparation, characterization, and use of illustrative anti-HER2 antibodies of the present technology.

Example 1: Introduction and Preliminary Experiments

[00270] Early bispecific T cell engager (TCE) efforts have mainly focused on maximizing cytotoxic activity based on *in vitro* cell-based assays without anticipating the biological consequences of high potency on cytokine release and T-cell exhaustion or depletion in the patient. These safety concerns were summarized at a recent FDA-sponsored workshop focused on CD3 TCE safety assessment (Kamperschroer *et al.*, *J Immunotoxicol.* 17(1):67-85 (2020)). Later generations of TCEs include Fcs or other similar domains for the purpose of extending half-life, but adverse events and clinical holds suggest that extending half-life with a high potency TCE could exacerbate serious adverse events associated with neurotoxicity and cytokine release syndrome (CRS) (Vafa *et al.*, *Front. Oncol.* 10: 446 (2020)).

[00271] One potential strategy for overcoming resistance to current targeted therapies is to harness the killing activity of the T cell to defeat cancer by employing BsAbs. These T-cell-engaging antibodies are designed to simultaneously bind antigens on tumor cells and T-cell

activators such as the co-receptor CD3. BsAb engagement of the T cell mediates the killing of tumor cells by activating T cells through binding of CD3 and forming a cytolytic synapse, redirecting the killing activity toward the antigen-expressing tumor cells in a major histocompatibility complex (MHC)-independent manner.

5 [00272] Trastuzumab × huOKT3 (ABP100) is a bispecific antibody developed to treat patients affected by HER2+ types of cancer, including but not limited to breast, gastric and colorectal. This molecule binds HER2+ tumors and brings natural immune T cells to the tumor to reduce it. This BsAb is based on two well-known molecules: trastuzumab, a fully humanized HER2-targeting Immunoglobulin G1 (IgG1), and humanized muromonab-CD3 (huOKT3), a CD3-
10 targeting IgG1. A key feature of ABP100 is that it was built using a symmetric bivalent BsAb platform IgG-[L]-scFv, in which a single-chain variable fragment (scFv) recognizing human CD3 is fused to the C terminus of each anti-tumor IgG antibody light chain (**FIG. 4**). The symmetric IgG-[L]-scFv design has provided potent *in vitro* and *in vivo* anti-tumor activity against multiple tumor antigens (GD2, CD3, GPA33, and HER2), and recent reports demonstrate
15 that the IgG-L-scFv platform valency and spatial configuration drive substantially more robust anti-tumor responses than many other BsAb formats (Santich *et al.*, *Sci. Transl. Med.* 12: eaax1315 (2020), **FIGs. 5A-5B**). To reduce the risk of CRS, the Fc domain function of ABP100 was silenced to eliminate potential antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) activity by introducing two well-characterized
20 mutations: N297A to remove glycosylation, and K322A to reduce complement activation.

[00273] Starting with ABP100 in the full format described above as a template, a dual strategy for the CD3×HER2 bispecific antibody program was proposed to limit the toxic effects that are commonly associated with first-generation TCEs and CAR T-cell therapeutics. The two products of this dual strategy are 1) ABP100a, a HER2 affinity-tuned BsAb with selective killing
25 of HER2-high expressing cells designed for ex-vivo loading of patient T cells for reinfusion, and 2) ABP102, a precisely redesigned BsAb for intravenous delivery with dual affinity-tuned arms for CD3 and HER2 binding (**FIG. 4**).

[00274] ABP100a was built by replacing the huOKT3 portion of ABP100 with a novel humanized huSP34 CD3 binding arm and introducing novel affinity-tuned HER2 binding arms (FIG. 4). The humanized huSP34 CD3 binding arm is cross-reactive with non-human primate (Cyno) CD3, which makes it a suitable model for assessing and predicting toxicities in humans.

5 The affinity-tuned HER2 binding arms allows ABP100a to selectively kill HER2 high-expressing cells, while sparing low, endogenous-level HER2-expressing cells. The potential of directly arming ABP100a onto patient-derived and activated T cells, a technology that has shown great potential in preclinical models, will be explored (FIG. 6). The approach is to be explored by using a relatively high-affinity CD3-binding arm with a low off-rate, bearing in mind that
10 CRS concerns should be minimal by directly arming pre-activated T cells.

[00275] In parallel, ABP102 was developed by additionally affinity tuning the CD3 T-cell binding arm to select for CD3 affinity with potent killing of HER2-amplified cancer cells with the limited T-cell generation of cytokines such as interferon gamma (IFN γ) and tumour necrosis factor alpha (TNF α). This approach invokes the key understanding that individually tuning each
15 binding arm of the BsAb will not necessarily improve the overall efficacy or safety profile of the molecule as a whole, so the dual affinity-tuned BsAb in the context of the full BsAb with all modifications fully implemented was evaluated. This dual modification of the HER2 and CD3 binding affinities on ABP102 yielded novel BsAbs for systemic delivery with selective killing of HER2-amplified cancer cells and is anticipated to show reduced cytokine production for better
20 safety in the clinic.

Example 2: Antibody humanization, affinity tuning, and characterization

[00276] Antibody humanization and back-mutation by rational design

[00277] The complementarity-determining regions (CDRs), hypervariable loops, and framework regions (FRs) of a mouse anti-CD3 antibody were analyzed within the variable
25 sequence and identified according to the KABAT delineation system. The CDRs of the mouse antibody were directly grafted to the human acceptor framework using the heavy chain variable (V_H) and light chain variable domain (V_L) that share the highest sequence identities to the mouse counterparts. Homology modeling was then performed to obtain the modeled structure of the

mouse antibody, and the solvent accessible surface area of framework residues was calculated to identify framework residues that are buried. Then, critical residues in the sequences of the V_H and V_L that are different in the grafted and mouse antibody framework sequences were identified and back-mutated. Finally, the grafted sequence were inspected for potential liability like N-glycosylation sites, post-translational modifications and unpaired cysteine residues which may affect the binding activity of the grafted antibody.

[00278] *Design and Construction of NNK Libraries, FASEBA Screening, and Affinity Ranking*

[00279] Initially, the affinity measurement of HER2 and the parental BsAb was determined using a Surface Plasmon Resonance (SPR) biosensor, Biacore 8K (GE Healthcare, Marlborough, MA). The equilibrium dissociation constant (KD) was calculated from the ratio of k_d over k_a . To determine the contribution of a specific residue to antibody affinity and expression, paratope mapping was performed by screening of NNK libraries. In brief, the V_H and V_L of the parental antibody were searched by using NCBI Ig-Blast (www.ncbi.nlm.nih.gov/projects/igblast/) and CDRs defined by the KABAT delineation system. All residues within CDRs were defined and mutated by NNK method. Each individual NNK library was generated per residue based on the FASEBA platform with a theoretical diversity at 20. Over 48 clones were randomly selected from each NNK library for expression in *E. coli* in 96-deep-well plates. All clones were sequenced, and the unique clones selected. The crude selected protein secreted in medium was analyzed by ELISA against bovine serum albumin (BSA) and human and cyno antigen protein for the assessment of expression and binding specificity, respectively. The “beneficial mutants” that decrease antibody affinity, without compromising antibody expression, were confirmed by GenScript’s Fast Screening for Expression level, Biophysical properties, and Affinities (FASEBA) platform via screening and affinity ranking.

[00280] *Selection and synthesis of affinity-tuned bispecific antibodies*

[00281] From the mutant library, a series of antibodies with varying affinities for human and cyno HER2 were constructed in a bispecific antibody format by subcloning into an expression vector for expression in Expi-CHO-S cells. The bispecific antibodies were purified using protein A columns.

[00282] Binding between the parental and affinity-tuned bispecific antibodies and human and cyno target proteins (HER2 and CD3) were validated by ELISA, and a Biacore 8K was used to study the kinetics of the interaction between the antigen and the bispecific antibodies. See **FIGs. 10, 12 and 17A-17B**.

5 **Example 3: In vitro Characterization of Anti-HER2 Antibodies of the Present Technology**

[00283] T cell and target-cell binding by flow cytometry

[00284] Purified CD3⁺ T cells and whole human PBMCs were stained in bulk with Live/Dead stain, then incubated with a range of concentrations of bispecific antibodies to assess for binding. Controls included appropriate monoclonal antibodies (humanized SP34-hIgG1) and isotype
10 control (hIgG1). A secondary antibody, anti-human IgG Fc specific PE conjugate, was used for detection.

[00285] Cell lines were grown per recommendations of the source of the cell line, followed by Accutase treatment to lift cells. Cells were resuspended in media, washed, and stained in bulk with Live/Dead stain, followed by staining with bispecific antibodies and a control monoclonal
15 antibody (trastuzumab hIgG1). A secondary antibody, anti-human IgG Fc specific PE conjugate, was used for detection. Data collection was performed using the HTS platform for the BD FACSCelesta, with data analysis performed in FlowJo software. **FIG. 7A** shows that the anti-HER2×CD3 BsAbs of the present technology show reduced binding to T cells compared to the humanized SP34-hIgG1 control.

20 [00286] T cell-dependent cellular cytotoxicity (TDCC) in vitro assay

[00287] To assess for biologic activity, established in-house TDCC assays (Cell Titer Glo 2.0 (Promega) and Caspase3/7 green apoptosis assay (IncuCyte)) using effector human CD3⁺ T cells were performed to test bispecific HER2 × CD3 antibodies with differing CD3 affinities against different target cell lines. Anti-HER2 × CD3 BsAbs showed selective killing of high HER2-
25 expressing target cells (SKBR-3 cell line) while sparing target cells expressing near endogenous HER2 levels (MCF-7 cell line) (**FIGs. 7B-7C**, and **FIGs. 9A-9B**). In this experiment, ABP100s.10.0 had WT-trastuzumab-like affinity, and ABP100s.10.5 and ABP100s.10.6 had

lower HER2 affinity (~64-fold and ~108-fold, respectively), whereas all bispecific constructs contained the same high-affinity CD3 scFv arm. Notably, ABP100s.10.5 and ABP100s.10.6 constructs with low HER2 affinity allowed for differential and selective killing of low HER2-expressing target cells (MCF-7), in contrast to the antibody with WT-like affinity
5 (ABP100s.10.0).

[00288] In contrast, the trastuzumab × huOKT3 parent antibody showed non-selective killing for both SKBR-3 and MCF-7 cell lines. See **FIGs. 8A-8B**. It is observed that killing of the HER2-high cell line is achieved at a lower concentration of trastuzumab × huOKT3 than the HER2-low cell line, but killing of the HER2-low cell line cannot be completely eliminated (**FIG.**
10 **8B**).

[00289] The CTG2.0 assay readout was on the Spectramax iD3, a standard luminescence 96-well plate reader, using white-colored well plates. The IncuCyte S3 platform and software for image-based analyses allowed determination of total green area/image from the Caspase3/7 green reagent using black clear-bottom 96-well tissue-culture treated plates. This assay provided
15 estimated relative frequency of apoptotic cells within each well.

[00290] These results demonstrate that reduction in CD3 scFv affinity has minimal impact on the killing of high HER2-expressing target cells (SKBR-3), while continuing to exhibit no killing of low HER2-expressing cells (MCF-7).

[00291] The effect of the various Her2 and CD3 affinities of each HER2×CD3 BsAbs of the
20 present technology on antibody binding, T cell activation, T cell-mediated killing, and cytokine production in the presence or absence of tumor cell lines expressing various levels of Her2 was investigated. SKBR3 and HCC1954 were selected as they represent high-density Her-2 expressing cell lines with similar Her2-expression levels (Ram *et al.*, *MAbs*. 2014 6(5): 1211–1219) which correspond to HercepTest results of 3+ for SKBR3 (Dako HercepTest Interpretation
25 Manual, www.agilent.com/cs/library/usermanuals/public/28630_herceptest_interpretation_manual-breast_ihc_row.pdf), and MCF-7 and HT55 as low-density Her2-expressing cells with similar expression levels to each other and to that of endogenous non-cancerous cells expressing Her2, with MCF-7 representing HercepTest scores of 0-1+ (Rhodes *et al.*, *Am J Clin Pathol* 2002

118(3):408-17; Subik *et al.*, *Breast Cancer* (Auckl). 2010; 4: 35–41; Slaga *et al.*, *Sci Transl Med* 2018 Oct 17;10(463):eaat5775). All p values from the data below for the NFAT T cell receptor activation assay, cytotoxicity assays, and cytokine assays were derived from a two-Way ANOVA with Tukey's multiple comparisons test ran on Graphpad Prism Version 9.4.0.

5 **[00292] CD3/TCR NFAT T cell activation reporter assay:** To assess for T cell activation by bispecific and monoclonal antibodies, the T cell activation Bioassay kit (Promega J1621 / J1625) was used with TCR/CD3 Jurkat effector cells (NFAT reporter), with detection using Bio-Glo Luciferase assay system (Promega G7941). Briefly, using White bottom/chimney (solid white) TC-treated plates (Corning 3917), 40,000 target cells (*Her2-high*: SK-BR-3, HCC1954; 10 *Her2-low*: MCF-7, HT55) were plated overnight in 100 μ L media, and also used a condition without target cells. Assay was then performed in accordance with detailed instructions provided with the assay kit, with 7 hours of incubation followed by luminescence readout.

[00293] Generally higher activation was seen with target cells expressing higher amounts of Her2 (**FIGs. 13A-13B**). Notably, 10.5.1 and 10.6.1 were only slightly decreased from the 15 parental construct (10.0) on Her2-high targets (SK-BR-3, HCC1954) with all activation readouts for all constructs being within 300,000-600,000 RLUs at the highest dose examined (40 nM) (**FIGs. 13A-13B**). On Her2-low targets, the 10.0 construct exhibited similar, if slightly lower activation (Approximately 300,000 RLU at 40 nM on both MCF-7 and HT-55 cell lines) while the 10.5 and 10.6 constructs showed significantly diminished activation (approximately 200,000 20 RLU) compared to the 10.0 on both cell lines ($p < 0.0001$ for both comparisons) (**FIGs. 13C-13D**). Furthermore, the 10.5.1 and 10.6.1 constructs exhibited significantly lower activation at 40 nM (approximately 100,000 RLU) than 10.5 and 10.6 ($p < 0.0001$ for both comparisons) on Her2-low target cells (HT55, MCF-7; **FIGs. 13C-13D**) and were statistically not different from the isotype control and were similar to the background activation seen in the absence of target 25 cells (**FIG. 13E**). Taken together, these data are consistent with the lack of 10.5.1 and 10.6.1-induced T cell activation in low-Her2-expressing cells.

[00294] T cell dependent cellular cytotoxicity (TDCC): To assess *in-vitro* functional capacity of the bispecific antibodies to mediate T cell-mediated killing of Her2-expressing target cells, T cell dependent cellular cytotoxicity (TDCC) assays were performed with CD3+ T cells.

Target cells were plated to white bottom/chimney tissue culture treated plates (Corning 3917) at 10,000 cells/well in 100 μ L media and incubated overnight (*Her2-high*: SK-BR-3, HCC1954; *Her2-low*: MCF-7, HT55). Bispecific antibodies were diluted (range: 30, 0.3, 0.003, 0.00003 nM final concentrations) in RPMI1640/10% heat-inactivated FBS. Culture media was removed from target cells and bispecific antibodies were added at 100 μ L, followed by addition of purified human cryopreserved T cells (StemCellTechnologies 70024) at an effector:target ratio of 5 to 1 (T cells : target cells). Detection was performed at 40 hours with CellTiterGlo2.0 (Promega) and luminescence detection on a SpectraMax iD3 plate reader. Results in the figure displayed are representative of experiments done with three distinct donor CD3+ T cell samples.

[00295] With SK-BR-3 target cells, 10.5.1 and 10.6.1 had only exhibited modest, yet significant (10.0 vs. 10.5.1: $p = 0.0042$; 10.0 vs 10.6.1: $p = 0.0002$) reductions in killing versus the parental construct (10.0) at 40 nM (**FIG. 14A**). There was no statistically significant difference between the Her2-mutated, CD3-unmutated 10.5 and 10.6 and the parental 10.0 (**FIG. 14A**) in the presence of SKBR3 cells, consistent with the intended targeting of high Her2-expressing cells by 10.5.1 and 10.6.1. On HCC1954 target cells, while there was significantly reduced killing with 10.5 and 10.6 compared to the 10.0 parent (**FIG. 14B**), this reduction was slight (approximately 10-20% reduction from the 10.0 killing levels for the SKBR3 and HCC1954) in comparison to the reduction seen in the Her2-low expressing cells (approximately 67-75% reduction from the 10.0 killing levels for the MCF-7 and HT55). On Her2-low (HT55, MCF-7) target cells at 40 nM, there was no significant difference between the isotype control killing levels and that of 10.5, 10.6, 10.5.1, and 10.6.1 (**FIGs. 14C-14D**) (with the exception of isotype vs. 10.5 for the MCF-7 cell line, **FIG. 14C**). In contrast, at the same dose in both MCF-7 and HT-55 cell lines, all of these constructs displayed significantly reduced killing compared to the 10.0 parent ($p < 0.0001$ for: 10.0 vs 10.5, 10.0 vs 10.6, 10.0 vs 10.5.1 and 10.0 vs 10.6.1) (**FIGs. 14C-14D**) indicating that there was little to no killing of the low Her2-expressing cell lines by the Her2- and CD3-mutated constructs. The significantly increased killing resulting from 10.5 on the MCF-7 cell line was consistently observed in multiple experiments indicating that it may likely be due to the slightly stronger affinity of 10.5 for Her2 (KD is approximately 45 nM) than 10.6 (KD is approximately 67 nM).

[00296] Multiplex Cytokine detection assay and associated cytotoxicity on Her2-high and Her2-low target cell lines with human PBMCs. To analyze cytokine release mediated by bispecific antibody constructs, human PBMCs were contacted with soluble antibody with SKBR-3 (Her2-high) and MCF-7 (Her2-low), as well as a "no target cell" condition. Monoclonal anti-CD3/anti-CD28 antibodies served as a control. Briefly, SKBR-3 (Her2-high) and MCF-7 (Her2-low) target cell lines were plated the night before assay. Bispecific antibodies were diluted (range: 30, 0.3, 0.003, 0.00003 nM final concentrations) in RPMI1640/10% heat-inactivated FBS. Culture media was removed from target cells and bispecific antibodies were added at 100 μ L, followed by addition of human PBMCs (1 donor, Stem Cell Technologies PBMCs $\sim$ 5 x 10⁷ cells/vial) (10:1 E:T ratio; 100,000 PBMC : 10,000 target cells) in white-chimney/bottom plates in RPMI/10% HI FBS. Culture supernatants were harvested at 24 hours and frozen -80°C for multiplexed bead-based cytokine release assay (R&D Systems Human High Sensitivity Cytokine Base Kit B: IFN- γ , IL-2, TNF- α , IL-6, GM-CSF) in conjunction with signal detection using a Magpix (Luminex) and quantification of cytokine in picograms/mL in comparison to standard wells using Luminex xMAP software. CellTiterGlo2.0 (Promega) was used to develop the assays for TDCC %cytotoxicity assessment with luminescence detection on a SpectraMax iD3 plate reader. Results in the figure displayed are representative of experiments done with three distinct donor PBMC samples. Cytotoxicity and cytokine release results were compiled in Excel and graphed in GraphPAD PRISM.

[00297] Since the excessive cytokine production associated with T cell engager administration can result in the primary toxicity of T cell engagers, cytokine release syndrome (CRS), the effect of weakened affinities for Her2 and CD3 on cytokine production was examined in the presence of tumor cell lines expressing high (SKBR3) or low (MCF-7) Her2 levels and humans PBMCs. The 10.0 parent, the Her2-reduced affinity 10.5 and 10.6 constructs, and the Her2- and CD3-weakened affinity 10.5.1 and 10.6.1 constructs all stimulated cytokine production that was significantly higher than the isotype control (control representing background cytokine production) in the presence of SKBR3 cells (**FIGs. 15A-15D**). However, in the presence of MCF-7 cells, only 10.0 displayed significantly increased cytokine production compared to the isotype control, indicating the reduced ability of the Her2- and/or CD3-weakened constructs to

stimulate cytokine production (**FIGs. 15E-15H**). At 30nM, all affinity-weakened constructs displayed significantly reduced IL-2, IFN- γ , and TNF- α production in the presence of MCF-7 cells ($p < 0.0001$ for: 10.0 vs 10.5, 10.0 vs 10.6, 10.0 vs 10.5.1 and 10.0 vs 10.6.1). Importantly, the production of IL-6, a cytokine that is a crucial mediator of CRS associated with immunomodulatory agents (Morris *et al.*, *Nat Rev Immunol* 2022;22(2):85-96), was significantly reduced with the Her2 and CD3-dually-weakened 10.5.1 and 10.6.1 constructs as compared to 10.0 ($p = 0.0432$ and $p = 0.029$, respectively) in the presence of MCF-7 cells (**FIG. 15F**). The lack of significant difference in IL-6 production observed with the Her2 affinity-weakened 10.5 and 10.6 constructs compared to the 10.0 parent highlights the importance of both Her2 and CD3 affinity weakening to achieve lowered IL-6 levels. The “no target cell” condition showed that PBMCs with bispecific antibodies did not alone promote cytokine release. For SKBR-3 (Her2-high) target cells, cytotoxicity was comparable for 10.5.1 and 10.6.1 relative to parental construct (10.0), with only minor differences observed.

[00298] These results demonstrate that these agents are useful for reducing the incidence of CRS when treating patients with high Her2 expressing (*e.g.* 3+ Herceptest score) cancers by selectively killing and producing cytokine in response to high Her2-expressing cells while sparing low Her2 expressing tissues with endogenous, noncancerous tissue levels of Her2 (0-1+ Herceptest scores), thus reducing on-target, off-tumor toxicities in the clinic.

[00299] **Flow cytometric analysis of bispecific antibody binding to activated T cells and Her-2 expressing target cells.** To assess flow cytometric binding of bispecific antibodies to activated T cells, human PBMCs (StemCell Technologies 70025.2) were stimulated with an OKT3/IL-2 stimulation protocol over 12 days. Briefly, PBMCs were activated with 100 IU/mL of recombinant human IL-2 (Stemcell Technologies, cat #78145.1) and 20 ng/mL of OKT3 (Biolegend, mouse IgG2a, Cat#317326) in soluble format for 3 days, then expanded/maintained thereafter by using fresh media and IL-2 only by normalizing cells to 1×10^6 cells/mL in RPMI1640/10%FBS. Activated T cells were cryopreserved and stored in liquid nitrogen freezer. On the day of assay, T cells were thawed and washed, followed by staining with diluted bispecific antibodies (initial working stock was 240nM (2x) for a final concentration of 120nM, serially diluted 1:10 in FACS buffer for a total of seven serial dilutions). Cells were stained for

30 minutes at 4°C in cold PBS/1%BSA with primary antibodies, followed by washing and addition of 1:250 secondary antibody (anti-human IgG-PE, Thermo Fisher Scientific) for 30 minutes at 4°C in cold PBS/1%BSA. Following wash step, resuspended cells in 150µL PBS/1%BSA and detect PE signal on a FACSCelesta HTS system with 96 well V bottom plate.

5 Live/dead was assessed using BV421 channel (Zombie violet, Thermo Fisher Scientific) or Trypan Blue prior to assay.

[00300] To assess flow cytometric binding of bispecific antibodies to cell lines, including Her2-high (SKBR-3, SKOV-3) and Her2-low (MCF-7, HT55), cells were grown in their respective culture media according to ATCC protocols to 70-80% confluence. Cell lines were treated with Accutase to preserve cell surface epitopes for flow cytometry. Cells were resuspended in 5mL PBS (no BSA) and stained with 1:1000 dilution of Live/Dead Zombie dye (Biolegend) at room temperature for 20 minutes. Cells were then washed and normalized to add 100,000 cells/well on a 96-well V-bottom plate with staining for 30 minutes at 4°C in cold PBS/1%BSA with primary antibodies. This was followed by washing and addition of 1:250 secondary antibody (anti-human IgG-PE, Thermo Fisher Scientific) for 30 minutes at 4°C in cold PBS/1%BSA. Following wash step, resuspended cells in 150µL PBS/1%BSA and detect PE signal on a FACSCelesta HTS system with 96 well V bottom plate. Live/dead was assessed using BV421 channel (Zombie violet, Thermo Fisher Scientific) or Trypan Blue prior to assay. Analysis was performed in FlowJo software to obtain PE (anti-human IgG-PE) Median (MFI) values, then data was organized and plotted in Microsoft Excel, and GraphPad PRISM software in GraphPAD Prism software.

[00301] Results: Activated T cell binding was reduced for 10.5.1 and 10.6.1 compared to the parental construct (10.0) (**FIG. 16E**). The reduced T cell binding observed in 10.5.1 and 10.6.1 can be attributed at least in part to having a CD3 arm exhibiting reduced affinity.

25 **[00302]** Target cell line binding to Her2-high (SK-BR-3, SK-OV-3) cell lines was slightly reduced with 10.5.1 and 10.6.1 constructs compared to the parental construct (10.0) (approximately 33% reduction in MFI for both 10.5.1 and 10.6.1 compared to 10.0 at about 100 nM concentration) (**FIGs. 16A-16B**). Target cell line binding to Her2-low (MCF-7, HT55) target cell lines was greatly reduced for the 10.5.1 and 10.6.1 constructs compared to the parental

construct (10.0) (approximately 84% reduction in MFI for both constructs compared to 10.0 at about 100 nM concentration) (**FIGs. 16C-16D**). Together, these results demonstrate that the dually affinity-weakened HER2 x CD3 constructs (10.5.1, 10.6.1) have properties that contribute to selectivity for Her2-high target cell lines and maintain the capability to bind to CD3+ T cells in order to potentiate cytotoxicity. Overall, the affinity-weakened constructs 10.5.1 and 10.6.1 show reduced cytotoxicity and cytokine release on Her2-low target cells when compared to the parental construct.

[00303] Taken together, the HER2 and CD3 reduced affinity bispecific antibody constructs described in **FIG. 10** having comparable affinities to 10.5.1 and 10.6.1 are expected to show similar behavior as 10.5.1 and 10.6.1 in at least one of NFAT activation, TDCC, and/or FACS.

Example 4: In Vivo Efficacy of the HER2 Affinity-tuned Bispecific Antibodies of the Present Technology

[00304] In order to evaluate the *in vivo* efficacy of the affinity tuned bispecific antibodies of the present technology, human tumor and peripheral blood mononuclear cell (PBMC) co-xenografts in mice will be performed. Given that low-affinity binding to HER2 results in selective killing of high-HER2 expressing tumors, sparing low-HER2-expressing tumors (used as a surrogate for endogenous HER2 expression of normal, non-cancerous tissues) *in vitro* (**FIG. 14**) we intend to evaluate if this selective killing would also be observed *in vivo*. NSG mice will be implanted subcutaneously with mixture of 1-5 million HER2-expressing tumors and human PBMCs (1:2 or 1:3 PBMC:tumor (E:T) ratio). A high-Her2-expressing tumor cell line, such as HCC1954 cells (representing expression levels of tumors expected in the clinic (*e.g.* those with 2+ or 3+ HecepTest scores) or a low Her-2-expressing tumor cell line, such as HT55 cells (representing Her2-levels in noncancerous tissues) will be implanted into sufficiently immunocompromised mice, such as Nod-Scid-gamma (NSG) mice or similar. Different dose levels (at least a range that includes from 5 mg/kg to 0.005 mg/kg) of various Her2- and CD3-affinity weakened constructs will be administered to mice after implantation of tumor cells and PBMCs. Doses will be administered parenterally (*e.g.*, i.v. or i.p.) once weekly or more for one or more weeks. Tumor volumes will be measured for the duration of the study.

[00305] It is expected that the 10.0 parent will non-selectively inhibit the growth of both high and low Her2-expressing tumor cells at doses where 10.5.1 and 10.6.1 will selectively inhibit the growth of the high-Her2 tumors but have little or no growth inhibiting activity on low Her2-expressing tumors. It is also expected that the levels of cytokines such as IL-2, IL-6, IFN- γ , and
5 TNF- α will be reduced in the animals dosed with 10.5.1 and 10.6.1 clones as compared to the 10.0 parent.

[00306] Accordingly, the immunoglobulin-related compositions of the present technology are useful to treat a HER2-associated cancer in a subject in need thereof.

EQUIVALENTS

10 [00307] The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within the scope of the present technology, in
15 addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the present technology. It is to be understood that this present technology is not limited to particular methods, reagents, compounds compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of
20 describing particular embodiments only, and is not intended to be limiting.

[00308] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

25 [00309] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range

discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language such as “up to,” “at least,” “greater than,” “less than,” and the like, include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood
5 by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

[00310] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to
10 the extent they are not inconsistent with the explicit teachings of this specification.

WHAT IS CLAIMED IS:

1. An antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein

5 (a) (i) the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 1, a V_H -CDR2 sequence of SEQ ID NO: 2 or SEQ ID NO: 7 and a V_H -CDR3 sequence of SEQ ID NO: 8; or

(ii) the V_H comprises a V_H -CDR1 sequence of SEQ ID NO: 1, a V_H -CDR2 sequence of SEQ ID NO: 7 and a V_H -CDR3 sequence of SEQ ID NO: 3 or SEQ ID NO: 8; and/or

10 (b) (i) the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 5, SEQ ID NO: 10, or SEQ ID NO: 11, and a V_L -CDR3 sequence of SEQ ID NO: 6 or SEQ ID NO: 12; or

(ii) the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 4 or SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 10, or SEQ ID NO: 11, and a V_L -CDR3 sequence of
15 SEQ ID NO: 6 or SEQ ID NO: 12; or

(iii) the V_L comprises a V_L -CDR1 sequence of SEQ ID NO: 4 or SEQ ID NO: 9, a V_L -CDR2 sequence of SEQ ID NO: 5, SEQ ID NO: 10, or SEQ ID NO: 11, and a V_L -CDR3 sequence of SEQ ID NO: 12.

2. An antibody or antigen binding fragment thereof comprising a heavy chain
20 immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein: (a) the V_H comprises an amino acid sequence selected from any one of SEQ ID NOs: 13, 15, or 17; and/or (b) the V_L comprises an amino acid sequence selected from any one of SEQ ID NOs: 14, 16, 18, 19, or 20.

3. An antibody or antigen binding fragment thereof comprising heavy chain
25 immunoglobulin variable domain (V_H) and light chain immunoglobulin variable domain (V_L) amino acid sequences selected from the group consisting of:

SEQ ID NOs: 13 and 14,

SEQ ID NOs: 15 and 16,

SEQ ID NOs: 17 and 14,
SEQ ID NOs: 15 and 18,
SEQ ID NOs: 15 and 19, and
SEQ ID NOs: 15 and 20, respectively.

- 5 4. An antibody comprising a first polypeptide chain, a second polypeptide chain, a third polypeptide chain and a fourth polypeptide chain, wherein the first and second polypeptide chains are covalently bonded to one another, the second and third polypeptide chains are covalently bonded to one another, and the third and fourth polypeptide chain are covalently bonded to one another, and wherein:
- 10 (a) each of the first polypeptide chain and the fourth polypeptide chain comprises in the N-terminal to C-terminal direction:
- (i) a light chain variable domain of a first immunoglobulin that is capable of specifically binding to a first epitope;
 - (ii) a light chain constant domain of the first immunoglobulin;
 - 15 (iii) a flexible peptide linker comprising the amino acid sequence (GGGGS)₃; and
 - (iv) a light chain variable domain of a second immunoglobulin that is linked to a complementary heavy chain variable domain of the second immunoglobulin, or a heavy chain variable domain of a second immunoglobulin that is linked to a complementary light chain variable domain of the second immunoglobulin, wherein the light chain and heavy chain variable
 - 20 domains of the second immunoglobulin are capable of specifically binding to a second epitope, and are linked together via a flexible peptide linker comprising the amino acid sequence (GGGGS)₆ to form a single-chain variable fragment; and
- (b) each of the second polypeptide chain and the third polypeptide chain comprises in the N-terminal to C-terminal direction:
- 25 (i) a heavy chain variable domain of the first immunoglobulin that is capable of specifically binding to the first epitope; and
 - (ii) a heavy chain constant domain of the first immunoglobulin; and
- wherein the heavy chain variable domain of the first immunoglobulin or the heavy chain variable domain of the second immunoglobulin comprises any one of SEQ ID NOs: 13, 15, or 17, and/or

the light chain variable domain of the first immunoglobulin or the light chain variable domain of the second immunoglobulin comprises any one of SEQ ID NOs: 14, 16, 18, 19, or 20.

5. An antibody or antigen binding fragment comprising a heavy chain (HC) and a light chain (LC) selected from the group consisting of

- 5 SEQ ID NOs: 21 and 22,
 SEQ ID NOs: 21 and 23,
 SEQ ID NOs: 21 and 24,
 SEQ ID NOs: 21 and 25,
 SEQ ID NOs: 21 and 26,
10 SEQ ID NOs: 21 and 27,
 SEQ ID NOs: 21 and 28,
 SEQ ID NOs: 21 and 29,
 SEQ ID NOs: 21 and 30,
 SEQ ID NOs: 21 and 31,
15 SEQ ID NOs: 21 and 32,
 SEQ ID NOs: 21 and 33,
 SEQ ID NOs: 34 and 33,
 SEQ ID NOs: 21 and 35,
 SEQ ID NOs: 36 and 33,
20 SEQ ID NOs: 21 and 37,
 SEQ ID NOs: 21 and 38,
 SEQ ID NOs: 21 and 39,
 SEQ ID NOs: 21 and 40,
 SEQ ID NOs: 21 and 41,
25 SEQ ID NOs: 21 and 42,
 SEQ ID NOs: 21 and 43,
 SEQ ID NOs: 21 and 44,
 SEQ ID NOs: 21 and 45,
 SEQ ID NOs: 21 and 46,

SEQ ID NOs: 21 and 47,
SEQ ID NOs: 21 and 48,
SEQ ID NOs: 21 and 49,
SEQ ID NOs: 21 and 50,
5 SEQ ID NOs: 21 and 51,
SEQ ID NOs: 21 and 52,
SEQ ID NOs: 21 and 53,
SEQ ID NOs: 21 and 54,
SEQ ID NOs: 21 and 55,
10 SEQ ID NOs: 21 and 56,
SEQ ID NOs: 21 and 57,
SEQ ID NOs: 21 and 58,
SEQ ID NOs: 21 and 59,
SEQ ID NOs: 21 and 60,
15 SEQ ID NOs: 21 and 61,
SEQ ID NOs: 21 and 62,
SEQ ID NOs: 21 and 63,
SEQ ID NOs: 21 and 64,
SEQ ID NOs: 21 and 65,
20 SEQ ID NOs: 21 and 66,
SEQ ID NOs: 21 and 67,
SEQ ID NOs: 21 and 68,
SEQ ID NOs: 21 and 69,
SEQ ID NOs: 21 and 70,
25 SEQ ID NOs: 21 and 71,
SEQ ID NOs: 21 and 72, and
SEQ ID NOs: 21 and 85, respectively.

6. The antibody or antigen binding fragment of any one of claims 1-5, further comprising a Fc domain of an isotype selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, and IgE.

7. The antibody of claim 6, comprising an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A, L234A, L235A, and K322A.

8. The antibody of claim 6, comprising an IgG4 constant region comprising a S228P mutation.

9. The antigen binding fragment of any one of claims 1-5, wherein the antigen binding fragment is selected from the group consisting of Fab, F(ab')₂, Fab', scFv, and Fv.

10. The antibody or antigen binding fragment of any one of claims 1-9, wherein the antibody is a monoclonal antibody, a chimeric antibody, a humanized antibody, a bispecific antibody, or multi-specific antibody.

11. The antibody of any one of claims 1-10, wherein the antibody lacks α -1,6-fucose modifications.

12. The antibody or antigen binding fragment of any one of claims 10-11, wherein the multi-specific antibody or antigen binding fragment binds to T cells, B-cells, myeloid cells, plasma cells, or mast-cells.

13. The antibody or antigen binding fragment of any one of claims 10-12, wherein the multi-specific antibody or antigen binding fragment binds to CD3, CD4, CD8, CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, CD22, CD14, CD15, CD16, CD123, TCR gamma/delta, NKp46, KIR, or a small molecule DOTA hapten.

14. A recombinant nucleic acid sequence encoding the antibody or antigen binding fragment of any one of claims 1-13.

15. A host cell or vector comprising the recombinant nucleic acid sequence of claim 14.

16. A composition comprising the antibody or antigen binding fragment of any one of claims 1-13 and a pharmaceutically-acceptable carrier, wherein the antibody or antigen binding fragment is optionally conjugated to an agent selected from the group consisting of isotopes,

dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

17. The composition of claim 16, wherein the pharmaceutical composition further comprises
5 an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

18. A method for treating cancer in a subject in need thereof, comprising administering to the
10 subject an effective amount of the antibody or antigen binding fragment of any one of claims 1-13 or the pharmaceutical composition of any one of claims 16-17, wherein the antibody or antigen binding fragment specifically binds to HER2.

19. The method of claim 18, wherein the cancer is a solid tumor.

20. The method of claim 18 or 19, wherein the cancer is breast cancer, gastric cancer, osteosarcoma, desmoplastic small round cell cancer, squamous cell carcinoma of head and neck
15 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, or neuroblastoma.

21. The method of any one of claims 18-20, wherein the antibody or antigen binding
20 fragment is administered to the subject separately, sequentially or simultaneously with an additional therapeutic agent.

22. The method of claim 21, wherein the additional therapeutic agent is one or more of alkylating agents, platinum agents, taxanes, vinca agents, anti-estrogen drugs, aromatase inhibitors, ovarian suppression agents, VEGF/VEGFR inhibitors, EGF/EGFR inhibitors, PARP
25 inhibitors, cytostatic alkaloids, cytotoxic antibiotics, antimetabolites, endocrine/hormonal agents, T cells, and bisphosphonate therapy agents.

23. The method of claim 22, wherein the additional therapeutic agent is an immuno-modulating/stimulating antibody.

24. The method of claim 23, wherein the immuno-modulating/stimulating antibody is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-TIM3 antibody, an anti-4-1BB antibody, an anti-CD73 antibody, an anti-GITR antibody, or an anti-LAG-3 antibody.

- 5 25. A method for detecting cancer in a subject *in vivo* comprising
- (a) administering to the subject an effective amount of the antibody or antigen binding fragment of any one of claims 1-13, wherein the antibody or antigen binding fragment is configured to localize to a cancer cell expressing HER2 and is labeled with a radioisotope; and
 - 10 (b) detecting the presence of a tumor in the subject by detecting radioactive levels emitted by the antibody or antigen binding fragment that are higher than a reference value.

26. The method of claim 25, wherein the subject is diagnosed with or is suspected of having cancer.

- 15 27. The method of claim 25 or 26, wherein the radioactive levels emitted by the antibody or antigen binding fragment are detected using positron emission tomography or single photon emission computed tomography.

28. The method of any one of claims 25-27, further comprising administering to the subject an effective amount of an immunoconjugate comprising the antibody or antigen binding
20 fragment of any one of claims 1-13 conjugated to a radionuclide.

29. The method of any one of claims 25-28, wherein the cancer is a solid tumor.

30. The method of any one of claims 25-29, wherein the cancer is breast cancer, gastric cancer, osteosarcoma, desmoplastic small round cell cancer, squamous cell carcinoma of head and neck cancer, ovarian cancer, prostate cancer, pancreatic cancer, glioblastoma multiforme,
25 gastric junction adenocarcinoma, gastroesophageal junction adenocarcinoma, cervical cancer, salivary gland cancer, soft tissue sarcoma, leukemia, melanoma, Ewing's sarcoma, rhabdomyosarcoma, or neuroblastoma.

31. A kit comprising the antibody or antigen binding fragment of any one of claims 1-13 and instructions for use.

32. The kit of claim 31, wherein the antibody or antigen binding fragment is coupled to at least one detectable label selected from the group consisting of a radioactive label, a fluorescent
5 label, and a chromogenic label.

33. The kit of claim 31 or 32, further comprising a secondary antibody that specifically binds to the antibody or antigen binding fragment of any one of claims 1-13.

34. A method for detecting HER2 protein expression levels in a biological sample comprising contacting the biological sample with the antibody or antigen binding fragment of
10 any one of claims 1-13, and detecting binding to HER2 protein in the biological sample.

35. The multi-specific antibody or antigen binding fragment of any one of claims 10-13, wherein the multi-specific antibody or antigen binding fragment binds to T cells and/or CD3.

36. A T cell that is armed *ex vivo* with the multi-specific antibody or antigen binding fragment of claim 35.

15 37. An *ex vivo* method of making a therapeutic T cell, comprising binding the multi-specific antibody or antigen binding fragment of claim 35 to a T cell, wherein the T cell is optionally a human T cell, and wherein the binding is noncovalent.

38. A method for treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of the T cell of claim 36.

FIG. 1

HER2-VH CDR1 (SEQ ID NO: 1): GFNIKDTYIH

HER2-VH CDR2 (SEQ ID NO: 2): RIYPTNGYTRYADSVKG

HER2-VH CDR2-Variant (SEQ ID NO: 7): RGYPTNGYTRYADSVKG

HER2-VH CDR3 (SEQ ID NO: 3): WGGDGFYAMDY

HER2-VH CDR3-Variant (SEQ ID NO: 8): WGDDGFYAMDY

HER2-VL CDR1 (SEQ ID NO: 4): RASQDVNTAVA

HER2-VL CDR1-Variant (SEQ ID NO: 9): RASQDVPTAVA

HER2-VL CDR2 (SEQ ID NO: 5): SASFLYS

HER2-VL CDR2-Variant-1 (SEQ ID NO: 10): SASDLYS

HER2-VL CDR2-Variant-2 (SEQ ID NO: 11): DASFLYS

HER2-VL CDR3 (SEQ ID NO: 6): QQHYTTPPT

HER2-VL CDR3-Variant (SEQ ID NO: 12): QQHYTAPPT

FIG. 2**ABP100s.10.1 HER2-VH (SEQ ID NO: 13):**

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

ABP100s.10.1 L HER2-VL (SEQ ID NO: 14):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS
GSRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIK

ABP100s.10.2 HER2-VH (SEQ ID NO: 15):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

ABP100s.10.2 HER2-VL (SEQ ID NO: 16):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS
GSRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIK

ABP100s.10.3 HER2-VH (SEQ ID NO: 17):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARGYPTNGYTRYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

ABP100s.10.3 HER2-VL (SEQ ID NO: 14):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS
GSRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIK

ABP100s.10.4 HER2-VH (SEQ ID NO: 15):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

ABP100s.10.4 HER2-VL (SEQ ID NO: 18):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASDLYSGVPSRFS
GSRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIK

ABP100s.10.5 HER2-VH (SEQ ID NO: 15):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

ABP100s.10.5 HER2-VL (SEQ ID NO: 19):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS
GSRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIK

ABP100s.10.6 HER2-VH (SEQ ID NO: 15):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYA
DSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS

ABP100s.10.6 HER2-VL (SEQ ID NO: 20):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFS
GSRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIK

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEFYAMDYW
GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKLSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQKPKGKAPKLLIYSASF¹LYSG
VPSRFGSGRSGTDFTLTIS²SLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPS³VF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK⁴DSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG⁵
SEVQLVESGGGLVQPGGSLRLSCAASGFTENTYAMNWVRQAPGKGLEWVARIRSKY
NNYATYYADSVKGRFTISRDDSQSSLYLQMNSLKTEDTAVYYCVRHGNFGNS⁶YVS
WFAYWGQGT⁷LVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGQAVVTQEP⁸SF
SVSPGGTVTLTCSSTGAVTTSNYANWVQQT⁹PGQAFRGLIGGTNKRAPGVPDRFSGS
LIGDKAALTITGAQADDES¹⁰DYFCALWYSN¹¹LWVFGGGTKLTVL

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
GQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWOOGNVFCFSVMHEALHNHYTOKLSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASF¹LYSG
VPSRFSGSRSGTDFTLTIS²SLQPEDFATYYCQOHY³TIPT⁴IFGQGTKVEIKRTVAAPS⁵VF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK⁶DSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG⁷
SEVQLVESGGGLVQPGGSLRLSCAASGFTENTYAMNWVRQAPGKGLEWVARIRSKY⁸
NNYATYYADSVKGRFTISRDDSKSSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVS⁹
WFAYWGQGT¹⁰LVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG¹¹QAVVTQEP¹²SF
SVSPGGTVTLT¹³CGSSITGAVTTSNYANWVQQTPGQAFRGLIGGTNKRAPGV¹⁴PDRFSGS
LIGDKAALTITGAQADDESDFCALWYSNLWVFGGGTKLTVL

FIG. 3 (Contd.)**ABP100s.3 HC** (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGENIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.3 LC (SEQ ID NO: 24):

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQOHYTIPTTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSSGGGGSGGGGSGGGG
SEVQLVESGGGLVKPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKY
NNYATYYADSVKGRFTISRDDSQSSLYLQMNSLRAEDTAVYYCVRHGNFEGNSYVS
WFAYWGQGTLVTVSSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQAVVTQEPSPF
 SVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAFRGLIGGTNKRAPGVDPDRFSGS
 LIGDKAALTITGAQADDESDFYFCALWYSNLWVFGGGTKLTVL

ABP100s.4 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGENIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.4 LC (SEQ ID NO: 25):

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQOHYTIPTTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSSGGGGSGGGGSGGGG
SEVQLVESGGGLVKPGGSLRLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKY
NNYATYYADSVKGRFTISRDDAKSSLYLQMNSLRAEDTAVYYCVRHGNFEGNSYVS
WFAYWGQGTLVTVSSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQAVVTQEPSPF
 SVSPGGTVTLTCGSSTGAVTTSNYANWVQQTPGQAFRGLIGGTNKRAPGVDPDRFSGS
 LIGDKAALTITGAQADDESDFYFCALWYSNLWVFGGGTKLTVL

FIG. 3 (Contd.)**ABP100s.5 HC** (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
 YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEFYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.5 LC (SEQ ID NO: 26):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISSLQPEDFATYYCQOHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
 SEVQLVESGGGLVQPGGSLKLSCAASGFTIENTYAMNWVRQAPGKGLEWVARIRSK
 YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
 SWEAYWGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 SQTIVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLGSGVQPEDEAIYFCALWYSNLWVFGGGTKLEIK

ABP100s.6 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
 YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEFYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.6 LC (SEQ ID NO: 27):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISSLQPEDFATYYCQOHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
 SEVQLVESGGGLVQPGGSLKLSCAASGFTIENTYAMNWVRQAPGKGLEWVARIRSK
 YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
 SWEAYWGQGTLLTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 SQTIVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLGSGVQPEDEAEYFCALWYSNLWVFGGGTKLEIK

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEFYAMDYW
GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASF_{LYSG}
VPSRFGSGRSGTDFTLTIS_{SLQPEDFATYYCQOHY}TTPPTFGQGTKVEIKRTVAAPS_{VF}
IFPPSDEQLKSGTASV_{VCLLN}NFYPREAKVQWKVDNALQSGNSQESVTEQDSK_{DSTY}
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTS_{GGGGSGGGGSGGGG}
SEVQLVESGGGLVQPGSLKLSCAASG_{ETENTYAMN}WVRQASGKGLEWVARIR_{SK}
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRH_{GNF}GN_{SYV}
SWFAYWGQTLVTVSS_{GGGGSGGGGSGGGGSGGGGSGGGGSGGGG}SQT_{VVTQEPS}
LTVSPGGTVTLTCR_{SSTGAVTTSNYAN}WVQQKPGQA_{FRGLIGGTNKR}APGVPARFS
GSLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGGGTKLEIK

EVQLVESGGGLVQPGGSLRLSCAASG**FN**IKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSR**WG**GDGFYAMDYW
GQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTV**PS**SLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV**VVD**VSHEDPEVKFNWYVD
GVEVHNAKTKPREEQY**A**STYRVVSVLTVLHQDWLNGKEYK**C****A**VS**N**KALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQOGNVFSCSV**M**HEALHNHYTOKLSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQKPGKAPKLLIYSASFLYSG
VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQOHYTIPTTFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGSLKLSCAASGFTENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFAYWGQGTLLTVVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
LTVSPGGTVTLTCRSSTGAVTTSNYANWVQKPGQAFRGLIGGTNKRAPGVPARFS
GSLGGKAALTLSGVQPEDEAIYFCALWYSNLTWVFGCGTKLEIK

FIG. 3 (Contd.)**ABP100s.5.2 HC (SEQ ID NO: 21):**

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYV
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.5.2 LC (SEQ ID NO: 30):

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFITENTYAMNWVRQAPGKGLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFAYWGCGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQCFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGGGTKLEIK

ABP100s.5.3 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYV
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.5.3 LC (SEQ ID NO: 31):

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFITENTYAMNWVRQAPGKGLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 SQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGGGTKLEIK

FIG. 3 (Contd.)**ABP100s.5.4 HC** (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.5.4 LC (SEQ ID NO: 32):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQOHYTIPPIFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTIENTYAMNWVRQAPGKGLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNEFGNSYV
SWEAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGGGTKLTVL

ABP100s.10.0 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.0 LC (SEQ ID NO: 33):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQOHYTIPPIFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTIENTYAMNWVRQAPGKGLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNEFGNSYV
SWEAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGGGTKLTVL

FIG. 3 (Contd.)**ABP100s.10.1 HC (SEQ ID NO: 34):**

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGDDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.1 LC (SEQ ID NO: 33):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISSLQPEDFATYYCQOHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNEFGNSYV
SWEAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTIVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

ABP100s.10.2 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGDDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.2 LC (SEQ ID NO: 35):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISSLQPEDFATYYCQOHYTAPPPTFGQGTKVEIKRTVAAPS
 VFIFPPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKDDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNEFGNSYV
SWEAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTIVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

FIG. 3 (Contd.)**ABP100s.10.3 HC** (SEQ ID NO: 36):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARGYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.3 LC (SEQ ID NO: 33):

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPS
 VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
 DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTINTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTVVTQEPS
 LTVSPGGTVTLTCRSSIGAVTTSNYANWVQQKPGQAFRGLIGGTNKRAPGVPARFS
 GSLLGGKAALTLGSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

ABP100s.10.4 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.4 LC (SEQ ID NO: 37):

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWYQQKPGKAPKLLIYSASDLYSG
 VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPS
 VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
 DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTINTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTVVTQEPS
 LTVSPGGTVTLTCRSSIGAVTTSNYANWVQQKPGQAFRGLIGGTNKRAPGVPARFS
 GSLLGGKAALTLGSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

FIG. 3 (Contd.)**ABP100s.10.5 HC** (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.5 LC (SEQ ID NO: 38):

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWYQQKPGKAPKLLIYDASFLYSG
 VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQOHYITPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTVVTQEPS
 LTVSPGGTVTLTCRSSIGAVTTSNYANWVQQKPGQAFRGLIGGTNKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

ABP100s.10.6 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.6 LC (SEQ ID NO: 39):

DIQMTQSPSSLSASVGDRVTITCRASODVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQOHYITPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTFTNTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTVVTQEPS
 LTVSPGGTVTLTCRSSIGAVTTSNYANWVQQKPGQAFRGLIGGTNKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
GQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKLSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASF~~LYSG~~
VPSRFGSGRSGTDFTLTIS~~SLQPEDFATYYCQOHY~~TTPPTFGQG~~TKVEIKRTVAAPS~~V
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK~~DSTY~~
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTS~~GGGGSGGGGSGGGG~~
SEVQLVESGGGLVQPGSLKLSCAASG~~ET~~ENTYAMN~~WVRQAPGKCLEWVARIRSK~~
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
~~SWFGY~~WGQGT~~LVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG~~SQT~~VVTQEPS~~
LTVSPGGTVTLTCRSSTGAYTTSNYANWVQQKPGQA~~FRGLIGGT~~INKRAPGV~~PARFS~~
GSLGGKAALTL~~SGVQPEDAIYFCALWYSNLWVFGCGTKLTVL~~

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
GQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWOOGNVFCFSVMHEALHNHYTOKLSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQKPGKAPKLLIYSASFLYSG
VPSRFSGRSGTDFTLTISSLQPEDFATYYCQOHYTIPTIFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDY
SLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGSLKLSCAASGFTENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYKADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNGFGNSYV
SWFAYWGQGTLLTVVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
LTVSPGGTVTLTCRSSTGAVTTSNYANWVQKPGQAFRGLIGGTNKRAPGVPARFS
GSLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

FIG. 3 (Contd.)**ABP100s.10.0.3 HC (SEQ ID NO: 21):**

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.0.3 LC (SEQ ID NO: 42):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQOHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
 SEVQLVESGGGLVQPGGSLKLSCAASGFIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADKVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWEAYWGQGLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLGSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

ABP100s.10.0.4 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.0.4 LC (SEQ ID NO: 43):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQOHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVCLLNFFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
 SEVQLVESGGGLVQPGGSLKLSCAASGFIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYWDSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWEAYWGQGLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLGSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

FIG. 3 (Contd.)**ABP100s.10.0.5 HC** (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.0.5 LC (SEQ ID NO: 44):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQOHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFITENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFMYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

ABP100s.10.0.6 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.0.6 LC (SEQ ID NO: 45):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQOHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFITENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFAYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCMLWYSNLWVFGCGTKLTVL

FIG. 3 (Contd.)**ABP100s.10.5.1 HC (SEQ ID NO: 21):**

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.5.1 LC (SEQ ID NO: 46):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYDASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPS
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNEFGNSYV
SWFGYWGQGTLVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSG
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

ABP100s.10.5.2 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.5.2 LC (SEQ ID NO: 85):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYDASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPS
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYKADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNEFGNSYV
SWFAYWGQGTLVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSG
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

FIG. 3 (Contd.)**ABP100s.10.5.3 HC** (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.5.3 LC (SEQ ID NO: 47):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYDASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADKVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWEAYWGQGTLVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGQTIVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLGSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

ABP100s.10.5.4 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.5.4 LC (SEQ ID NO: 48):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYDASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYWDSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWEAYWGQGTLVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGQTIVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLGSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEFYAMDYW
GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYDASFLYSGV
VPSRFGSGRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSIV
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSLTLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGSLKLSCAASGETENTYAMNWVRQAPGKCLEWVARIRSK
YNRYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFMYWGQGTLLTVVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTIVTTQEPS
LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTNKRAPGVPARFS
GSLLGGAALTL SGVQPEDAIFYFCALWYSNLWFVGCCTKLT

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
GQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTOKLSLSLSPGK

[illegible]

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKLSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVPTAVA WYQKPKGKAPKLLIYSASFLYSG
VPSRFGSGRSGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPS
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGETFTNTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFGYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
SQT VVTQEPS
LTVSPGGTVTLTCRSSTGAYTTSNYANWVQKPGQAFRGLIGGTNKRAPGVPARFS
GSLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
GQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTOKLSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVPTAVAWYQKPKGKAPKLLIYSASFLYSG
VPSRFGSGRSGTDFTLTISSLQPEDFATYYCQQHYTTPPIFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
SLSSITLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGSGGGG
SEVQLVESGGGLVQPGSLKLSCAASGETENTYAMNWRQAPGKCLEWVARIRSK
YNNYATYKADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWFAYWGQGLTVTVSSGGGGSGGGSGGGSGGGSGGGSGGGSGGGSGGGSGQTIVTQEPS
LTVSPGGTVTLTCSRSTGAVTTSNYANWVQKPGQAFRGLIGGTNKRAFGVPARFS
GSLGGKAALTLSGVQPEDEAIYFCALWYSNLTWVFGCGTKLTVL

FIG. 3 (Contd.)**ABP100s.10.6.3 HC** (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.6.3 LC (SEQ ID NO: 53):

DIQMTQSPSSLSASVGDRVTITCRASODVPTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADKVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWEAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

ABP100s.10.6.4 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.6.4 LC (SEQ ID NO: 54):

DIQMTQSPSSLSASVGDRVTITCRASODVPTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFTIENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYWDSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYV
SWEAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

FIG. 3 (Contd.)**ABP100s.10.6.5 HC** (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.6.5 LC (SEQ ID NO: 55):

DIQMTQSPSSLSASVGDRVTITCRASODVPTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFITENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNEFGNSYV
SWFMYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCALWYSNLWVFGCGTKLTVL

ABP100s.10.6.6 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.10.6.6 LC (SEQ ID NO: 56):

DIQMTQSPSSLSASVGDRVTITCRASODVPTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQQHYTIPPTFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLVESGGGLVQPGGSLKLSCAASGFITENTYAMNWVRQAPGKCLEWVARIRSK
YNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCVRHGNEFGNSYV
SWFAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQTVVTQEPS
 LTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAFRGLIGGTINKRAPGVPARFS
 GSLLGGKAALTLSGVQPEDEAIYFCMLWYSNLWVFGCGTKLTVL

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEFYAMDYW
GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPETCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASF¹LYSG
VPSRFGSGRSGTDFTLTIS²SLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPS³VF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK⁴DSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG⁵
SEVQLLES⁶GGGLVQPGGSLRLSCAASGFTENTYAMN⁷WVRQAPGKGLEWVSRIRSKY
NNYATYYADSVKDRFTISRDN⁸SKNTLYLQMNSLRAEDTAVYYCAKHG⁹NFGNSYVS
WFAYWGQGTITVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG¹⁰SQAVVTQEP¹¹SL
TVSPGGTVTLTCSRSTGAVTTSNYANWFQ¹²QKPGQAPRTLIYGTNKR¹³APWTPARFSGS
LLGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYW
 GQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTOKLSLSLSPGK

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQKPGKAPKLLIYDASFLYSG
VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQOHYTIPTIFGQGTKVEIKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLLESGGGLVQPGGSLRLSCAASGFTENTYAMNWVRQAPGKGLEWVSRIRSKY
NNYATYYADSVKDRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKHGNGNSYVS
WFAYWGQGTITVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
TVSPGGTVTLTCRSSITGAVTTSNYANWFQKPGQAPRTLIYGTNKRAPWTPARFSGS
LLGGKAALTLGAOPEDEAEYYCALWYSNLWVFGGGTKLTVL

FIG. 3 (Contd.)**ABP100s.12.0 HC** (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.12.0 LC (SEQ ID NO: 59):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQOHYTIPPIFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLLESGGGLVQPGGSLRLSCAASGFNTENTYAMNWVRQAPGKCLEWVSRIRSKY
NNYATYYADSVKDRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGNEFGNSYVS
WFAYWGQGTIVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQAVVTQEPSL
 TVSPGGTVTLTCRSSTGAVTTSNYANWFQQKPGQAPRTLIYGTNKRAPWTPARFSGS
 LLGGAALTLGAQPEDEAEYYCALWYSNLWVFGCGTKLTVL

ABP100s.12.5 HC (SEQ ID NO: 21):

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNG
YTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGEYAMDYW
 GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
 SGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK
 THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYAASTYRVVSVLTVLHQDWLNGKEYKCAAVSNKALPAPIEKTI
 SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
 TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

ABP100s.12.5 LC (SEQ ID NO: 60):

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYDASFLYSG
 VPSRFGSGRSGTDFTLTISLQPEDFATYYCQOHYTIPPIFGQGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKIDSTY
 SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGG
SEVQLLESGGGLVQPGGSLRLSCAASGFNTENTYAMNWVRQAPGKCLEWVSRIRSKY
NNYATYYADSVKDRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKHGNEFGNSYVS
WFAYWGQGTIVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGQAVVTQEPSL
 TVSPGGTVTLTCRSSTGAVTTSNYANWFQQKPGQAPRTLIYGTNKRAPWTPARFSGS
 LLGGAALTLGAQPEDEAEYYCALWYSNLWVFGCGTKLTVL

FIG. 4

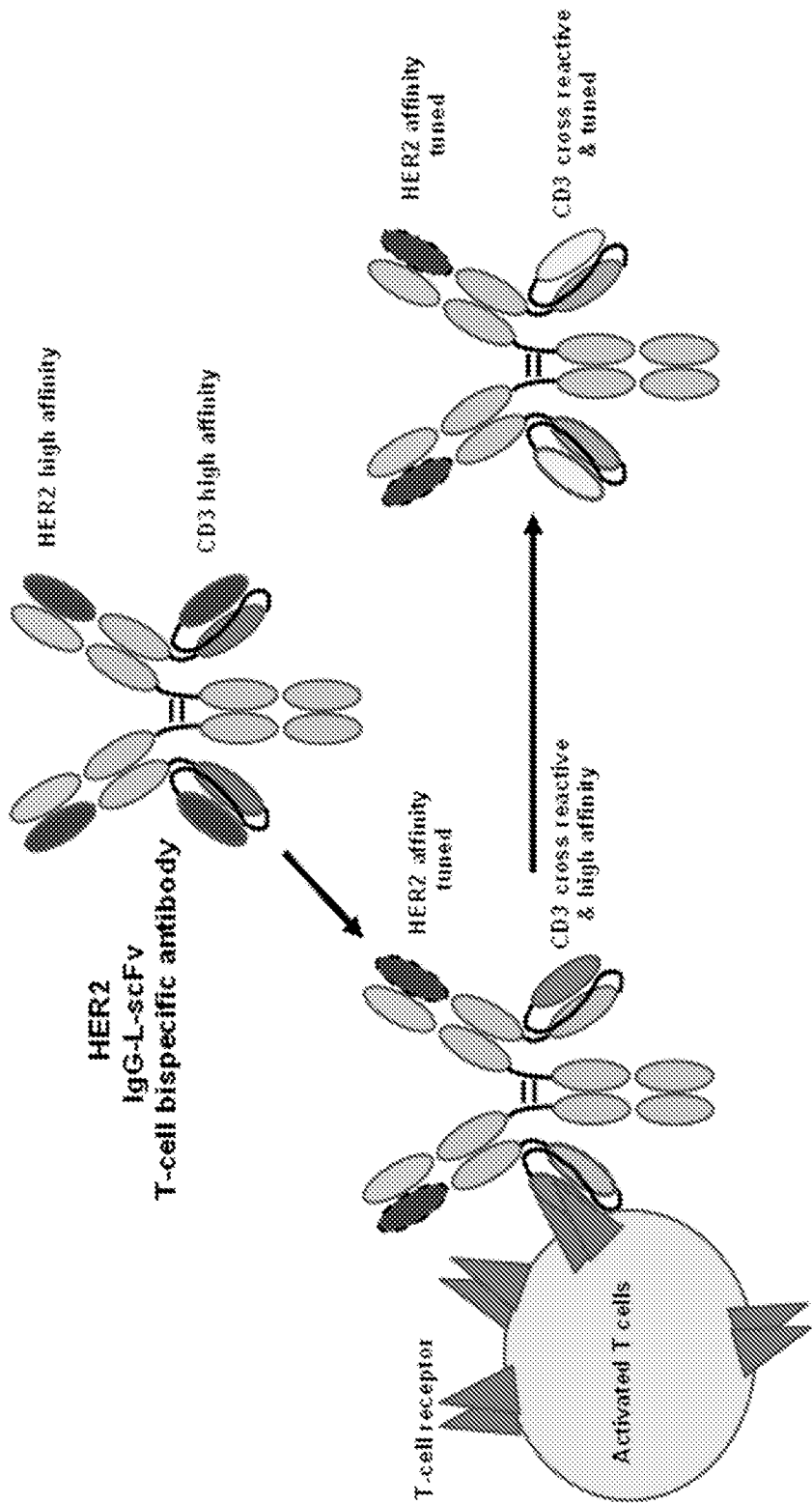


FIG. 5A

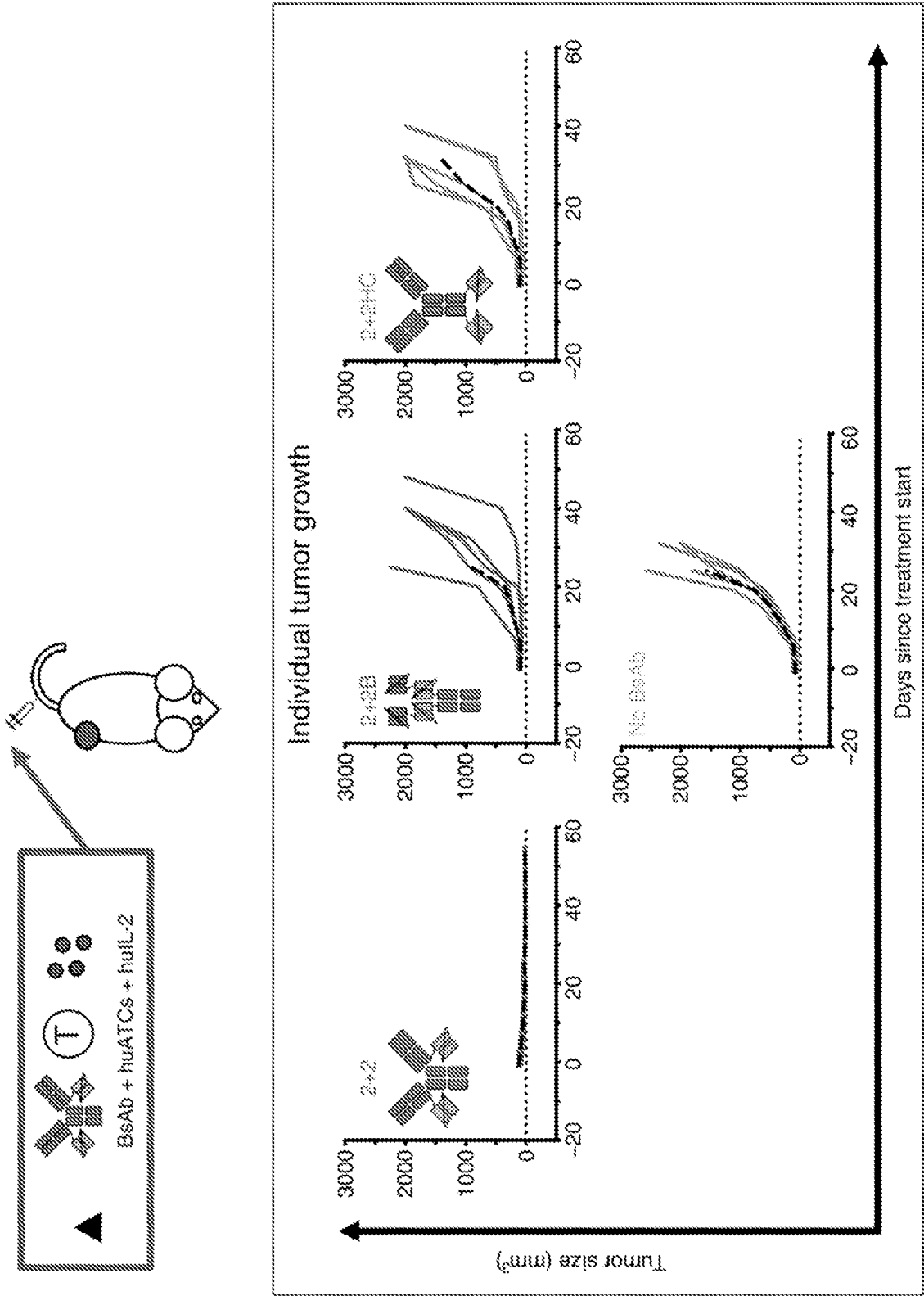


FIG. 5B

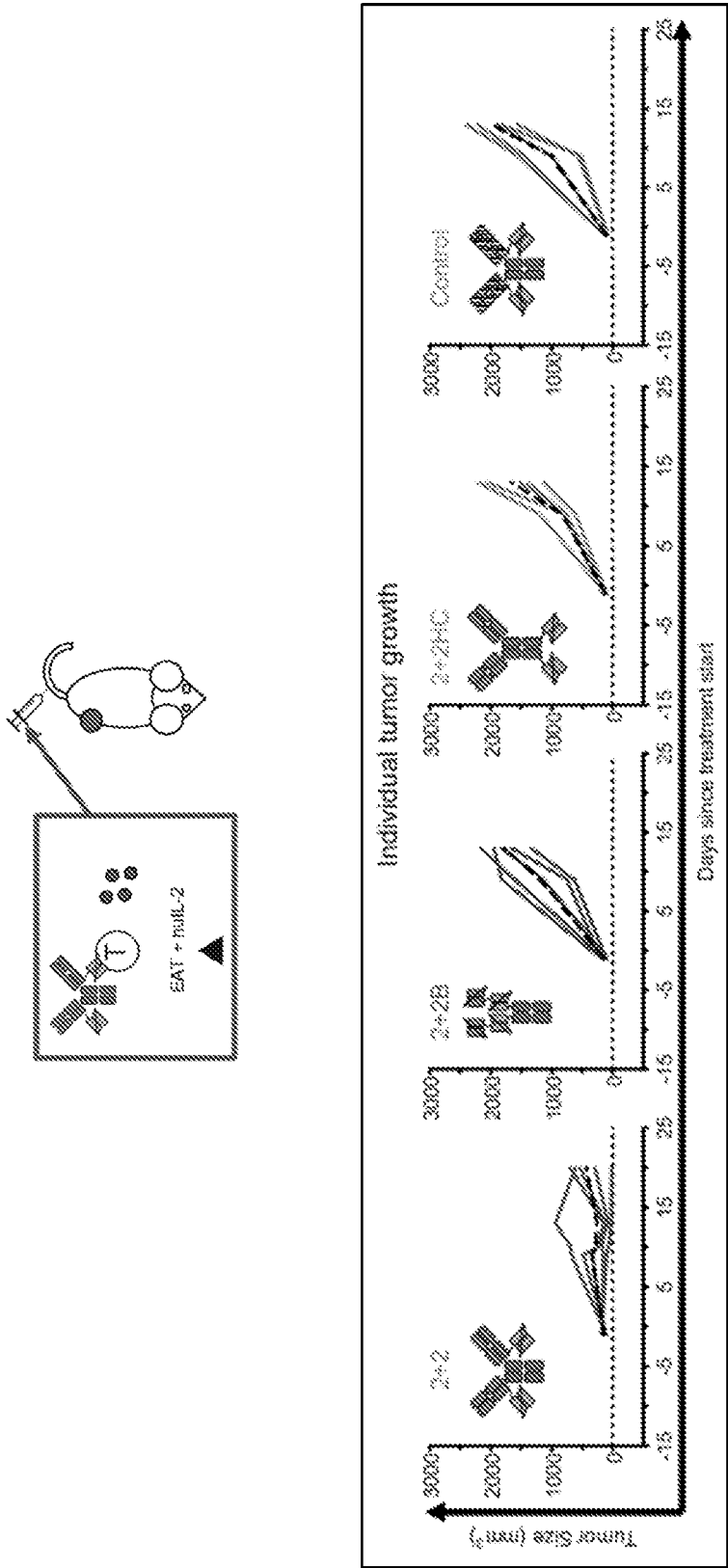


FIG. 6

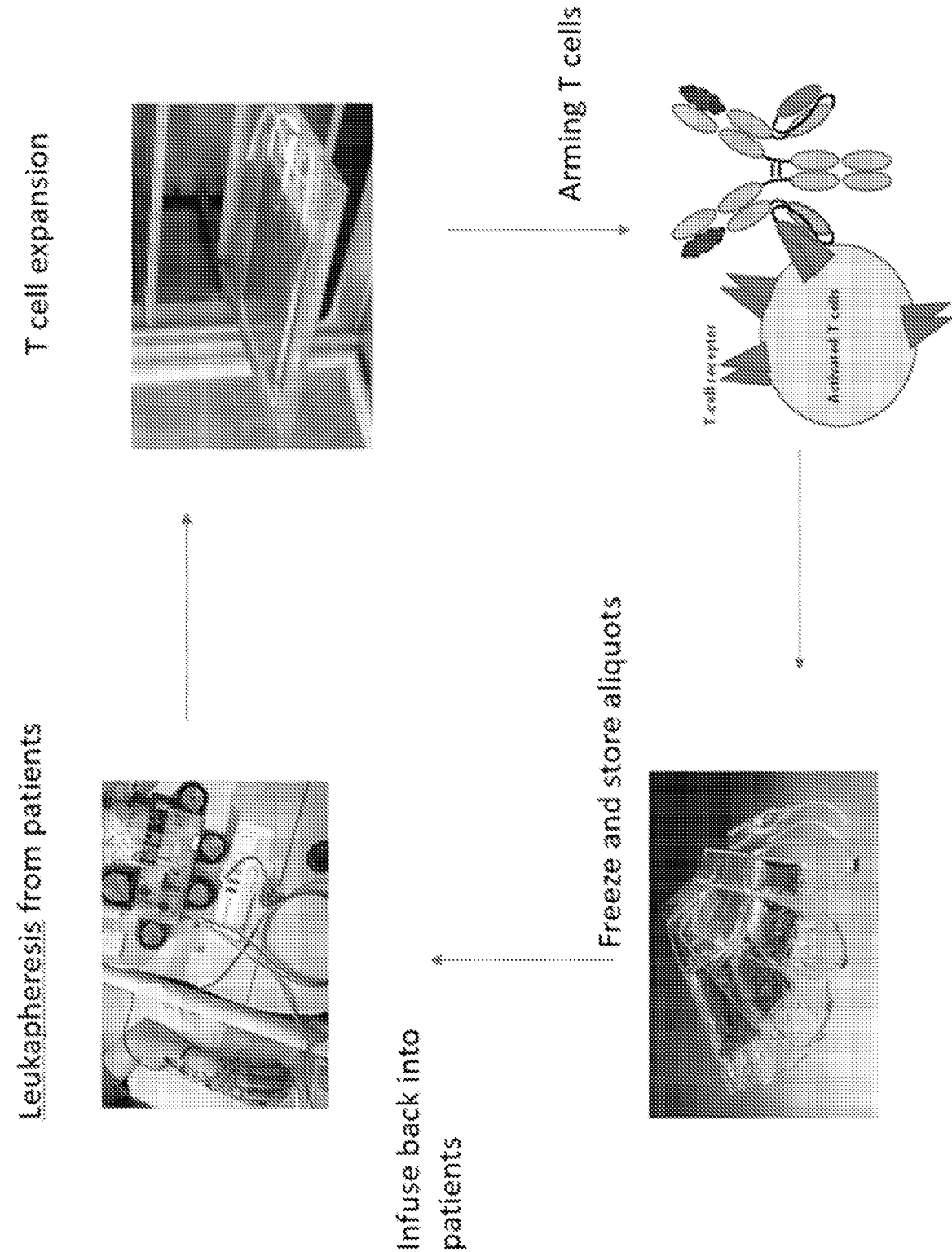


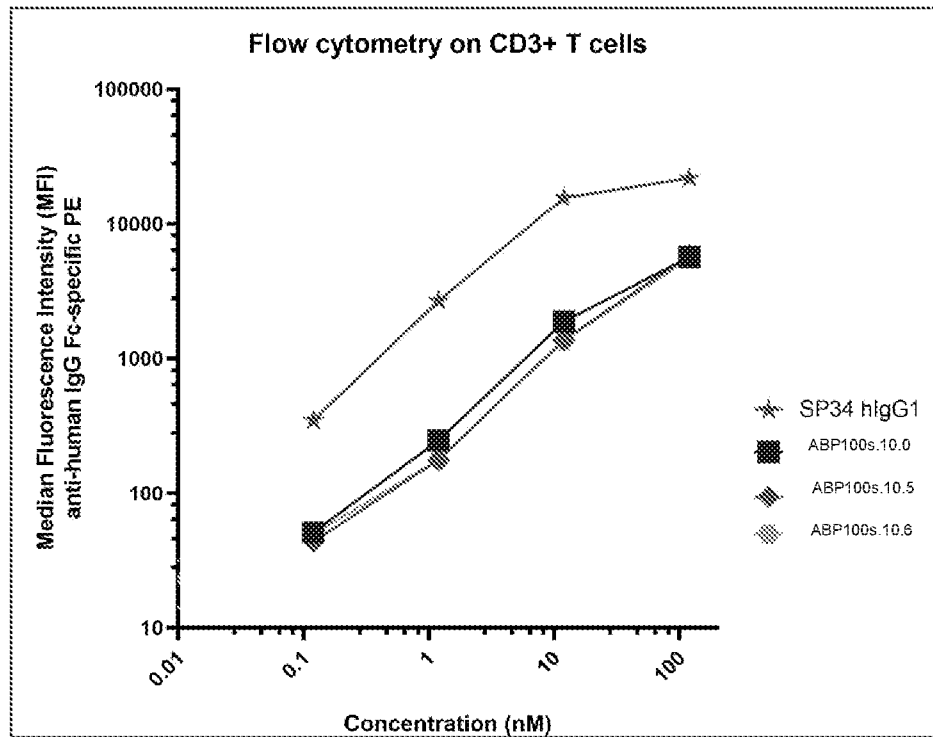
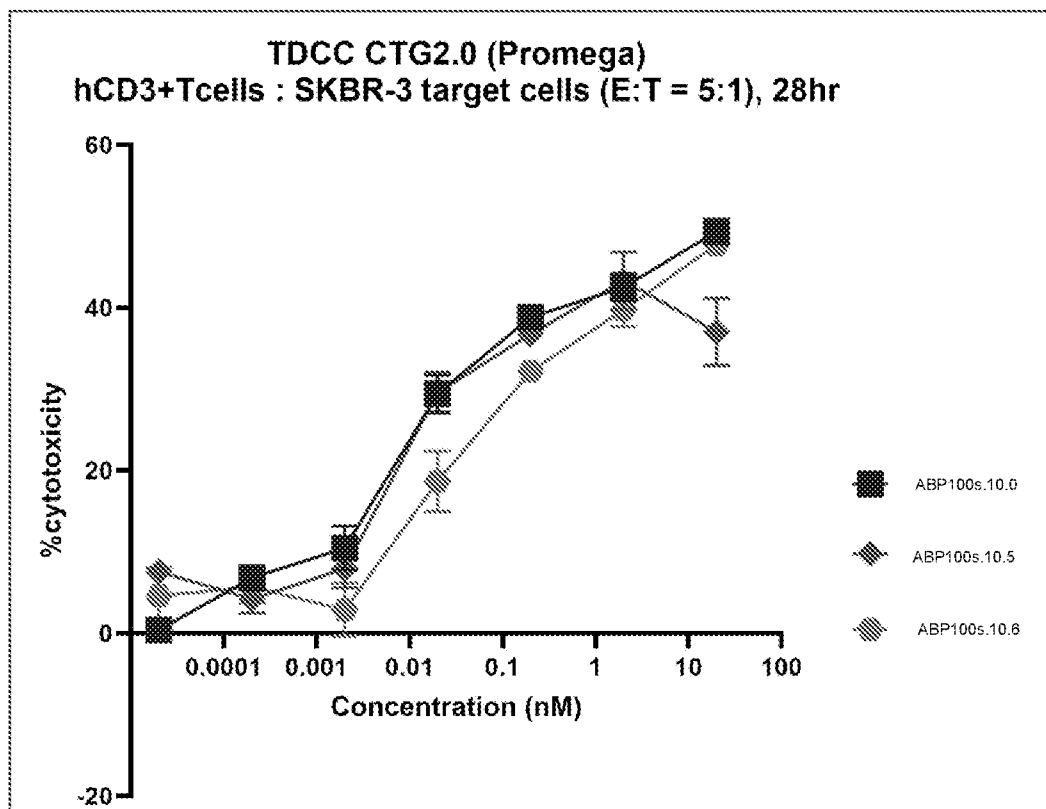
FIG. 7A**FIG. 7B**

FIG. 7C

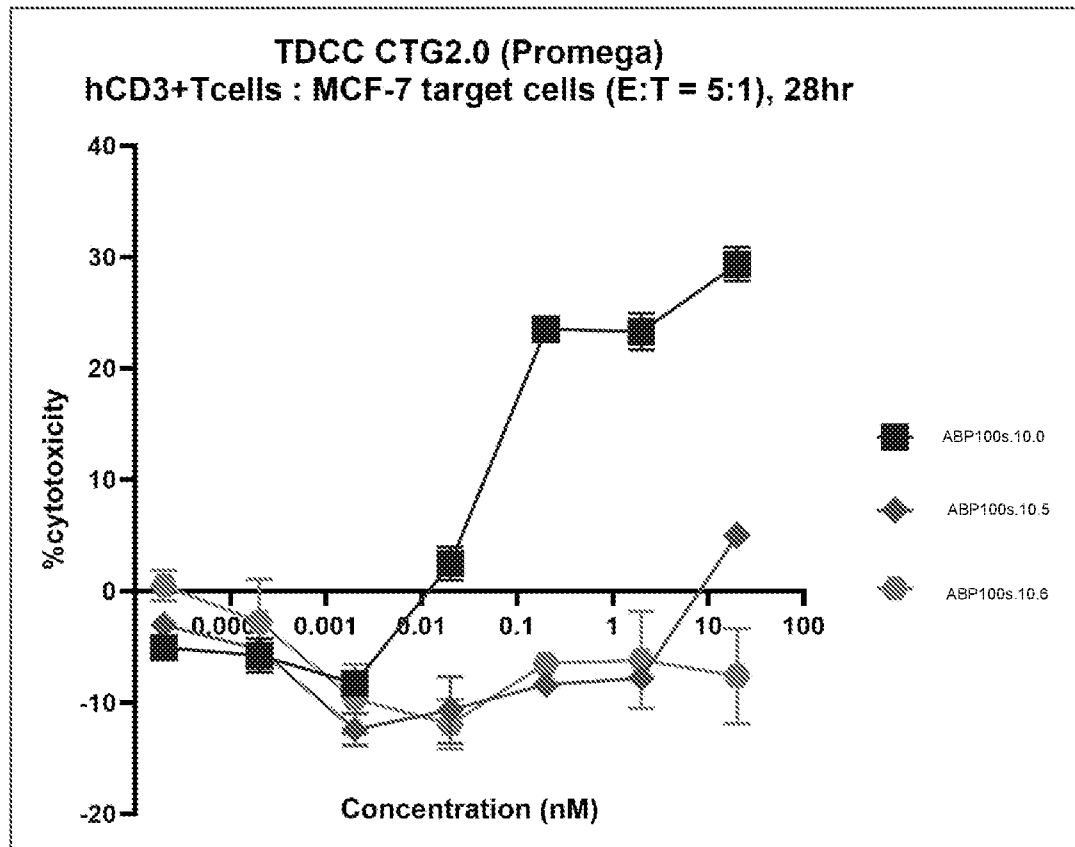
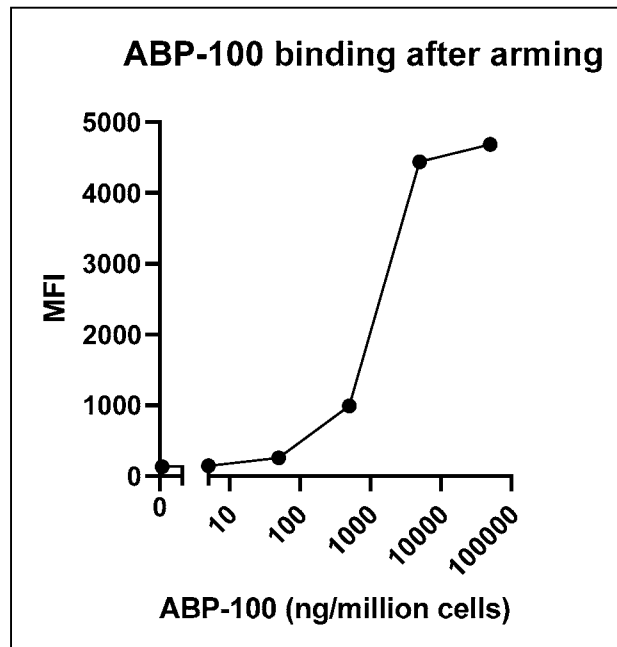


FIG. 8A**FIG. 8B**

**ABP100 TDCC assay (Cell Titer Glo 2.0)
SK-BR-3 and MCF-7 target cells**

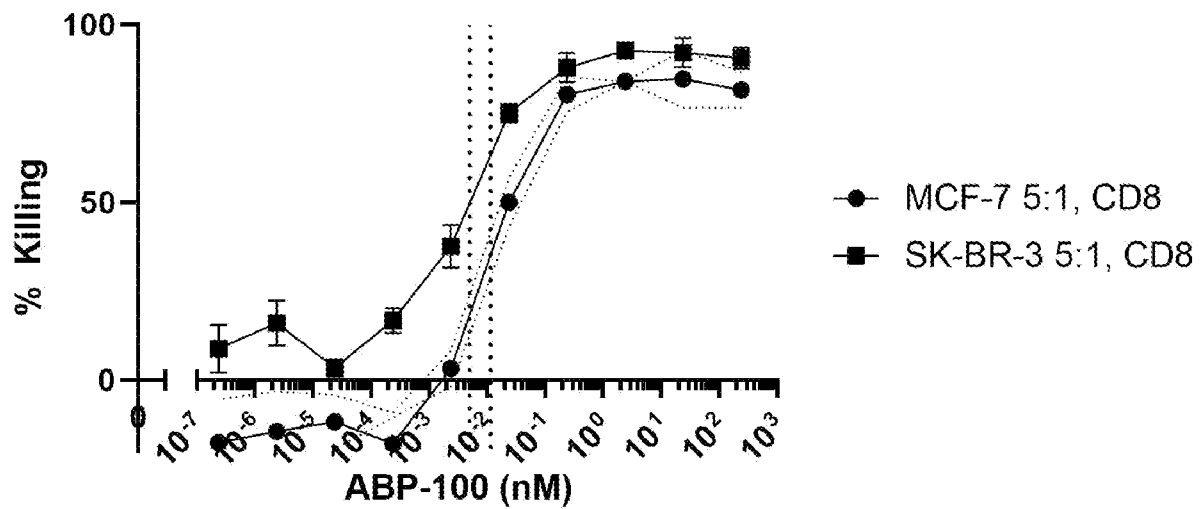


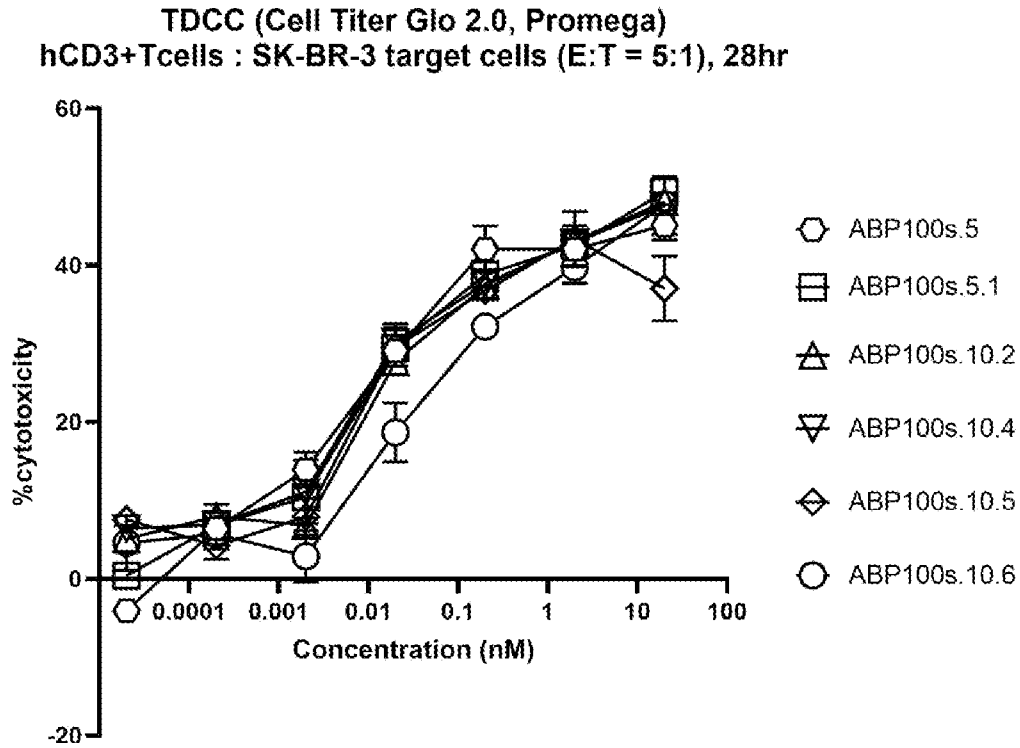
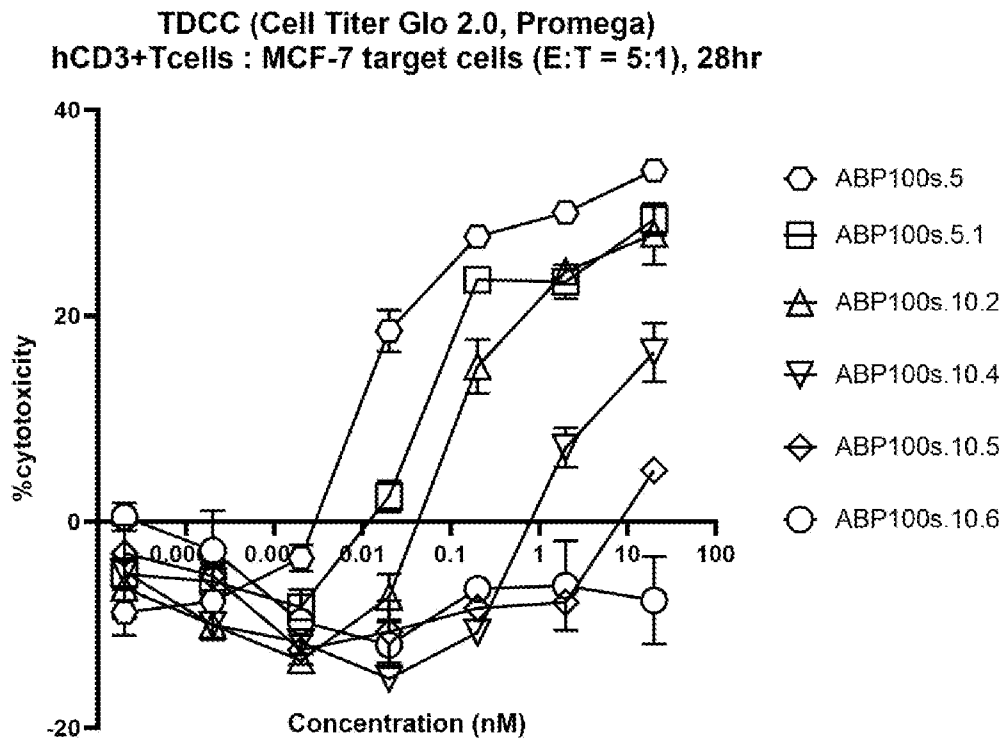
FIG. 9A**FIG. 9B**

FIG. 10

Clone name	CD3 Affinity (hi/low/medium)	Kd	HER2 Affinity (hi/low/medium)	Kd
ABP100s.1	Hi	<10nM	Hi	<2nM
ABP100s.2	Hi	<10nM	Hi	<2nM
ABP100s.3	Hi	<10nM	Hi	<2nM
ABP100s.4	Hi	<10nM	Hi	<2nM
ABP100s.5	Hi	<10nM	Hi	<2nM
ABP100s.6	Hi	<10nM	Hi	<2nM
ABP100s.7	Hi	<10nM	Hi	<2nM
ABP100s.5.1	Hi	<10nM	Hi	<2nM
ABP100s.5.2	Hi	<10nM	Hi	<2nM
ABP100s.5.3	Low	>100nM	Hi	<2nM
ABP100s.5.4	Hi	<10nM	Hi	<2nM
ABP100s.10.0	Hi	<10nM	Hi	<2nM
ABP100s.10.1	Hi	<10nM	Hi	<2nM
ABP100s.10.2	Hi	<10nM	Med	2 - 30nM
ABP100s.10.3	Hi	<10nM	Med	2 - 30nM
ABP100s.10.4	Hi	<10nM	Med	2 - 30nM
ABP100s.10.5	Hi	<10nM	Low	>30nM
ABP100s.10.6	Hi	<10nM	Low	>30nM
ABP100s.10.0.1	Hi	<10nM	Hi	<2nM
ABP100s.10.0.2	Med	10 - 100nM	Hi	<2nM

FIG. 10 (Contd.)

Clone name	CD3 Affinity (hi/low/medium)	Kd	HER2 Affinity (hi/low/medium)	Kd
ABP100s.10.0.3	Med	10 - 100nM	Hi	<2nM
ABP100s.10.0.4	Med	10 - 100nM	Hi	<2nM
ABP100s.10.0.5	Low	>100nM	Hi	<2nM
ABP100s.10.0.6	Low	>100nM	Hi	<2nM
ABP100s.10.5.1	Hi	<10nM	Low	>30nM
ABP100s.10.5.2	Med	10 - 100nM	Low	>30nM
ABP100s.10.5.3	Med	10 - 100nM	Low	>30nM
ABP100s.10.5.4	Med	10 - 100nM	Low	>30nM
ABP100s.10.5.5	Low	>100nM	Low	>30nM
ABP100s.10.5.6	Low	>100nM	Low	>30nM
ABP100s.10.6.1	Hi	<10nM	Low	>30nM
ABP100s.10.6.2	Med	10 - 100nM	Low	>30nM
ABP100s.10.6.3	Med	10 - 100nM	Low	>30nM
ABP100s.10.6.4	Med	10 - 100nM	Low	>30nM
ABP100s.10.6.5	Low	≥100nM	Low	>30nM
ABP100s.10.6.6	Low	≥100nM	Low	>30nM
ABP100s.11.0	Hi	<10nM	Hi	<2nM
ABP100s.11.5	Hi	<10nM	Low	<2nM
ABP100s.12.0	Hi	<10nM	Hi	<2nM
ABP100s.12.5	Hi	<10nM	Low	<2nM

FIG. 11

	HC aa sequence	LC aa sequence
ABP 100s .13	EVQLVESGGGLVQPGGSLRLSCAASGFNIDQYIHVVQRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKHTCTPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSEFLYS KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 21)	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGSRSGTD FTLTISLQPEDFATYYCQQHYTTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTL TLKADYEKHKVYACEVTHQGLSPVTKSFNRGECT SGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGS LRLSCAASGFTFNTYAMNWWVRQAPGKGLEWVAR IRSKYNNYATYYADSVKGRFTISRDDSQSSLYLQM NSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQG TLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSAVVVTQEPSTVSPGGTVTLTCSSTGAVTT SNYANWVQKPGQAFRGLIGGTNKRAPWTPARF SGSLIGDKAALTLSGAQPEDEAEYFCALWYSNLWV FGGGTKLTVL (SEQ ID NO: 61)
ABP 100s .14	EVQLVESGGGLVQPGGSLRLSCAASGFNIDQYIHVVQRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKHTCTPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSEFLYS KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 21)	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGSRSGTD FTLTISLQPEDFATYYCQQHYTTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTL TLKADYEKHKVYACEVTHQGLSPVTKSFNRGECT SGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGS LRLSCAASGFTFNTYAMNWWVRQAPGKGLEWVAR IRSKYNNYATYYADSVKGRFTISRDDSKSSLYLQM NSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQG TLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSAVVVTQEPSTVSPGGTVTLTCSSTGAVTT SNYANWVQKPGQAFRGLIGGTNKRAPWTPARF SGSLIGDKAALTLSGAQPEDEAEYFCALWYSNLWV FGGGTKLTVL (SEQ ID NO: 62)
ABP 100s .15	EVQLVESGGGLVQPGGSLRLSCAASGFNIDQYIHVVQRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKHTCTPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGSEFLYS KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 21)	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGSRSGTD FTLTISLQPEDFATYYCQQHYTTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTL TLKADYEKHKVYACEVTHQGLSPVTKSFNRGECT SGGGGSGGGGSGGGGSEVQLVESGGGLVKPGGSL RLSCAASGFTFNTYAMNWWVRQAPGKGLEWVAR RSKYNNYATYYADSVKGRFTISRDDSQSSLYLQMN SLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGT LTVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSAVVVTQEPSTVSPGGTVTLTCSSTGAVTTS NYANWVQKPGQAFRGLIGGTNKRAPWTPARF GSLIGDKAALTLSGAQPEDEAEYFCALWYSNLWV FGGGTKLTVL (SEQ ID NO: 63)

FIG. 11

	HC aa sequence	LC aa sequence
ABP 100s .16	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAV SNKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 21)	DIQMTQSPSSLSASVGDRVITITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGSRSGTD FTLTISLQPEDFATYYCQQHYTTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTL TLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECT SGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSL RLSCAASGFTFNTYAMNWWVRQAPGKGLEWVARI RSKYNNYATYYADSVKGRFTISRDDAKSLLYLQMN SLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGGSAVVVTQEPSTLVSPGGTVTLTCSSTGAVTTS NYANWVQQKPGQAFRGLIGGTNKRAPWTPARFS GSLIGDKAALTLSGAQPEDEAEYFCALWYSNLWVF GGGKLTVL (SEQ ID NO: 64)
ABP 100s .17	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAV SNKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 21)	DIQMTQSPSSLSASVGDRVITITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGSRSGTD FTLTISLQPEDFATYYCQQHYTTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTL TLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECT SGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGS LRLSCAASGFTFNTYAMNWWVRQAPGKGLEWVAR IRSKYNNYATYYADSVKGRFTISRDDSQSSLYLQM NSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQ TLVTSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGGSAVVVTQEPSTLVSPGGTVTLTCSSTGAVTT SNYANWVQQKPGQAFRGLIGGTNKKASWTPARF SGSLLGDKAALTLSGAQPEDEAEYFCALWYSNLW VFGGGKLTVL (SEQ ID NO: 65)
ABP 100s .18	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAV SNKALPAIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 21)	DIQMTQSPSSLSASVGDRVITITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGSRSGTD FTLTISLQPEDFATYYCQQHYTTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTL TLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECT SGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGS LRLSCAASGFTFNTYAMNWWVRQAPGKGLEWVAR IRSKYNNYATYYADSVKGRFTISRDDSKSSLYLQM NSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQ TLVTSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGGSAVVVTQEPSTLVSPGGTVTLTCSSTGAVTT SNYANWVQQKPGQAFRGLIGGTNKKASWTPARF SGSLLGDKAALTLSGAQPEDEAEYFCALWYSNLW VFGGGKLTVL (SEQ ID NO: 66)
ABP 100s .19	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGLTVTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN	DIQMTQSPSSLSASVGDRVITITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGSRSGTD FTLTISLQPEDFATYYCQQHYTTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTL

FIG. 11

	HC aa sequence	LC aa sequence
	VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGFSFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 21)	TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECTSGGGGSGGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGFTFTNTYAMNWWVRQAPGKGLEWVARI RSKYNNYATYYADSVKGRFTISRDDSQSSLYLQMN SLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSQAVVTQEPSTLTVSPGGTVTLTCSSTGAVTTS NYANWVQQKPGQAFRGLIGGTNKKASWTPARFSGSLLGDKAALTLSGAQPEDEAEYCALWYSNLWV FGGGTCLTVL (SEQ ID NO: 67)
ABP 100s .20	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGFSFLYS KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 21)	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGRSGTD FTLTISSLQPEDFATYYCQGHYTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECT SGGGGSGGGGSGGGGSEVQLVESGGGLVKPGGSL RLSCAASGFTFTNTYAMNWWVRQAPGKGLEWVARI RSKYNNYATYYADSVKGRFTISRDDAKSSLYLQMN SLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSQAVVTQEPSTLTVSPGGTVTLTCSSTGAVTTS NYANWVQQKPGQAFRGLIGGTNKKASWTPARFSGSLLGDKAALTLSGAQPEDEAEYCALWYSNLWV FGGGTCLTVL (SEQ ID NO: 68)
ABP 100s .21	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGFSFLYS KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 21)	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGRSGTD FTLTISSLQPEDFATYYCQGHYTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECT SGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGS LRLSCAASGFTFTNTYAMNWWVRQAPGKGLEWVAR I RSKYNNYATYYADSVKGRFTISRDDSQSSLYLQM NSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQG TLVTSSGGGGSGGGGSGGGGSGGGGSGGGGSGG GGSQAVVTQEPSTLTVSPGGTVTLTCSSTGAVTT S NYANWVQQTPGQAFRGLIGGTNKRASGVPDFRSGSLLGDKAALTITGAQADESDYYCALWYSNLW VFGGGTCLTVL (SEQ ID NO: 69)
ABP 100s .22	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQA PGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYL QMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGT LTVS SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYASTYRVVSVLTVLHQDWLNGKEYKCAV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGFSFLYS	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVA WYQQKPGKAPKLLIYSASFLYSVPSRFSGRSGTD FTLTISSLQPEDFATYYCQGHYTPPTFGQGTKEIK RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDYSLSTL TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECT SGGGGSGGGGSGGGGSEVQLVESGGGLVQPGGS LRLSCAASGFTFTNTYAMNWWVRQAPGKGLEWVAR I RSKYNNYATYYADSVKGRFTISRDDSKSSLYLQM NSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQG

[illegible]

FIG. 12

Clone name	CD3 Affinity (hi/low/medium)	Kd	HER2 Affinity (hi/low/medium)	Kd
ABP100s.13	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.14	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.15	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.16	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.17	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.18	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.19	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.20	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.21	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.22	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.23	Hi	<10nM	Hi (HER2)	<2nM
ABP100s.24	Hi	<10nM	Hi (HER2)	<2nM

FIG. 13A

SK-BR-3
CD3/TCR NFAT Luciferase Bioassay

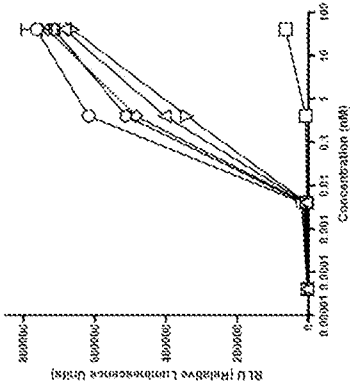


FIG. 13B

HCC1952
CD3/TCR NFAT Luciferase Bioassay

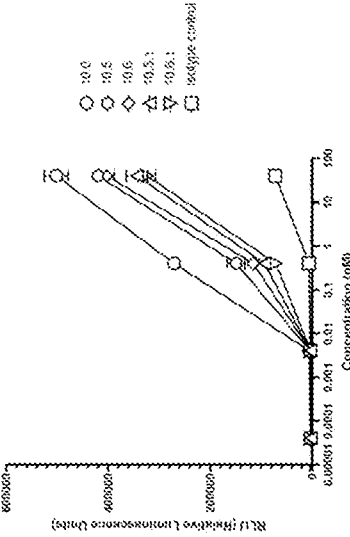


FIG. 13C

MCF-7
CD3/TCR NFAT Luciferase Bioassay

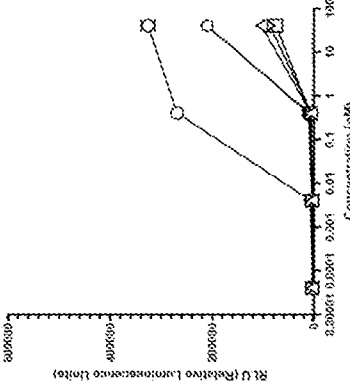


FIG. 13D

HT55
CD3/TCR NFAT Luciferase Bioassay

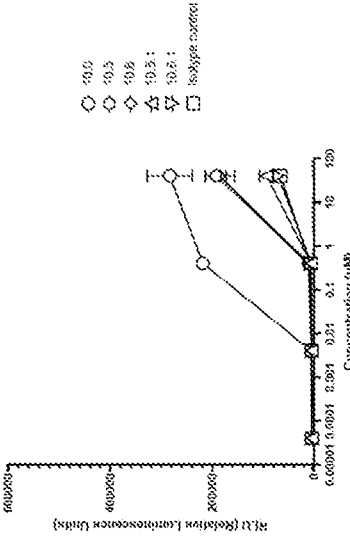


FIG. 13E

no target cells
CD3/TCR NFAT Luciferase Bioassay

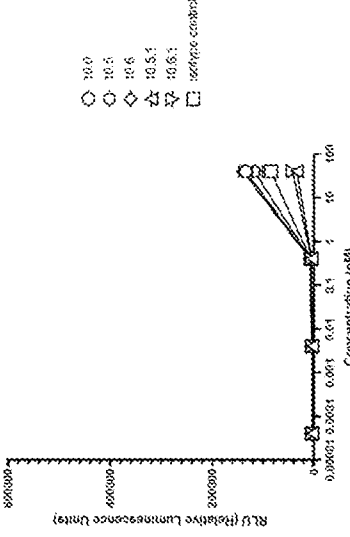


FIG. 14A

CD3+ T cells: SKBR3 target cells
TDCC, 5:1 E:T, 40 hr

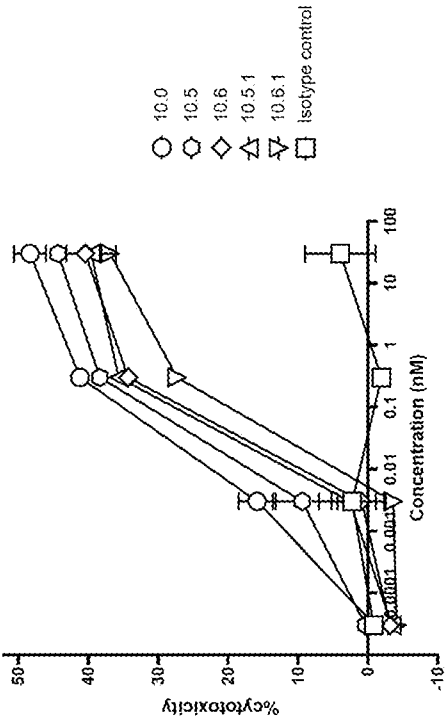


FIG. 14B

CD3+ T cells: HCC1954 target cells
TDCC, 5:1 E:T, 40 hr

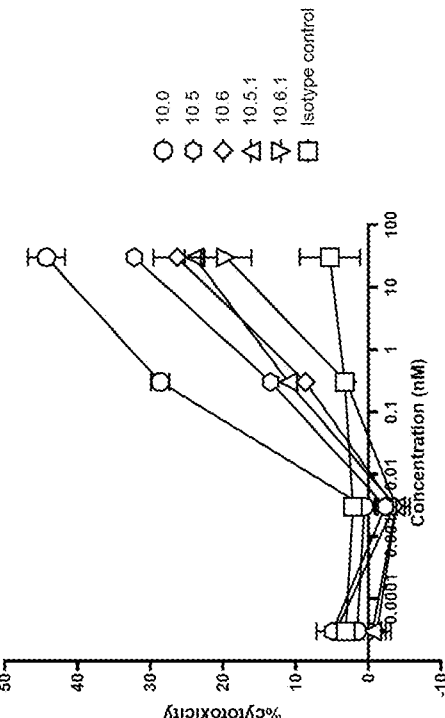


FIG. 14C

CD3+ T cells: MCF-7 target cells
TDCC, 5:1 E:T, 40 hr

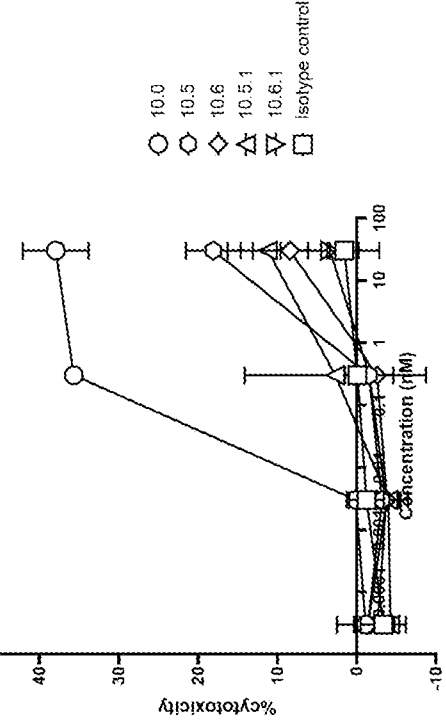


FIG. 14D

CD3+ T cells: HT55 target cells
TDCC, 5:1 E:T, 40 hr

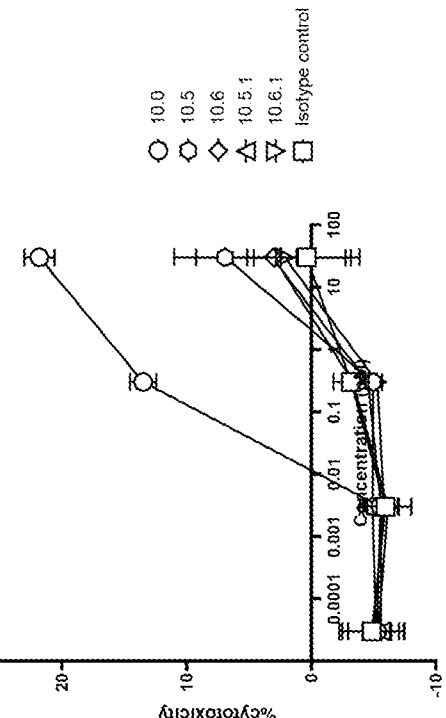




FIG. 16A

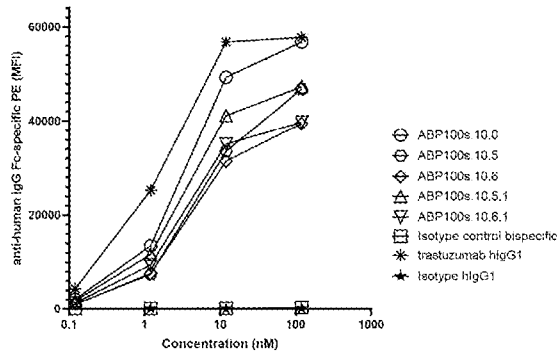
SK-BR-3
flow cytometry

FIG. 16B

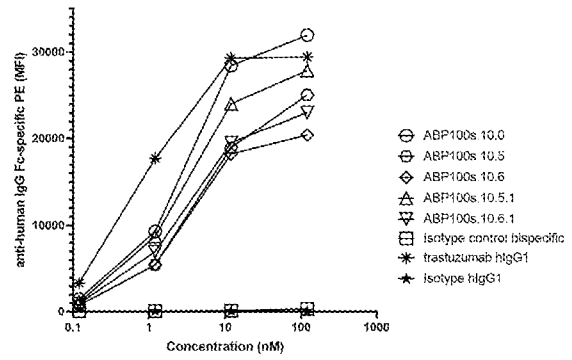
SK-OV-3
flow cytometry

FIG. 16C

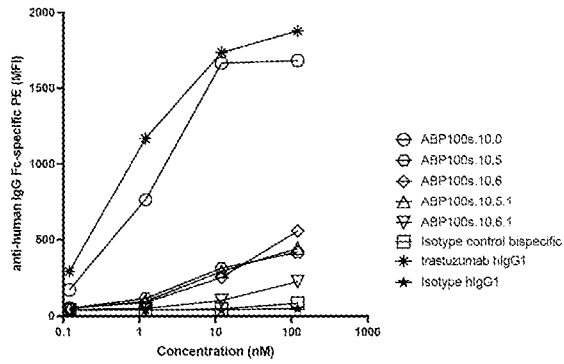
MCF-7
flow cytometry

FIG. 16D

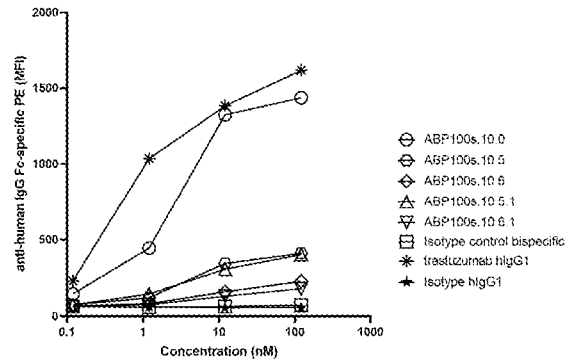
HT55
flow cytometry

FIG. 16E

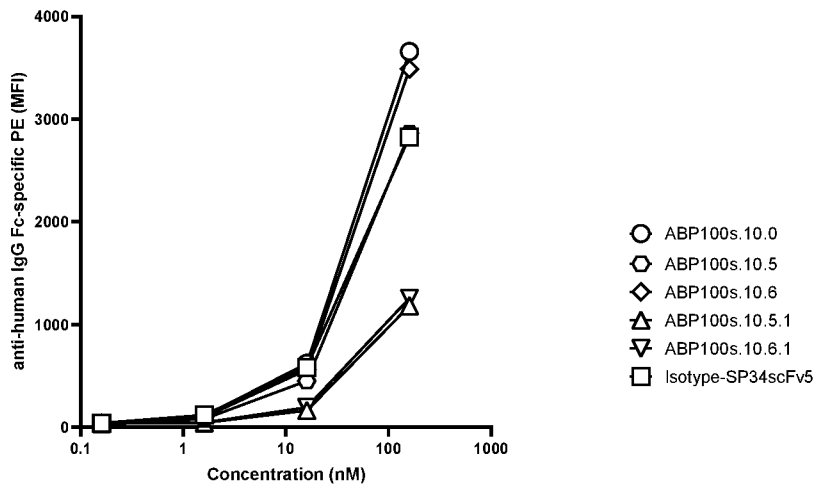
Activated CD3+ T cells
flow cytometry

FIG. 17A

Capture	Analyte	Chi ² (RU ²)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
10.0 (Fab-hIgG1)	HuHer2	1.01E+01	1.81E+05	1.13E-04	6.22E-10	132.1
10.0 (Fab-hIgG1)	CynoHer2	6.14E+00	1.81E+05	1.46E-03	8.09E-09	129.8

Capture	Analyte	Chi ² (RU ²)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
10.5	HuHer2	1.11E+01	2.04E+05	8.24E-03	4.04E-08	543.5
10.5	CynoHer2	8.60E+00	1.66E+05	5.53E-03	3.33E-08	565.1

Capture	Analyte	Chi ² (RU ²)	ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
10.6	HuHer2	3.88E+00	2.98E+05	1.99E-02	6.69E-08	176.1
10.6	CynoHer2	4.99E+00	1.76E+06	1.13E-01	6.45E-08	114.1

FIG. 17B

Capture	Analyte	Chi ² (RU ²)	k _a (1/Ms)	k _d (1/s)	KD (M)	R _{max} (RU)
10.0 (Fab-hIgG1)	HuCD3	1.67E+00	8.49E+05	1.74E-03	2.06E-09	30.1
10.0 (Fab-hIgG1)	HuCD3	1.97E-01	2.36E+05	3.38E-04	1.44E-09	25.7
10.0 (Fab-hIgG1)	HuCD3	1.43E-01	2.11E+05	3.81E-04	1.81E-09	25
10.0 (Fab-hIgG1)	HuCD3	1.43E-01	2.02E+05	3.65E-04	1.81E-09	25

10.0 (Fab-hIgG1)	CynoCD3	1.60E-01	2.66E+05	1.05E-03	3.95E-09	20.9
10.0 (Fab-hIgG1)	CynoCD3	2.89E-01	2.87E+05	1.05E-03	3.64E-09	25
10.0 (Fab-hIgG1)	CynoCD3	1.87E-01	2.69E+05	1.02E-03	3.78E-09	23.8
10.0 (Fab-hIgG1)	CynoCD3	1.41E+00	1.39E+05	7.36E-04	5.31E-09	30.1