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(73) Patenthaver: **Zymeworks BC Inc., Suite 800 - 114 East 4th Avenue, Vancouver, BC V5T 1G4, Canada**

(72) Opfinder: **WICKMAN, Grant Raymond, c/o ZYMEWORKS INC., 1385 West 8th Avenue, Suite 540, Vancouver, British Columbia V6H 3V9, Canada**
NG, Gordon Yiu Kon, c/o ZYMEWORKS INC., 1385 West 8th Avenue, Suite 540, Vancouver, British Columbia V6H 3V9, Canada
WEISSER, Nina E., c/o ZYMEWORKS INC., 1385 West 8th Avenue, Suite 540, Vancouver, British Columbia V6H 3V9, Canada

(74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**

(54) Benævnelse: **FREMGANGSMÅDER TIL ANVENDELSE AF BISPECIFIKKE ANTIGENBINDINGSKONSTRUKTER MÅLRETTET HER2**

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THOMAS SPRETER VON KREUDENSTEIN ET AL: "Supplemental Material to: Improving biophysical properties of a bispecific antibody scaffold to aid developability", MABS, vol. 5, no. 5, 1 September 2013 (2013-09-01), US, pages 646 - 654, XP055280747, ISSN: 1942-0862, DOI: 10.4161/mabs.25632
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Fortsættes ...

DESCRIPTION

Description

Background

[0001] The majority of current marketed antibody therapeutics are bivalent monospecific antibodies optimized and selected for high affinity binding and avidity conferred by the two antigen-binding domains. Afucosylation or enhancement of FcgR binding by mutagenesis have been employed to render antibodies more efficacious via antibody Fc dependent cell cytotoxicity mechanisms. Afucyosylated antibodies or antibodies with enhanced FcgR binding still suffer from incomplete therapeutic efficacy in clinical testing and marketed drug status has yet to be achieved for any of these antibodies. Typical bivalent antibodies conjugated to toxins (antibody drug conjugates) are more efficacious but broader clinical utility is limited by dose-limiting toxicity.

[0002] Therapeutic antibodies would ideally possess certain minimal characteristics, including target specificity, biostability, bioavailability and biodistribution following administration to a subject patient, and sufficient target binding affinity and high target occupancy to maximize antibody dependent therapeutic effects. Typically therapeutic antibodies are monospecific. Monospecific targeting however does not address other target epitopes that may be relevant in signaling and disease pathogenesis, allowing for drug resistance and escape mechanism. Some of the current therapeutic paradigms call for the use of combination of two therapeutic monospecific antibodies targeting two different epitopes of the same target antigen. One example is the use of a combination of Trastuzumab and Pertuzumab, both targeting the HER2 receptor protein on the surface of some cancer cells, but patients still progress with disease while others with lower HER2 receptor levels (HER2 <3+ by Hercept test) show no therapeutic benefit. Therapeutic antibodies targeting HER2 are disclosed in WO 2012/143523 to GenMab and WO 2009/154651 to Genentech. For example, WO 2012/143523 describes bispecific antibodies which comprise antigen-binding regions binding to two different epitopes of HER2, and related antibody-based compositions and molecules. Pharmaceutical compositions comprising the antibodies and methods of preparing and using the antibodies are also disclosed. Antibodies are also described in WO 2009/068625 and WO 2009/068631.

[0003] Co-owned patent application publication number WO2015077891describes HER2 antibodies. Co-owned patent application number PCT/US2014/037401 (WO 2014/182970) describes HER2 antibodies. Co-owned patent application number PCT/CA2013/050358 (WO 2013/166604) describes single arm monovalent antibodies. Co-owned patent application publication numbers WO2012/058768, filed November 4, 2011, WO2013/063702, filed November 2, 2012, WO2013/166594, filed May 10, 2013, and WO2013/166604, filed May 8, 2013 describe therapeutic antibodies.

Summary

[0004] The invention is defined by the claims.

[0005] The invention provides an antigen-binding construct for use in a method of inhibiting tumor growth in a human subject, wherein the human subject is administered at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 doses of the antigen binding construct, and/or wherein each dose is administered at least every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days. The antigen-binding construct provided for use according to the invention comprises a first antigen-binding polypeptide construct which monovalently and specifically binds a HER2 (human epidermal growth factor receptor 2) ECD2 (extracellular domain 2) antigen on a HER2-expressing cell and a second antigen-binding polypeptide construct which monovalently and specifically binds a HER2 ECD4 (extracellular domain 4) antigen on a HER2-expressing cell, first and second linker polypeptides, wherein the first linker polypeptide is operably linked to the first antigen-binding polypeptide construct, and the second linker polypeptide is operably linked to the second antigen-binding polypeptide construct; wherein the linker polypeptides are covalently linked with each other, and wherein the linker polypeptides are operatively linked to a human IgG1 Fc region. The ECD2-binding polypeptide construct is a Fab, wherein the Fab-linker-IgG1 Fc comprises a heavy chain comprising SEQ ID NO:97 and a light chain comprising SEQ ID NO:69. The ECD4 binding polypeptide construct is an scFv, wherein the scFv-linker-IgG1 Fc comprises SEQ ID NO:295.

[0006] The antigen-binding construct provided for use according to the invention is often referred to herein as the "v 10000" construct. References to other constructs are for reference and illustration, and do not form part of the claimed invention.

Brief Description of the Drawings

[0007]

Figure 1A depicts the structure of a biparatopic antibody in a Fab-Fab format. Figures 1B to 1E depict the structure of possible versions of a biparatopic antibody in an scFv-Fab format. In Figure 1B, antigen-binding domain 1 is an scFv, fused to Chain A, while antigen-binding domain 2 is a Fab, fused to Chain B. In Figure 1C, antigen-binding domain 1 is a Fab, fused to Chain A, while antigen-binding domain 2 is an scFv, fused to Chain B. In Figure 1D, antigen-binding domain 2 is a Fab, fused to Chain A, while antigen-binding domain 1 is an scFv, fused to Chain B. In Figure 1E, antigen-binding domain 2 is an scFv, fused to Chain A, while antigen-binding domain 1 is a Fab, fused to Chain B. In Figure 1F, both antigen-binding domains are scFvs.

Figure 2 depicts the characterization of expression and purification of exemplary anti-HER2 biparatopic antibodies. Figure 2A and Figure 2B depict

the SEC chromatograph of the protein A purified antibody, and non-reducing SDS-PAGE analysis of 10L expression and purification of v5019. Figure 2C depicts the SDS-PAGE analysis of a 25L expression and purification of v10000.

Figure 3 depicts the results of UPLC-SEC analysis of exemplary anti-HER2 biparatopic antibodies purified by protein A and SEC. Figure 3A shows the results for v5019, where the upper panel shows the results of the purification and the lower panel shows the same result with an expanded scale for the y-axis. A summary of the data obtained is provided below the UPLC-SEC results. Figure 3B shows the results for v10000.

Figure 4 depicts LCMS analysis of the heterodimer purity of exemplary anti-HER2 biparatopic antibodies. Figure 4A depicts results from LC-MS analysis of the pooled SEC fractions of v5019. Figure 4B depicts the results from LC-MS analysis of the pooled protein A fractions of v10000.

Figure 5 depicts analysis of a 25L-scale preparation of an exemplary anti-HER2 biparatopic antibody. Figure 5A depicts the SDS-PAGE profile of an exemplary anti-HER2 biparatopic following MabSelect™ and HiTrap™ SP FF purification. Figure 5B depicts LCMS analysis of the purified antibody.

Figure 6 compares the ability of an exemplary biparatopic anti-HER2 antibodies to bind to HER2+ whole cells displaying different HER2 receptor density compared to control antibodies, as measured by FACS. Figure 6A and Figure 6E depict binding to SKOV3 cells; Figure 6B depicts binding to JIMT1 cells; Figure 6C and Figure 6F depict binding to MCF7 cells; Figure 6D depicts binding to MDA-MB-231 cells; and Figure 6G depicts binding to WI-38 cells.

Figure 7 depicts the ability of exemplary anti-HER2 biparatopic antibodies to inhibit the growth of HER2+ cells. Figure 7A and Figure 7D shows growth inhibition in SKOV3 cells; Figure 7B shows growth inhibition in BT-474 cells; Figure 7C shows growth inhibition in SKBR3 cells, and Figure 7E shows growth inhibition in JIMT-1 cells.

Figure 8 depicts the SPR binding data relating to the paratopes of an exemplary anti-HER2 biparatopic antibodies. Figure 8A illustrates the K_D values (nM) of a monovalent anti-Her2 antibody (v1040; representing the antigen-binding domain on CH-B of exemplary anti-Her2 biparatopic antibody), for binding to immobilized Her2 ECD or dimeric Her2-Fc. Figure 8B illustrates the K_D values (nM) of a monovalent anti-Her2 antibody (v4182; representing the antigen-binding domain on CH-A of exemplary anti-Her2 biparatopic antibody) for binding to immobilized Her2 ECD or dimeric Her2-Fc.

Figure 9 depicts the ability of exemplary anti-HER2 biparatopic antibody to internalize in HER2+ cells. Figure 9A depicts internalization in BT-474 cells, while Figure 9b depicts internalization in JIMT-1 cells.

Figure 10 depicts surface binding and internalization of exemplary anti-HER2 biparatopic antibodies. Figure 10A (v5019) depicts the result in BT-474 cells; Figure 10B (v5019) and Figure 10F (v5019 and v10000) depict the result in JIMT1 cells; Figure 10C (v5019) and Figure 10E (v5019 and v10000) depict the result in SKOV3 cells, and Figure 10D (v5019) depicts the result in MCF7 cells.

Figure 11 depicts the ability of an exemplary anti-HER2 biparatopic antibody to mediate ADCC in SKOV3 cells. In Figure 11A, the assay was carried out using an effector to target cell ratio of 5:1; in Figure 11B, the assay was carried out using an effector to target cell ratio of 3:1; and in Figure 11C, the assay was carried out using an effector to target cell ratio of 1:1.

Figure 12 depicts the characterization of affinity and binding kinetics of monovalent anti-HER2 (v630 and v4182) and an exemplary biparatopic anti-Her2 antibody (v5019) to recombinant human HER2. Figure 12A shows the measurement of ka (1/Ms). Figure 12B shows the measurement of kd (1/s). Figure 12C shows the measurement of K_D (M).

Figure 13 depicts affinity and binding characteristics of an exemplary biparatopic anti-HER2 antibody to recombinant human HER2 over a range of antibody capture levels. Figure 13A depicts the measurement of kd (1/s) to HER2 ECD determined over a range of antibody capture levels for exemplary biparatopic anti-Her2 antibody (v5019). Figure 13B depicts the measurement of kd (1/s) to HER2 ECD determined over a range of antibody capture levels for monovalent anti-Her2 antibody (v4182). Figure 13C depicts the measurement of kd (1/s) to HER2 ECD determined over a range of antibody capture levels for monovalent anti-Her2 antibody (v630).

Figure 14 shows a comparison of the mechanism of binding of a monospecific anti-ECD4 HER2 antibody (left), and a Fab-scFv biparatopic anti-ECD2x ECD4 HER2 antibody (right). The monospecific anti-ECD4 HER2 antibody is capable of binding one antibody molecule to two HER2 molecules; whereas the biparatopic anti-ECD2 x ECD4 HER2 antibody is capable of binding one antibody to two HER2 molecule, as well as 2 antibodies to one HER2 molecule and combinations therein which results in HER2 receptor cross-linking and lattice formation followed by downstream biological effects such as internalization and/or growth inhibition as indicated by the arrows. IEC represents "immune effector cells." The four extracellular domains of HER2 are numbered as 1, 2, 3, or 4 where 1=ECD1, 2=ECD2, 3=ECD3, and 4=ECD4.

Figure 15 depicts the effect of an exemplary anti-HER2 biparatopic antibody on AKT phosphorylation in BT-474 cells.

Figure 16 depicts the effect of an exemplary anti-HER2 biparatopic antibody on cardiomyocyte viability. Figure 16A depicts the effect of v5019 and the corresponding ADC v6363 on cardiomyocyte viability; Figure 16B depicts the effect of v5019, v7091, and v10000 and corresponding ADCs v6363, 7148, 10553 on cardiomyocyte viability, and Figure 16C depicts the effect of v5019, v7091, and v10000 and corresponding ADCs v6363, 7148, 10553 on the viability of doxorubicin-pretreated cardiomyocytes.

Figure 17 depicts the ability of exemplary anti-HER2 biparatopic antibody drug conjugates to inhibit the growth of HER2+ cells. Figure 17A shows the ability of the ADC v6363 to inhibit the growth of JIMT1 cells. Figure 17B shows the ability of the ADC v6363 to inhibit the growth of SKOV3 cells. Figure 17C shows the ability of the ADC v6363 to inhibit the growth of MCF7 cells. Figure 17D shows the ability of the ADC v6363 to inhibit the growth of MDA-MB-231 cells. Figure 17E shows the ability of ADCs v6363, v10553, and v1748 to inhibit the growth of SKOV3 cells. Figure 17F shows the ability of ADCs v6363, v10553, and v1748 to inhibit the growth of JIMT-1 cells. Figure 17G shows the ability of ADCs v6363, v10553, and v1748 to inhibit the growth of NCI-N87 cells.

Figure 18 depicts the effect of a biparatopic anti-HER2 antibody in a human ovarian cancer line xenograft model (SKOV3). Figure 18A shows the effect of the antibody on mean tumor volume. Figure 18B shows the effect of the antibody on percent survival of the animals.

Figure 19 depicts the effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) in a human ovarian cancer line xenograft model (SKOV3).

Figure 19A shows the effect of the antibody on mean tumor volume. Figure 19B shows the effect of the antibody on percent survival of the animals.

Figure 20 depicts the effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) on mean tumour volume in a human breast primary cell xenograft model (HBCx-13b).

Figure 21 depicts the effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) on mean tumour volume in a human breast primary cell xenograft model (T226).

Figure 22 depicts the effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) on mean tumour volume in a human breast primary cell xenograft model (HBCx-5).

Figure 23 depicts the effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) on anti-HER2 treatment resistant tumors in a human cell line xenograft model (SKOV3).

Figure 24 depicts the effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) to anti-HER2 treatment resistant tumors in human primary cell xenograft model (HBCx-13b).

Figure 25 depicts the thermal stability of exemplary anti-HER2 biparatopic antibodies. Figure 25A depicts the thermal stability of v5019. Figure 25B depicts the thermal stability of v10000. Figure 25C depicts the thermal stability of v7091.

Figure 26 depicts the thermal stability of exemplary anti-HER2 biparatopic antibody drug conjugates. Figure 26A depicts the thermal stability of v6363. Figure 26B depicts the thermal stability of v10553. Figure 26C depicts the thermal stability of v7148.

Figure 27 depicts the ability of anti-HER2 biparatopic antibodies to mediate ADCC in HER2+ cells. The legend shown in Figure 27C applies to Figure 27A and Figure 27B. Figure 27A depicts this ability in SKBR3 cells; Figure 27B depicts this ability in JIMT-1 cells; Figure 27C depicts this ability in MDA-MB-231 cells; and Figure 27D depicts this ability in WI-38 cells.

Figure 28 depicts the effect of afucosylation on the ability of anti-HER2 biparatopic antibodies to mediate ADCC. The legend shown in Figure 28B applies to Figure 28A as well. Figure 28A compares the ability of an afucosylated version of v5019 to mediate ADCC to that of Herceptin™ in SKOV3 cells. Figure 28B compares the ability of an afucosylated version of v5019 to mediate ADCC to that of Herceptin™ in MDA-MB-231 cells. Figure 28C compares the ability of v10000 and an afucosylated version of v10000 to mediate ADCC against that of Herceptin™ in ZR-75-1 cells.

Figure 29 depicts the ability of v5019 to inhibit growth of BT-474 cells in the presence or absence of growth-stimulatory ligands.

Figure 30 depicts the effect of an afucosylated version of v5019 (v7187) on tumor volume in a human breast cancer xenograft model (HBCx13B).

Figure 31 depicts the ability of anti-HER2 biparatopic antibodies and anti-HER2 biparatopic-ADCs to bind to HER2+ tumor cells. Figure 31A compares the binding of v6363 to a T-DM1 analog, v6246, in SKOV3 cells. Figure 31B compares the binding of v6363 to a T-DM1 analog, v6246, in JIMT-1 cells. Figure 31C compares the binding of several exemplary anti-HER2 biparatopic antibodies and anti-HER2 biparatopic-ADCs to controls, in SKOV3 cells. Figure 31D compares the binding of several exemplary anti-HER2 biparatopic antibodies and anti-HER2 biparatopic-ADCs to controls, in JIMT-1 cells.

Figure 32 depicts Dose-Dependent Tumour Growth Inhibition of an exemplary anti-HER2 biparatopic-ADC in a HER2 3+ (ER-PR negative) patient derived xenograft model (HBCx13b). Figure 32A shows the effect of v6363 on tumor volume, while Figure 32B shows the effect on percent survival.

Figure 33 depicts the effect of Biparatopic anti-HER2-ADC v6363 compared to Standard of Care Combinations in a Trastuzumab Resistant PDX HBCx-13b xenograft model. Figure 33A depicts the effect of treatment on tumor volume, while Figure 33B depicts the effect of treatment on survival.

Figure 34 depicts the efficacy of a biparatopic anti-HER2-ADC in HER2+ trastuzumab-resistant breast cancer cell derived tumour xenograft model (JIMT-1).

Figure 35 depicts the efficacy of exemplary anti-HER2 biparatopic antibodies *in vivo* in a trastuzumab sensitive ovarian cancer cell derived tumour xenograft model (SKOV3). Figure 35A depicts the effect of treatment on tumor volume, while Figure 35B depicts the effect of treatment on survival.

Figure 36 depicts the dose-dependent efficacy of exemplary anti-HER2 biparatopic antibodies *in vivo* in a trastuzumab sensitive ovarian cancer cell derived tumour xenograft model (SKOV3).

Figure 37 depicts the ability of an anti-HER2 biparatopic antibody and an anti-HER2 biparatopic-ADC to inhibit growth of cell lines expressing HER2, and EGFR and/or HER3 at the 3+, 2+ or 1+ levels. Figure 37A depicts the ability of v10000 to inhibit growth selected cell lines. Figure 37B depicts the ability of v10553 to inhibit growth of selected cell lines.

Figure 38 depicts a summary of the ability of v10000 and v10553 to inhibit growth in a panel of cell lines. Hyphenated values (e.g. 1/2) indicate discrepant erbb receptor levels as reported in the literature; Erbb IHC values were obtained internally or from the literature. Where no value is reported the receptor quantities are unknown and/or not reported. * IHC level estimate based on erBb2 gene expression data (Crown BioSciences). Numbered references are described below.

Figure 39 depicts the ability of v10000 to mediate ADCC in HER2+ cells. Figure 39A depicts the results in FaDu cells. Figure 39B depicts the results in A549 cells. Figure 39C depicts the results in BxPC3 cells. Figure 39D depicts the results in MiaPaca2 cells.

Figure 40 depicts the ability of anti-HER2 biparatopic antibodies to mediate ADCC in HER2+ cells. Figure 40A depicts the results in A549 cells. Figure 40B depicts the results in NCI-N87 cells. Figure 40C depicts the results in HCT-116 cells.

Figure 41 depicts the effect of anti-HER2 biparatopic antibody format on binding HER2+ cells. Figure 41A depicts the effect of format on binding to BT-474 cells. Figure 41B depicts the effect of format on binding to JIMT-1 cells. Figure 41C depicts the effect of format on binding to MCF7 cells.

Figure 41D depicts the effect of format on binding to MDA-MB-231 cells.

Figure 42 depicts the effect of anti-HER2 biparatopic antibody format on internalization of antibody in HER2+ cells. Figure 42A depicts the effect on internalization in BT-474 cells. Figure 42B depicts the effect on internalization in JIMT-1 cells. Figure 42C depicts the effect on internalization in MCF7 cells.

Figure 43 depicts the effect of anti-HER2 biparatopic antibody format on the ability to mediate ADCC in HER2+ cells. Figure 43A depicts the effect in JIMT-1 cells. Figure 43B depicts the effect in MCF7 cells. Figure 43C depicts the effect in HER2 0/1+ MDA-MB-231 breast tumor cells.

Figure 44 depicts the effect of anti-HER2 biparatopic antibody format on the ability of the antibodies to inhibit HER2+ tumor cell growth in BT-474 cells in the presence or absence of growth-stimulatory ligands.

Figure 45 depicts the effect of anti-HER2 biparatopic antibody format on the ability of the antibodies to inhibit growth of SKBR3 cells.

Figure 46 depicts the effect of anti-HER2 biparatopic antibody format on the ability of antibodies to inhibit growth of HER2+ tumor cells. Figure 46A depicts growth inhibition in SKOV3 cells. Figure 46B depicts growth inhibition in JIMT-1 cells. Figure 46C depicts growth inhibition in MCF7 cells.

Figure 47 depicts a comparison of binding characteristics of anti-HER2 biparatopic antibodies of differing format as measured by SPR. Figure 47A depicts the plot and linear regression analysis for the kd (1/s) at different antibody capture levels with v6903 and v7091. Figure 47B depicts the plot and linear regression analysis for the KD (M) at different antibody capture levels with v6903 and v7091.
References found in Figure 38 are as follows: 1. Labouret et al. 2012, Neoplasia 14:121-130 ; 2. Ghasemi et al. 2014, Oncogenesis doi:10.1038/oncsis.2014.31; 3. Gaborit et al. 2011 J Bio Chem, 286:1133-11345; 4. Kimura et al. 2006, Clin Cancer Res; 12:4925-4932; 5. Komoto et al. 2009, Cane Sci; 101:468-473; 6. Cretella et al. 2014, Molecular Cancer 13:143-155; 7. Bunn et al. 2001, Clin Cancer Res; 7:3239-3250; 8. Lewis Phillips et al. 2013, Clin Cancer Res, 20:456-468; 9. McDonagh et al. 2012, 11:582-593; 10. Coldren et al. 2006, Mol Cancer Res:521-528; 11. Cavazzoni et al. 2012 Mol Cancer, 11:91-115; 12. Li et al. 2014, Mol Cancer Res, doi:10.1158/1541-7786.MCR-13-0396; 13. Chmielewski et al. 2004, Immunology, 173:7647-7653; 14. Kuwada et al. 2004, Int J Cancer, 109:291-301; 15. Fujimoto-Ouchi et al. 2007, Clin Chemother Pharmacol, 59:795-805; 16. Chavez-Blanco et al. 2004, BMC Cancer, 4:59; 17. Campiglio et al. 2004, J Cellular Physiology. 198:259-268; 18. Lehmann et al. 2011, J Clin Investigation, 121:2750-2767; 19. Collins et al. 2011, Annals Oncology, 23:1788-1795; 20. Takai et al. 2005, Cancer, 104:2701-2708; 21. Rusnack et al. 2007, Cell Prolif, 40:580-594; 22. Ma et al. 2013, PLOS ONE, 8:e73261-e73261; 23. Meira et al. 2009, British J Cancer, 101:782-791; 24. Hayashi MP28-14 poster; 25. Wang et al. 2005 J Huazhong Univ Sci Technolog Med Sci. 25:326-8; 26. Makhja et al. 2010. J Clinc Oncolo 28:1215-1223.

Figure 48A-B depicts the effect of a biparatopic anti-HER2 antibody in a xenograft model of HER2-low, non-small cell lung cancer. Figure 48A shows the effect of the antibody on tumor volume. Figure 48B shows the effect of the antibody on percent survival of the animals.

Figure 49A-B depicts the effect of a biparatopic anti-HER2 antibody in a xenograft model of HER2-low, head and neck squamous cell carcinoma. Figure 49A shows the effect of the antibody on tumor volume. Figure 49B shows the effect of the antibody on percent survival of the animals.

Figure 50A-B depicts the effect of a biparatopic anti-HER2 antibody in a xenograft model of HER2-low, ER+ breast cancer. Figure 50A shows the effect of the antibody on tumor volume. Figure 50B shows the effect of the antibody on percent survival of the animals.

Figure 51A-B shows tumor volume and survival in a xenograft model of pancreatic cancer.

Figure 52 shows tumor volume in a xenograft model of gastric cancer.

DETAILED DESCRIPTION

[0008] The invention is as set out in the claims.

[0009] Described herein are methods of using bispecific antigen-binding constructs that bind HER2.

Antigen-binding constructs

[0010] Provided herein are antigen-binding constructs, e.g., antibodies, that bind HER2. The antigen-binding construct for use according to the invention includes a first antigen-binding polypeptide construct binding a HER2 ECD2 antigen and a second antigen-binding polypeptide construct binding a HER2 ECD4 antigen. As set out in more detail in claim 1, the first antigen-binding polypeptide construct is a Fab (fragment antigen-binding) and the second antigen-binding polypeptide construct is an scFv (single chain Fv). Another antigen-binding polypeptide construct that is not claimed, is a sdab (single domain antibody). The antigen-binding construct provided for use according to the invention includes a scaffold, which is a human IgG1 Fc region.

[0011] The term "antigen-binding construct" refers to any agent, e.g., polypeptide or polypeptide complex capable of binding to an antigen. In some aspects an antigen-binding construct is a polypeptide that specifically binds to an antigen of interest. An antigen-binding construct can be a monomer, dimer, multimer, a protein, a peptide, or a protein or peptide complex; an antibody, an antibody fragment, or an antigen-binding fragment thereof; an scFv and the like. An antigen-binding construct can be monospecific, bispecific, or multispecific. An antigen-binding construct can include, e.g., one or more antigen-binding polypeptide constructs (e.g., Fabs or scFvs) linked to one or more Fc. Further examples of antigen-binding constructs are described below and provided in the Examples. The antigen-binding construct provided for use according to the invention, is as defined in claim 1.

[0012] An antigen-binding construct may be monospecific, although this is not claimed. A monospecific antigen-binding construct refers to an antigen-binding construct with one binding specificity. In other words, the antigen-binding polypeptide construct binds to the same epitope on the same antigen. Examples of monospecific antigen-binding constructs include trastuzumab and pertuzumab.

[0013] A bispecific antigen binding construct has two antigen binding polypeptide constructs, each with a unique binding specificity. For example, a first antigen binding polypeptide construct binds to an epitope on a first antigen, and a second antigen binding polypeptide construct binds to an epitope on a second antigen. The term "biparatopic" as used herein, refers to a bispecific antibody where the first antigen binding moiety and the second antigen binding moiety bind to different epitopes on the same antigen.

[0014] An antigen-binding construct can be an antibody or antigen-binding portion thereof. As used herein, an "antibody" or "immunoglobulin" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically bind and recognize an analyte (e.g., antigen). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. The "class" of an antibody or immunoglobulin refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG_2, IgG_3, IgG_4, IgA1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[0015] An exemplary immunoglobulin (antibody) structural unit is composed of two pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminal domain of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (VL) and variable heavy chain (VH) refer to these light and heavy chain domains respectively. The IgG1 heavy chain comprises of the VH, CH1, CH2 and CH3 domains respectively from the N to C-terminus. The light chain comprises of the VL and CL domains from N to C terminus. The IgG1 heavy chain comprises a hinge between the CH1 and CH2 domains.

[0016] The term "hypervariable region" or "HVR", as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the complementarity determining regions (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. Hypervariable regions (HVRs) are also referred to as "complementarity determining regions" (CDRs), and these terms are used herein interchangeably in reference to portions of the variable region that form the antigen-binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, Sequences of Proteins of Immunological Interest (1983) and by Chothia et al., J Mol Biol 196:901-917 (1987), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

[0017] "Humanized" forms of non-human (e.g., rodent) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, 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 FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will 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); Riechmann et al., Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992).

[0018] Humanized HER2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 or Trastuzumab (HERCEPTIN®) as described in Table 3 of U.S. Pat. No. 5,821,337; humanized 520C9 (WO93/21319) and humanized 2C4 antibodies as described in US Patent Publication No. 2006/0018899.

Antigen-binding polypeptide construct

[0019] The antigen-binding constructs described herein comprise an e antigen-binding polypeptide construct that binds to a HER2 ECD2 antigen. The antigen-binding construct provided for use according to the invention, is as defined in claim 1, and includes a second antigen-binding polypeptide construct that binds to a HER2 ECD4 antigen. The antigen-binding polypeptide construct of the invention comprises the sequences disclosed in the examples below for the v10000 construct.

[0020] The antigen-binding polypeptide construct is bivalent (binding to two epitopes).

[0021] The antigen binding construct includes two antigen-binding polypeptide constructs. The format of the antigen-binding construct is Fab-scFv (first antigen-binding polypeptide construct-second antigen-binding polypeptide respectively).

[0022] A Fab (also referred to as fragment antigen-binding) contains the constant domain (CL) of the light chain and the first constant domain

(CH1) of the heavy chain along with the variable domains VL and VH on the light and heavy chains respectively. The variable domains comprise the complementarity determining loops (CDR, also referred to as hypervariable region) that are involved in antigen-binding. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

[0023] A "single-chain Fv" or "scFv" includes the VH and VL domains of an antibody, wherein these domains are present in a single polypeptide chain. In one embodiment, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen-binding. For a review of scFv see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994). HER2 antibody scFv fragments are described in WO93/16185; U.S. Pat. No. 5,571,894; and U.S. Pat. No. 5,587,458.

[0024] A "single domain antibody" or "sdAb" format, not claimed, is an individual immunoglobulin domain. SdAbs are fairly stable and easy to express as fusion partner with the Fc chain of an antibody (Harmsen MM, De Haard HJ (2007). "Properties, production, and applications of camelid single-domain antibody fragments". Appl. Microbiol Biotechnol. 77(1): 13-22).

[0025] Other antigen binding polypeptide constructs that are not claimed, can bes derived from an antibody, a fibronectin, an affibody, anticalin, cysteine knot protein, DARPin, avimer, Kunitz domain or variant or derivative thereof.

[0026] The antigen binding polypeptide constructs described herein can be converted to different formats. For example, a Fab can be converted to an scFv or an scFv can be converted to a Fab. Methods of converting between types of antigen-binding domains are known in the art (see for example methods for converting an scFv to a Fab format described at, e.g., Zhou et al (2012) Mol Cancer Ther 11:1167-1476.).

[0027] The antigen binding constructs described herein specifically bind HER2. "Specifically binds", "specific binding" or "selective binding" means that the binding is selective for the antigen and can be discriminated from unwanted or non-specific interactions. The ability of an antigen-binding construct to bind to a specific antigenic determinant can be measured either through an enzyme-linked immunosorbent assay (ELISA) or other techniques familiar to one of skill in the art, e.g. surface plasmon resonance (SPR) technique (analyzed on a BIAcore instrument) (Liljeblad et al, Glyco J 17, 323-329 (2000)), and traditional binding assays (Heeley, Endocr Res 28, 217-229 (2002)).

[0028] In one embodiment, the extent of binding of an antigen-binding moiety to an unrelated protein is less than about 10% of the binding of the antigen-binding construct to the antigen as measured, e.g., by SPR.

HER2

[0029] The antigen-binding constructs described herein include an antigen-binding polypeptide construct that binds to the ECD2 of HER2.

[0030] The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein described, for example, in Semba et al., PNAS (USA) 82:6497-6501 (1985) and Yamamoto et al. Nature 319:230-234 (1986) (Genebank accession number X03363). The term "erbB2" and "neu" refers to the gene encoding human ErbB2 protein, p185 or p185neu refers to the protein product of the neu gene.

[0031] HER2 is a HER receptor. A "HER receptor" is a receptor protein tyrosine kinase which belongs to the human epidermal growth factor receptor (HER) family and includes EGFR, HER2, HER3 and HER4 receptors. A HER receptor will generally comprise an extracellular domain, which may bind an HER ligand; a lipophilic transmembrane domain; a conserved intracellular tyrosine kinase domain; and a carboxyl-terminal signaling domain harboring several tyrosine residues which can be phosphorylated. By "HER ligand" is meant a polypeptide which binds to and/or activates an HER receptor.

[0032] The extracellular (ecto) domain of HER2 comprises four domains, Domain I (ECD1, amino acid residues from about 1-195), Domain II (ECD2, amino acid residues from about 196-319), Domain III (ECD3, amino acid residues from about 320-488), and Domain IV (ECD4, amino acid residues from about 489-630) (residue numbering without signal peptide). See Garrett et al. Mol. Cell. 11: 495-505 (2003), Cho et al. Nature 421: 756-760 (2003), Franklin et al. Cancer Cell 5:317-328 (2004), Tse et al. Cancer Treat Rev. 2012 Apr;38(2): 133-42 (2012), or Plowman et al. Proc. Natl. Acad. Sci. 90:1746-1750 (1993).

[0033] The sequence of HER2 is as follows; ECD boundaries are Domain I: 1-165; Domain II: 166-322; Domain III: 323-488; Domain IV: 489-607.

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  1 tqvctgtdmk lrlpaspeth ldmlrhlyqg cqvvqgnlel tylptnasls flqdiqevqg
 61 yvliahnqvr qvplqrlriv rgtqlfedny alavldngdp lnnttpvtga spgglrelql
121 rslteilkgg vliqrnpqlc yqdtilwkdi fhknnqlalt lidtnrsrac hpcspmckgs
181 rcwgessedc qsltrtvcag gcarckgplp tdccheqcaa gctgpkhsdc laclhfnhsg
241 icelhcpalv tyntdtfesm pnpegrytfg ascvtacpyn ylstdvgsct lvcplhnqev
301 taedgtqrce kcskpcarvc yglgmehlre vravtsaniq efagckkifg slaflpesfd
361 gdpasntapl qpeqlqvfet leeitgylyi sawpdslpdl svfqnlqvir grilhngays
421 ltlqglgisw lglrslrelg sglalihhnt hlcfvhtvpw dqlfrnphqa llhtanrped
481 ecvgeglach qlcarghcwg pgptqcvncs qflrgqecve ecrvlqglpr eyvnarhclp
541 chpecqpqng svtcfgpead qcvacahykd ppfcvarcps gvkpdlsymp iwkfpdeega
601 cqpcpin (SEQ ID NO:349)
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[0034] The "epitope 2C4" is the region in the extracellular domain of HER2 to which the antibody 2C4 binds. Epitope 2C4 comprises residues from domain II in the extracellular domain of HER2. 2C4 and Pertuzumab bind to the extracellular domain of HER2 at the junction of domains I, II and III.

Franklin et al. Cancer Cell 5:317-328 (2004). In order to screen for antibodies which bind to the 2C4 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. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 2C4 epitope of HER2 using methods known in the art and/or one can study the antibody-HER2 structure (Franklin et al. Cancer Cell 5:317-328 (2004)) to see what domain(s) of HER2 is/are bound by the antibody.

[0035] The "epitope 4D5" is the region in the extracellular domain of HER2 to which the antibody 4D5 (ATCC CRL 10463) and Trastuzumab bind. This epitope is close to the transmembrane domain of HER2, and within Domain IV of HER2. To screen for antibodies which bind to the 4D5 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. Alternatively, epitope mapping can be performed to assess whether the antibody binds to the 4D5 epitope of HER2 (e.g. any one or more residues in the region from about residue 529 to about residue 625, inclusive, see FIG. 1 of US Patent Publication No. 2006/0018899).

Exemplary anti-HER2 antigen binding constructs

[0036] Exemplary anti-HER2 antibodies (or antigen-binding constructs) and controls are provided herein. Representations of exemplary biparatopic formats are shown in Figure 1. In all of the formats shown in Figure 1, the heterodimeric Fc is depicted with one chain (Chain A) shown in black and the other (Chain B) shown in grey, while one antigen-binding domain (1) is shown in hatched fill and the other antigen-binding domain (2) is shown in white.

[0037] The construct of the invention is set out in claim 1, which defines the v10000 construct. All other constructs described herein and below are for reference and are not part of the claimed invention.

[0038] Figure 1A depicts the structure of a biparatopic antibody in a Fab-Fab format. Figures 1B to 1E depict the structure of possible versions of a biparatopic antibody in an scFv-Fab format. In Figure 1B, antigen-binding domain 1 is an scFv, fused to Chain A, while antigen-binding domain 2 is a Fab, fused to Chain B. In Figure 1C, antigen-binding domain 1 is a Fab, fused to Chain A, while antigen-binding domain 2 is an scFv, fused to Chain B. In Figure 1D, antigen-binding domain 2 is a Fab, fused to Chain A, while antigen-binding domain 1 is an scFv, fused to Chain B. In Figure 1E, antigen-binding domain 2 is an scFv, fused to Chain A, while antigen-binding domain 1 is a Fab, fused to Chain B. In Figure 1F, both antigen-binding domains are scFvs.

[0039] The sequences of the following variants are provided in the Sequence Table found after the Examples. CDR regions were identified using a combination of the Kabat and Chothia methods. Regions may vary slightly based on method used for identification.

Exemplary anti-HER2 biparatopic antibodies

[0040] Exemplary anti-HER2 biparatopic antibodies are shown in Table 1.

Table 1: Exemplary anti-HER2 biparatopic antibodies (nb: only v10000 is according to the invention)

Variant		Chain A	Chain B
5019	domain containing the epitope	ECD2	ECD4
	Format	Fab	scFv
	Antibody name	Pertuzumab	Trastuzumab
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T366I_N390R_K392M_T394W
5020	domain containing the epitope	ECD4	ECD2
	format	scFv	Fab
	Antibody name	Trastuzumab	Pertuzumab
	CH3 sequence substitutions	L351Y_S400E_F405A_Y407V	T350V_T366L_K392L_T394W
7091	domain containing the epitope	ECD2	ECD4
	format	Fab	scFv
	Antibody name	Pertuzumab	Trastuzumab
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T350V_T366L_K392L_T394W
10000	domain containing the epitope	ECD2	ECD4
	format	Fab	scFv
	Antibody name CH3 sequence substitutions	Pertuzumab - with Y96A in VL region and T30A/A49G/L69F in VH region T350V_L351Y_F405A_Y407V	Trastuzumab T350V_T366L_K392L_T394W
6902	domain containing the	ECD2	ECD4

Variant		Chain A	Chain B
	epitope		
	format	Fab	Fab
	Antibody name	Trastuzumab	Pertuzumab
	Fab substitutions	HC: L143E K145T	HC: D146G Q179K
		LC: Q124R	LC: Q124E_Q160E_T180E
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T350V_T366L_K392L_T394W
6903	domain containing the epitope	ECD2	ECD4
	format	Fab	Fab
	Fab substitutions	HC: L143E_K145T	HC: D146G_Q179K
		LC: Q124R_Q1160K_T178R	LC: Q124E_Q160E_T180E
	Antibody name	Trastuzumab	Pertuzumab
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T350V_T366L_K392L_T394W
6717	domain containing the epitope	ECD4	ECD2
	format	scFv	scFv
	Antibody name	Pertuzumab	Trastuzumab
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T366I_N390R_K392M_T394W

Notes:
• CH3 numbering according to EU index as in Kabat referring to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85);
• Fab or variable domain numbering according to Kabat (Kabat and Wu, 1991; Kabat et al, Sequences of proteins of immunological interest. 5th Edition - US Department of Health and Human Services, NIH publication n° 91-3242, p 647 (1991))
• "domain containing the epitope"=domain of HER2 to which antigen-binding moiety binds;
• "Antibody name"=antibody from which antigen-binding moiety is derived, includes substitutions compared to wild-type when present;
• "Fab substitutions"=substitutions in Fab that promote correct light chain pairing;
• "CH3 sequence substitutions"=substitutions in CH3 domain that promote formation of heterodimeric Fc

Exemplary anti-HER2 monovalent control antibodies - not part of the claimed invention

[0041] v1040: a monovalent anti-HER2 antibody, where the HER2 binding domain is a Fab derived from trastuzumab on chain A, and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A_Y407V in Chain A, T350V_T366L_K392L_T394W in Chain B, and the hinge region of Chain B having the mutation C226S; the antigen-binding domain binds to domain 4 of HER2.

[0042] v630 - a monovalent anti-HER2 antibody, where the HER2 binding domain is an scFv derived from trastuzumab on Chain A, and the Fc region is a heterodimer having the mutations L351Y_S400E_F405A_Y407V in Chain A, T366I_N390R_K392M_T394W in Chain B; and the hinge region having the mutation C226S (EU numbering) in both chains; the antigen-binding domain binds to domain 4 of HER2.

[0043] v4182: a monovalent anti-HER2 antibody, where the HER2 binding domain is a Fab derived from pertuzumab on chain A, and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A_Y407V in Chain A, T350V_T366L_K392L_T394W in Chain B, and the hinge region of Chain B having the mutation C226S; the antigen-binding domain binds to domain 2 of HER2.

Exemplary anti-HER2 monospecific bivalent antibody controls (full-sized antibodies, FSAs) - not part of the claimed invention

[0044] v506 is a wild-type anti HER2 produced in-house in Chinese Hamster Ovary (CHO) cells, as a control. Both HER2 binding domains are derived from trastuzumab in the Fab format and the Fc is a wild type homodimer; the antigen-binding domain binds to domain 4 of HER2. This antibody is also referred to as a trastuzumab analog.

[0045] v792, is wild-type trastuzumab with a IgG1 hinge, where both HER2 binding domains are derived from trastuzumab in the Fab format, and the and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A_Y407V in Chain A, and T350V_T366L_K392L_T394W Chain B; the antigen-binding domain binds to domain 4 of HER2. This antibody is also referred to as a trastuzumab analog.

[0046] v4184, a bivalent anti-HER2 antibody, where both HER2 binding domains are derived from pertuzumab in the Fab format, and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A_Y407V in Chain A, and T350V_T366L_K392L_T394W Chain B. The antigen-binding domain binds to domain 2 of HER2. This antibody is also referred to as a pertuzumab analog.

Exemplary anti-HER2 biparatopic antibody drug conjugates (ADCs)

[0047] The following are exemplary anti-HER2 biparatopic antibody drug conjugates (anti-HER2 biparatopic-ADCs). ADCs of variants 5019, 7091, 10000 and 506 are identified as follows, wherein only ADCs of v 10000 form part of the claimed invention:

v6363 (v5019 conjugated to DM1)

v7148 (v7091 conjugated to DM1)

v10553 (v10000 conjugated to DM1)

v6246 (v506 conjugated to DM1, analogous to T-DM1, trastuzumab-emtansine)

v6249 (human IgG conjugated to DM1)

Fc of antigen-binding constructs.

[0048] The antigen-binding constructs for use according to th invention comprise a dimeric Fc. A dimeric Fc can be homodimeric or heterodimeric. The Fc according to the claimed invention is heterodimeric.

[0049] The term "Fc domain" or "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991. An "Fc polypeptide" of a dimeric Fc as used herein refers to one of the two polypeptides forming the dimeric Fc domain, i.e. a polypeptide comprising C-terminal constant regions of an immunoglobulin heavy chain, capable of stable self-association. For example, an Fc polypeptide of a dimeric IgG Fc comprises an IgG CH2 and an IgG CH3 constant domain sequence.

[0050] An Fc domain comprises either a CH3 domain or a CH3 and a CH2 domain. The CH3 domain comprises two CH3 sequences, one from each of the two Fc polypeptides of the dimeric Fc. The CH2 domain comprises two CH2 sequences, one from each of the two Fc polypeptides of the dimeric Fc.

[0051] The Fc according to the invention comprises two CH3 sequences. As defined in claim 1, the Fc is coupled, with or without one or more linkers, to a first antigen-binding construct and a second antigen-binding construct. The Fc is a human IgG1 Fc. The Fc is a heterodimeric Fc. The Fc comprises two CH2 sequences.

[0052] In some aspects, an Fc is a single polypeptide. In some aspects, an Fc is multiple peptides, e.g., two polypeptides.

[0053] An Fc is an Fc described in patent applications WO2012/058768, filed November 4, 2011 or WO2013/063702, filed November 2, 2012.

Modified CH3 Domains

[0054] The antigen-binding construct provided for use according to the invention comprises a heterodimeric Fc comprising a modified CH3 domain that has been asymmetrically modified. The heterodimeric Fc comprises two heavy chain constant domain polypeptides: a first Fc polypeptide and a second Fc polypeptide.

[0055] Two CH3 sequences that comprise one or more amino acid modifications introduced in an asymmetric fashion generally results in a heterodimeric Fc, rather than a homodimer, when the two CH3 sequences dimerize. As used herein, "asymmetric amino acid modifications" refers to any modification where an amino acid at a specific position on a first CH3 sequence is different from the amino acid on a second CH3 sequence at the same position, and the first and second CH3 sequence preferentially pair to form a heterodimer, rather than a homodimer. This heterodimerization can be a result of modification of only one of the two amino acids at the same respective amino acid position on each sequence; or modification of both amino acids on each sequence at the same respective position on each of the first and second CH3 sequences. The first and second CH3 sequence of a heterodimeric Fc can comprise one or more than one asymmetric amino acid modification.

[0056] Table A provides the amino acid sequence of the human IgG1 Fc sequence, corresponding to amino acids 231 to 447 of the full-length human IgG1 heavy chain. The CH3 sequence comprises amino acid 341-447 of the full-length human IgG1 heavy chain.

[0057] Typically an Fc can include two contiguous heavy chain sequences (A and B) that are capable of dimerizing. One or both sequences of an Fc include one or more mutations or modifications at the following locations: L351, F405, Y407, T366, K392, T394, T350, S400, and/or N390, using EU numbering. According to the claimed invention, the human IgG1 Fc region that is operatively linked to the first antigen-binding polypeptide construct comprises the mutations: T350V_L351Y_F405A_Y407V. The human IgG1 Fc region that is operatively linked to the second antigen-binding polypeptide construct comprises the mutations: T350V_T366L_K392L_T394W. Also described isan Fc includes a mutant sequence shown in Table X. One described Fc includes the mutations of Variant 1 A-B. One described Fc includes the mutations of Variant 2 A-B. One described Fc includes the mutations of Variant 3 A-B. One described Fc includes the mutations of Variant 4 A-B. One described Fc includes the mutations of Variant 5 A-B. Only the mutations present in v10000 are claimed, as noted above.

Table A: IgG1 Fc sequences

Human IgG1 Fc sequence 231-447 (EU-numbering)	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 350)

Variant IgG1 Fc sequence (231-447)	Chain	Mutations
1	A	L351Y_F405A_Y407V
1	B	T366L_K392M_T394W
2	A	L351Y_F405A_Y407V
2	B	T366L_K392L_T394W
3	A	T350V_L351Y_F405A_Y407V
3	B	T350V_T366L_K392L_T394W
4	A	T350V_L351Y_F405A_Y407V
4	B	T350V_T366L_K392M_T394W
5	A	T350V_L351Y_S400E_F405A_Y407V
5	B	T350V_T366L_N390R_K392M_T394W

[0058] A number of CH3 mutations are now described further, but are only part of the claimed invention to the extent that they describe the T350V_L351Y_F405A_Y407V // T350V_T366L_K392L_T394W mutations present in the claimed v10000 construct.

[0059] The first and second CH3 sequences can comprise amino acid mutations as described herein, with reference to amino acids 231 to 447 of the full-length human IgG1 heavy chain. In one embodiment, the heterodimeric Fc comprises a modified CH3 domain with a first CH3 sequence having amino acid modifications at positions F405 and Y407, and a second CH3 sequence having amino acid modifications at position T394. In one embodiment, the heterodimeric Fc comprises a modified CH3 domain with a first CH3 sequence having one or more amino acid modifications selected from L351Y, F405A, and Y407V, and the second CH3 sequence having one or more amino acid modifications selected from T366L, T366I, K392L, K392M, and T394W.

[0060] In one embodiment, a heterodimeric Fc comprises a modified CH3 domain with a first CH3 sequence having amino acid modifications at positions L351, F405 and Y407, and a second CH3 sequence having amino acid modifications at positions T366, K392, and T394, and one of the first or second CH3 sequences further comprising amino acid modifications at position Q347, and the other CH3 sequence further comprising amino acid modification at position K360. In another embodiment, a heterodimeric Fc comprises a modified CH3 domain with a first CH3 sequence having amino acid modifications at positions L351, F405 and Y407, and a second CH3 sequence having amino acid modifications at position T366, K392, and T394, one of the first or second CH3 sequences further comprising amino acid modifications at position Q347, and the other CH3 sequence further comprising amino acid modification at position K360, and one or both of said CH3 sequences further comprise the amino acid modification T350V.

[0061] In one embodiment, a heterodimeric Fc comprises a modified CH3 domain with a first CH3 sequence having amino acid modifications at positions L351, F405 and Y407, and a second CH3 sequence having amino acid modifications at positions T366, K392, and T394 and one of said first and second CH3 sequences further comprising amino acid modification of D399R or D399K and the other CH3 sequence comprising one or more of T411E, T411D, K409E, K409D, K392E and K392D. In another embodiment, a heterodimeric Fc comprises a modified CH3 domain with a first CH3 sequence having amino acid modifications at positions L351, F405 and Y407, and a second CH3 sequence having amino acid modifications at positions T366, K392, and T394, one of said first and second CH3 sequences further comprises amino acid modification of D399R or D399K and the other CH3 sequence comprising one or more of T411E, T411D, K409E, K409D, K392E and K392D, and one or both of said CH3 sequences further comprise the amino acid modification T350V.

[0062] In one embodiment, a heterodimeric Fc comprises a modified CH3 domain with a first CH3 sequence having amino acid modifications at positions L351, F405 and Y407, and a second CH3 sequence having amino acid modifications at positions T366, K392, and T394, wherein one or both of said CH3 sequences further comprise the amino acid modification of T350V.

[0063] In one embodiment, a heterodimeric Fc comprises a modified CH3 domain comprising the following amino acid modifications, where "A" represents the amino acid modifications to the first CH3 sequence, and "B" represents the amino acid modifications to the second CH3 sequence: A:L351Y_F405A_Y407V, **B**:T366L_K392M_T394W, **A**:L351Y_F405A_Y407V, **B**:T366L_K392L_T394W, **A**:T350V_L351Y_F405A_Y407V, **B**:T350V_T366L_K392L_T394W, **A**:T350V_L351Y_F405A_Y407V, **B**:T350V_T366L_K392M_T394W, **A**:T350V_L351Y_S400E_F405A_Y407V, and/or **B**:T350V_T366L_N390R_K392M_T394W.

[0064] The one or more asymmetric amino acid modifications can promote the formation of a heterodimeric Fc in which the heterodimeric CH3 domain has a stability that is comparable to a wild-type homodimeric CH3 domain. In an embodiment, the one or more asymmetric amino acid modifications promote the formation of a heterodimeric Fc domain in which the heterodimeric Fc domain has a stability that is comparable to a wild-type homodimeric Fc domain. In an embodiment, the one or more asymmetric amino acid modifications promote the formation of a heterodimeric Fc domain in which the heterodimeric Fc domain has a stability observed via the melting temperature (Tm) in a differential scanning calorimetry study, and where the melting temperature is within 4°C of that observed for the corresponding symmetric wild-type homodimeric Fc domain. In

some aspects, the Fc comprises one or more modifications in at least one of the C_{H3} sequences that promote the formation of a heterodimeric Fc with stability comparable to a wild-type homodimeric Fc.

[0065] In one embodiment, the stability of the CH3 domain can be assessed by measuring the melting temperature of the CH3 domain, for example by differential scanning calorimetry (DSC). Thus, in a further embodiment, the CH3 domain has a melting temperature of about 68°C or higher. In another embodiment, the CH3 domain has a melting temperature of about 70°C or higher. In another embodiment, the CH3 domain has a melting temperature of about 72°C or higher. In another embodiment, the CH3 domain has a melting temperature of about 73°C or higher. In another embodiment, the CH3 domain has a melting temperature of about 75°C or higher. In another embodiment, the CH3 domain has a melting temperature of about 78°C or higher. In some aspects, the dimerized CH3 sequences have a melting temperature (Tm) of about 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 77.5, 78, 79, 80, 81, 82, 83, 84, or 85°C or higher.

[0066] In some embodiments, a heterodimeric Fc comprising modified CH3 sequences can be formed with a purity of at least about 75% as compared to homodimeric Fc in the expressed product. In another embodiment, the heterodimeric Fc is formed with a purity greater than about 80%. In another embodiment, the heterodimeric Fc is formed with a purity greater than about 85%. In another embodiment, the heterodimeric Fc is formed with a purity greater than about 90%. In another embodiment, the heterodimeric Fc is formed with a purity greater than about 95%. In another embodiment, the heterodimeric Fc is formed with a purity greater than about 97%. In some aspects, the Fc is a heterodimer formed with a purity greater than about 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% when expressed. In some aspects, the Fc is a heterodimer formed with a purity greater than about 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% when expressed via a single cell.

[0067] Additional methods for modifying monomeric Fc polypeptides to promote heterodimeric Fc formation are described in International Patent Publication No. WO 96/027011 (knobs into holes), in Gunasekaran et al. (Gunasekaran K. et al. (2010) J Biol Chem. 285, 19637-46, electrostatic design to achieve selective heterodimerization), in Davis et al. (Davis, JH. et al. (2010) Prot Eng Des Sel ;23(4): 195-202, strand exchange engineered domain (SEED) technology), and in Labrijn et al [Efficient generation of stable bispecific IgG1 by controlled Fab-arm exchange. Labrijn AF, Meesters JI, de Goeij BE, van den Bremer ET, Neijssen J, van Kampen MD, Strumane K, Verploegen S, Kundu A, Gramer MJ, van Berkel PH, van de Winkel JG, Schuurman J, Parren PW. Proc Natl Acad Sci USA. 2013 Mar 26;110(13):5145-50.

CH2 domains

[0068] The Fc of the antigen-binding construct provided for use according to the invention comprises a CH2 domain. Several effector functions are mediated by Fc receptors (FcRs), which bind to the Fc of an antibody.

[0069] The terms "Fc receptor" and "FcR" are used to describe a receptor that binds to the Fc region of an antibody. For example, an FcR can be a native sequence human FcR. Generally, an FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Immunoglobulins of other isotypes can also be bound by certain FcRs (see, e.g., Janeway et al., Immuno Biology: the immune system in health and disease, (Elsevier Science Ltd., NY) (4th ed., 1999)). Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (reviewed in Daëron, Annu. Rev. Immunol. 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991); Capel et al., Immunomethods 4:25-34 (1994); and de Haas et al., J. Lab. Clin. Med. 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976); and Kim et al., J. Immunol. 24:249 (1994)).

[0070] Modifications in the CH2 domain can affect the binding of FcRs to the Fc. A number of amino acid modifications in the Fc region are known in the art for selectively altering the affinity of the Fc for different Fcgamma receptors. In some aspects that are described but not claimed, the Fc comprises one or more modifications to promote selective binding of Fc-gamma receptors.

[0071] Exemplary mutations, that are described but not claimed, that alter the binding of FcRs to the Fc are listed below:

[0072] S298A/E333A/K334A, S298A/E333A/K334A/K326A (Lu Y, Vernes JM, Chiang N, et al. J Immunol Methods. 2011 Feb 28;365(1-2):132-41);

[0073] F243L/R292P/Y300L/V305I/P396L, F243L/R292P/Y300L/L235V/P396L (Stavenhagen JB, Gorlatov S, Tuaillon N, et al. Cancer Res. 2007 Sep 15;67(18):8882-90; Nordstrom JL, Gorlatov S, Zhang W, et al. Breast Cancer Res. 2011 Nov 30; 13(6):R123);

[0074] F243L (Stewart R, Thom G, Levens M, et al. Protein Eng Des Sel. 2011 Sep;24(9):671-8.), S298A/E333A/K334A (Shields RL, Namenuk AK, Hong K, et al. J Biol Chem. 2001 Mar 2;276(9):6591-604);

[0075] S239D/I332E/A330L, S239D/I332E (Lazar GA, Dang W, Karki S, et al. Proc Natl Acad Sci USA. 2006 Mar 14;103(11):4005-10);

[0076] S239D/S267E, S267E/L328F (Chu SY, Vostiar I, Karki S, et al. Mol Immunol. 2008 Sep;45(15):3926-33);

[0077] S239D/D265S/S298A/I332E, S239E/S298A/K326A/A327H, G237F/S298A/A33 0L/I332E, S239D/I332E/S298A, S239D/K326E/A330L/I332E/S298A, G236A/S239D/D270L/I3 32E, S239E/S267E/H268D, L234F/S267E/N325L, G237F/V266L/S267D and other mutations listed in WO2011/120134 and WO2011/120135. *Therapeutic Antibody Engineering* (by William R. Strohl and Lila M. Strohl, Woodhead Publishing series in Biomedicine No 11, ISBN 1 907568 37 9, Oct 2012) lists mutations on page 283.

[0078] In some embodiments an antigen-binding construct described herein comprises an antigen-binding polypeptide construct which binds an antigen; and a dimeric Fc that has superior biophysical properties like stability and ease of manufacture relative to an antigen-binding construct which does not include the same dimeric Fc. In some embodiments a CH2 domain comprises one or more asymmetric amino acid modifications. Exemplary asymmetric mutations are described in International Patent Application Publication No. WO2014/190441.

Additional modifications to improve effector function.

[0079] An antigen-binding construct described herein may include modifications to improve its ability to mediate effector function. Such modifications are known in the art and include afucosylation, or (described but not claimed) engineering of the affinity of the Fc towards an activating receptor, mainly FCGR3a for ADCC, and towards C1q for CDC. The following Table B summarizes various designs reported in the literature for effector function engineering.

[0080] Methods of producing antigen-binding constructs with little or no fucose on the Fc glycosylation site (Asn 297 EU numbering) without altering the amino acid sequence are well known in the art. The GlymaX® technology (ProBioGen AG) is based on the introduction of a gene for an enzyme which deflects the cellular pathway of fucose biosynthesis into cells used for antigen-binding construct production. This prevents the addition of the sugar "fucose" to the N-linked antibody carbohydrate part by antigen-binding construct-producing cells. (von Horsten et al. (2010) Glycobiology. 2010 Dec; 20 (12):1607-18. Another approach to obtaining antigen-binding constructs with lowered levels of fucosylation can be found in U.S. patent 8,409,572, which teaches selecting cell lines for antigen-binding construct production for their ability to yield lower levels of fucosylation on antigen-binding constructs Antigen-binding constructs can be fully afucosylated (meaning they contain no detectable fucose) or they can be partially afucosylated, meaning that the isolated antibody contains less than 95%, less than 85%, less than 75%, less than 65%, less than 55%, less than 45%, less than 35%, less than 25%, less than 15% or less than 5% of the amount of fucose normally detected for a similar antibody produced by a mammalian expression system.

[0081] Thus, an antigen-binding construct described herein (but not claimed) can include a dimeric Fc that comprises one or more amino acid modifications as noted in Table B that confer improved effector function. In another embodiment, the antigen-binding construct can be afucosylated to improve effector function.

Table B: CH2 domains and effector function engineering.

Reference	Mutations	Effect

hinge or CH2 regions of the Fc. For example, US Patent Publication No. 2011/0212087 (Strohl), International Patent Publication No. WO 2006/105338 (Xencor), US Patent Publication No. 2012/0225058 (Xencor), US Patent Publication No. 2012/0251531 (Genentech), and Strop et al ((2012) J. Mol. Biol. 420: 204-219) describe specific modifications to reduce FcyR or complement binding to the Fc.

[0082] Specific, non-limiting examples of known amino acid modifications to reduce FcyR or complement binding to the Fc include those identified in the following table:

Table C: modifications to reduce FcyR or complement binding to the Fc

Company	Mutations

digestion, albeit in the context of an antigen-binding construct with 2 antigen-binding polypeptide constructs.

[0083] The linker polypeptides maintain the relative spatial conformation of the paratopes of a F(ab') fragment, and are capable of forming a covalent bond equivalent to the disulphide bond in the core hinge of IgG. The linker polypeptides according to the invention are IgG hinge regions from IgG1. Modified versions of these exemplary linkers are known but not claimed. For example, modifications to improve the stability of the IgG4 hinge are known in the art (see for example, Labrijn et al. (2009) Nature Biotechnology 27, 767 - 771).

[0084] The linker polypeptides are operatively linked to a scaffold as described here, which is a human IgG1Fc.

[0085] It is also described, but not claimed, that the linker polypeptides can be operatively linked to scaffolds other than an Fc. A number of alternate protein or molecular domains are know in the art and can be used to form selective pairs of two different antigen-binding polypeptides. An example is the leucine zipper domains such as Fos and Jun that selectively pair together [S A Kostelny, M S Cole, and J Y Tso. Formation of a bispecific antibody by the use of leucine zippers. J Immunol 1992 148:1547-53; Bernd J. Wranik, Erin L. Christensen, Gabriele Schaefer, Janet K. Jackman, Andrew C. Vendel, and Dan Eaton. LUZ-Y, a Novel Platform for the Mammalian Cell Production of Full-length IgG-bispecific AntibodiesJ. Biol. Chem. 2012 287: 43331-43339]. Alternately, other selectively pairing molecular pairs such as the barnase barstar pair [Deyev, S. M., Waibel, R., Lebedenko, E. N., Schubiger, A. P., and Plückthun, A. (2003). Design of multivalent complexes using the barnase*barstar module. Nat Biotechnol 21, 1486-1492], DNA strand pairs [Zahida N. Chaudri, Michael Bartlet-Jones, George Panayotou, Thomas Klonisch, Ivan M. Roitt, Torben Lund, Peter J. Delves, Dual specificity antibodies using a double-stranded oligonucleotide bridge, FEBS Letters, Volume 450, Issues 1-2, 30 April 1999, Pages 23-26], split fluorescent protein pairs [Ulrich Brinkmann, Alexander Haas. Fluorescent antibody fusion protein, its production and use, WO 2011135040 A1] can also be employed.

Affinity

[0086] In some embodiments, affinity is determined by SPR (surface plasmon resonance) and/or FACS (fluorescence activated cell sorting). In some embodiments, affinity is determined by SPR and/or FACS as described below.

Dissociation constant (K_D) and maximal binding (Bmax)

[0087] In some embodiments, an antigen-binding construct is described by functional characteristics including but not limited to a dissociation constant and a maximal binding.

[0088] The term "dissociation constant (K_D)" as used herein, is intended to refer to the equilibrium dissociation constant of a particular ligand-protein interaction. As used herein, ligand-protein interactions refer to, but are not limited to protein-protein interactions or antibody-antigen interactions. The K_D measures the propensity of two proteins (e.g. AB) to dissociate reversibly into smaller components (A+B), and is define as the ratio of the rate of dissociation, also called the "off-rate (k_{off})", to the association rate, or "on-rate (k_{on})". Thus, K_D equals k_{off}/k_{on} and is expressed as a molar concentration (M). It follows that the smaller the K_D, the stronger the affinity of binding. Therefore, a K_D of 1 mM indicates weak binding affinity compared to a K_D of 1 nM. K_D values for antigen-binding constructs can be determined using methods well established in the art. One method for determining the K_D of an antigen-binding construct is by using surface plasmon resonance (SPR), typically using a biosensor system such as a Biacore® system. Isothermal titration calorimetry (ITC) is another method that can be used to determine.

[0089] The binding characteristics of an antigen-binding construct can be determined by various techniques. One of which is the measurement of binding to target cells expressing the antigen by flow cytometry (FACS, Fluorescence-activated cell sorting). Typically, in such an experiment, the target cells expressing the antigen of interest are incubated with antigen-binding constructs at different concentrations, washed, incubated with a secondary agent for detecting the antigen-binding construct, washed, and analyzed in the flow cytometer to measure the median fluorescent intensity (MFI) representing the strength of detection signal on the cells, which in turn is related to the number of antigen-binding constructs bound to the cells. The antigen-binding construct concentration vs. MFI data is then fitted into a saturation binding equation to yield two key binding parameters, Bmax and apparent K_D.

[0090] Apparent K_D, or apparent equilibrium dissociation constant, represents the antigen-binding construct concentration at which half maximal cell binding is observed. Evidently, the smaller the K_D value, the smaller antigen-binding construct concentration is required to reach maximum cell binding and thus the higher is the affinity of the antigen-binding construct. The apparent K_D is dependent on the conditions of the cell binding experiment, such as different receptor levels expressed on the cells and incubation conditions, and thus the apparent K_D is generally different from the K_D values determined from cell-free molecular experiments such as SPR and ITC. However, there is generally good agreement between the different methods.

[0091] The term "Bmax", or maximal binding, refers to the maximum antigen-binding construct binding level on the cells at saturating concentrations of antigen-binding construct. This parameter can be reported in the arbitrary unit MFI for relative comparison, or converted into an absolute value corresponding to the number of antigen-binding constructs bound to the cell with the use of a standard curve.

Testing of antigen-binding constructs: HER2 binding

[0092] The antigen-binding constructs or pharmaceutical compositions described herein are tested in vitro, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, *in vitro* assays which can be used to determine whether administration of a specific antigen-binding construct is indicated, include in vitro cell culture assays, or *in vitro* assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered antigen-binding construct, and the effect of such antigen-binding construct upon the tissue sample is observed.

[0093] Candidate antigen-binding constructs can be assayed using cells, e.g., breast cancer cell lines, expressing HER2. The following Table D describes the expression level of HER2 in several representative cancer cell lines.

Table D - Relative expression levels of HER2 in cell lines of interest.

Cell Line	Description	IHC scoring	HER2 receptors/cell
NCI-N87	Human gastric carcinoma	3+	Not assessed
A549	Human lung alveolar carcinoma (non-small cell lung cancer)	0/1+	Not assessed
BxPC-3	Human pancreatic adenocarcinoma	1+	Not assessed
MIA PaCa-2	Human pancreatic ductal adenocarcinoma	2+	Not assessed
FaDu	Human pharyngeal squamous cell carcinoma	2+	Not assessed
HCT-116	Human colorectal epithelial carcinoma	1+	Not assessed
WI-38	Normal fetal lung	0	1.0x10E4
MDA-MB-231	Human triple negative breast epithelial adenocarcinoma	0/1+	1.7x10E4 - 2.3x10E4
MCF-7	Human estrogen receptor positive breast epithelial adenocarcinoma	1+	4x10E4 - 7x10E4
JIMT-1	Trastuzumab resistant breast epithelial carcinoma, amplified HER2 oncogene, insensitive to HER2-inhibiting drugs (*i.e.* Herceptin™)	2+	2x10E5 - 8x10E5
ZR-75-1	Estrogen receptor positive breast ductal carcinoma	2+	3x10E5

Cell Line	Description	IHC scoring	HER2 receptors/cell
SKOV-3	Human ovarian epithelial adenocarcinoma, HER2 gene amplified	2/3+	5x10E5 - 1x10E6
SK-BR-3	Human breast epithelial adenocarcinoma	3+	> 1x10E6
BT-474	Human breast epithelial ductal carcinoma,	3+	> 1x10E6

[0094] McDonagh et al Mol Cancer Ther. 2012 Mar;11(3):582-93; Subik et al. (2010) Breast Cancer: Basic Clinical Research:4; 35-41; Carter et al. PNAS, 1994:89;4285-4289; Yarden 2000, HER2: Basic Research, Prognosis and Therapy; Hendricks et al Mol Cancer Ther 2013; 12: 1816-28.

[0095] As is known in the art, a number of assays may be employed in order to identify antigen-binding constructs suitable for use in the methods described herein. These assays can be carried out in cancer cells expressing HER2. Examples of suitable cancer cells are identified in Table A5. Examples of assays that may be carried out are described as follows.

[0096] For example, to identify growth inhibitory candidate antigen-binding constructs that bind HER2, one may screen for antibodies which inhibit the growth of cancer cells which express HER2. In one embodiment, the candidate antigen-binding construct of choice is able to inhibit growth of cancer cells in cell culture by about 20-100% and preferably by about 50-100% at compared to a control antigen-binding construct.

[0097] To select for candidate antigen-binding constructs which induce cell death, loss of membrane integrity as indicated by, e.g., PI (phosphatidylinositol), trypan blue or 7AAD uptake may be assessed relative to control.

[0098] In order to select for candidate antigen-binding constructs which induce apoptosis, an annexin binding assay may be employed. In addition to the annexin binding assay, a DNA staining assay may also be used.

[0099] In one embodiment, the candidate antigen-binding construct of interest may block heregulin dependent association of ErbB2 with ErbB3 in both MCF7 and SK-BR-3 cells as determined in a co-immunoprecipitation experiment substantially more effectively than monoclonal antibody 4D5, and preferably substantially more effectively than monoclonal antibody 7F3.

[0100] To screen for antigen-binding constructs which bind to an epitope on ErbB2 bound by an antibody of interest, 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. Alternatively, or additionally, epitope mapping can be performed by methods known in the art.

[0101] Competition between antigen-binding constructs can be determined by an assay in which an antigen-binding construct under test inhibits or blocks specific binding of a reference antigen-binding construct to a common antigen (see, e.g., Junghans et al., Cancer Res. 50:1495, 1990; Fendly et al. Cancer Research 50: 1550-1558; US 6,949,245). A test antigen-binding construct competes with a reference antigen-binding construct if an excess of a test antigen-binding construct (e.g., at least 2x, 5x, 10x, 20x, or 100x) inhibits or blocks binding of the reference antigen-binding construct by, e.g., at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% as measured in a competitive binding assay. Antigen-binding constructs identified by competition assay (competing antigen-binding construct) include antigen-binding constructs binding to the same epitope as the reference antigen-binding construct and antigen-binding constructs binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antigen-binding construct for steric hindrance to occur. For example, a second, competing antigen-binding construct can be identified that competes for binding to HER2 with a first antigen-binding construct described herein. In certain instances, the second construct can block or inhibit binding of the first construct by, e.g., at least 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% as measured in a competitive binding assay. In certain instances, the second construct can displace the first construct by greater than 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%.

[0102] In some embodiments, antigen-binding constructs described herein are assayed for function in vivo, e.g., in animal models. In some embodiments, the animal models are those described in Table E. In some embodiments, the animal models are those described in the Examples. In some embodiments, the antigen-binding constructs display an increase in efficacy of treatment in an animal model compared to a reference antigen-binding construct.

Table E: Animal models for testing HER2 binding antigen-binding constructs

Xenograft Model	Description	Reference
SKOV3 human ovarian cancer	HER2+/3+, gene amplified, moderately sensitive to trastuzumab	Rhodes et al. 2002. American Journal of Pathology 118:408-417; Sims et al. 2012. British Journal of Cancer 106: 1779-1789
HBCx-13b human metastatic breast cancer	HER2 3+, estrogen receptor negative, progesterone receptor negative ; Invasive ductal breast carcinoma; Chemotherapy resistant, Trastuzumab resistant	Marangoni et al. 2007. Clinical Cancer Research 13:3989-3998; Reyal et al. 2012. Breast Cancer Research 14:R11
T226 human breast cancer	HER2 3+, estrogen receptor negative, progesterone receptor negative; Inflammatory breast cancer; Trastuzumab resistant, Docetaxel and capecitabine moderately sensitive, Adriamycin/cyclophosphamide sensitive	
HBCx-5 human breast cancer	HER2 3+, estrogen receptor negative, progesterone receptor negative; Invasive ductal carcinoma, luminal B; Trastuzumab resistant, Docetaxel moderately sensitive, Capecitabine, Adriamycin/Cyclophosphamide sensitive	Marangoni et al. 2007. Clinical Cancer Research 13:3989-3998; Reyal et al. 2012. Breast Cancer Research 14:R11
JIMT-1 human	HER2 2+, HER2 gene amplified, Trastuzumab and pertuzumab	Tanner et al. 2004. Molecular Cancer

Xenograft Model	Description	Reference
breast cancer	resistant	Therapeutics 3: 1585-1592

Reference antigen-binding construct

[0103] In some embodiments, the functional characteristics of the antigen-binding constructs described herein are compared to those of a reference antigen-binding construct. The identity of the reference antigen-binding construct depends on the functional characteristic being measured or the distinction being made. For example, when comparing the functional characteristics of antigen-binding constructs described herein, the reference antigen-binding construct may be a trastuzumab (for example v6336), or analog thereof, or may be a control IgG, for example a non-specific polyclonal human antibody.

Antigen-binding constructs and antibody drug conjugates (ADC)

[0104] In certain embodiments an antigen-binding construct is conjugated to a drug, e.g., a toxin, a chemotherapeutic agent, an immune modulator, or a radioisotope. Several methods of preparing ADCs (antibody drug conjugates or antigen-binding construct drug conjugates) are known in the art and are described below.

[0105] In some embodiments, the drug is selected from a maytansine, auristatin, calicheamicin, or derivative thereof. In other embodiments, the drug is a maytansine selected from DM1 and DM4. Further examples are described below.

[0106] In some embodiments the drug is conjugated to the isolated antigen-binding construct with an SMCC linker (DM1), or an SPDB linker (DM4). Additional examples are described below. The drug-to-antigen-binding protein ratio (DAR) can be, e.g., 1.0 to 6.0 or 3.0 to 5.0 or 3.5-4.2.

[0107] In some embodiments the antigen-binding construct is conjugated to a cytotoxic agent. The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, and Lu177), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof. Further examples are described below.

Drugs

[0108] Non-limiting examples of drugs or payloads used in various embodiments of ADCs include DM1 (maytansine, N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)- or N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)-maytansine), mc-MMAD (6-maleimidocaproyl-monomethylauristatin-D or N-methyl-L-valyl-N-[(1S,2R)-2-methoxy-4-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[[(1S)-2-phenyl-1-(2-thiazolyl)ethyl]amino]propyl]-1-pyr rolidinyl]-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-(9Cl)-L-valinamide), mc-MMAF (maleimidocaproyl-monomethylauristatin F or N-[6-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-1-oxohexyl]-N-methyl-L-valyl-L-valyl-(3R,4S,5S)-3-methoxy-5-methyl-4-(methylamino)heptanoyl-(αR, βR,2S)-β-methoxy-α-methyl-2-pyrrolidinepropanoyl-L-phenylalanine) and mc-Val-Cit-PABA-MMAE (6-maleimidocaproyl-ValcCit-(p-aminobenzyloxycarbonyl)-monomethylauristatin E or N-[[[4-[[N-[6-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-1-oxohexyl]-L-valyl-N5-(aminocarbonyl)-L-ornithyl]amino]phenyl]methoxy]carbonyl]-N-methyl-L-valyl-N-[(1S,2R)-4-[(2S)-2-[(1R,2R)-3-[[(1R,2S)-2-hydroxy-1-methyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]-1-pyrrolidinyl] -2-methoxy-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-L-valinamide). DM1 is a derivative of the tubulin inhibitor maytansine while MMAD, MMAE, and MMAF are auristatin derivatives.

Maytansinoid Drug Moieties

[0109] As indicated above, in some embodiments the drug is a maytansinoid. Exemplary maytansinoids include DM1, DM3 ($N^{2'}$-deacetyl-$N^{2'}$-(4-mercapto-1-oxopentyl) maytansine), and DM4 ($N^{2'}$-deacetyl-$N^{2'}$-(4-methyl-4-mercapto-1-oxopentyl)methylmaytansine) (see US20090202536).

[0110] Many positions on maytansine compounds are known to be useful as the linkage position, depending upon the type of link. For example, for forming an ester linkage, the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group and the C-20 position having a hydroxyl group are all suitable.

[0111] All stereoisomers of the maytansinoid drug moiety are contemplated for the ADCs described herein, i.e. any combination of R and S configurations at the chiral carbons of D.

Auristatins

[0112] In some embodiments, the drug is an auristatin, such as auristatin E (also known in the art as a derivative of dolastatin-10) or a derivative thereof. The auristatin can be, for example, an ester formed between auristatin E and a keto acid. For example, auristatin E can be reacted with paraacetyl benzoic acid or benzoylvaleric acid to produce AEB and AEVB, respectively. Other typical auristatins include AFP, MMAF, and MMAE. The synthesis and structure of exemplary auristatins are described in U.S. Pat. Nos. 6,884,869, 7,098,308, 7,256,257, 7,423,116, 7,498,298 and

7,745,394.

Chemotherapeutic agents

[0113] In some embodiments the antigen-binding construct is conjugated to a chemotherapeutic agent. Examples include but are not limited to Cisplantin and Lapatinib. A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer.

[0114] Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, trietylenephosphoramide, triethylenethiophosphaoramide and trimethylolomelamine; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK7; razoxane; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2',2'=-trichlorotriethylamine; urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxanes, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, N.J.) and doxetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; esperamicins; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

Conjugate Linkers

[0115] In some embodiments, the drug is linked to the antigen-binding construct, e.g., antibody, by a linker. Attachment of a linker to an antibody can be accomplished in a variety of ways, such as through surface lysines, reductive-coupling to oxidized carbohydrates, and through cysteine residues liberated by reducing interchain disulfide linkages. A variety of ADC linkage systems are known in the art, including hydrazone-, disulfide- and peptide-based linkages.

[0116] Suitable linkers include, for example, cleavable and non-cleavable linkers. A cleavable linker is typically susceptible to cleavage under intracellular conditions. Suitable cleavable linkers include, for example, a peptide linker cleavable by an intracellular protease, such as lysosomal protease or an endosomal protease. In exemplary embodiments, the linker can be a dipeptide linker, such as a valine-citrulline (val-cit), a phenylalanine-lysine (phe-lys) linker, or maleimidocapronic-valine-citruline-p-aminobenzyloxycarbonyl (mc-Val-Cit-PABA) linker. Another linker is Sulfosuccinimidyl-4-(N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC). Sulfo-smcc conjugation occurs via a maleimide group which reacts with sulfhydryls (thiols, - SH), while its Sulfo-NHS ester is reactive toward primary amines (as found in Lysine and the protein or peptide N-terminus). Yet another linker is maleimidocaproyl (MC). Other suitable linkers include linkers hydrolyzable at a specific pH or a pH range, such as a hydrazone linker. Additional suitable cleavable linkers include disulfide linkers. The linker may be covalently bound to the antibody to such an extent that the antibody must be degraded intracellularly in order for the drug to be released e.g. the MC linker and the like.

Preparation of ADCs

[0117] The ADC may be prepared by several routes, employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group or an electrophilic group of an antibody with a bivalent linker reagent, to form antibody-linker intermediate Ab-L, via a covalent bond, followed by reaction with an activated drug moiety D; and (2) reaction of a nucleophilic group or an electrophilic group of a drug moiety with a linker reagent, to form drug-linker intermediate D-L, via a covalent bond, followed by reaction with the nucleophilic group or an electrophilic group of an antibody. Conjugation methods (1) and (2) may be employed with a variety of antibodies, drug moieties, and linkers to prepare the antibody-drug conjugates described here.

[0118] Several specific examples of methods of preparing ADCs are known in the art and are described in US patents 8,624,003 (pot method), 8,163,888 (one-step), and 5,208,020 (two-step method).

Methods of Preparation of Antigen-binding constructs

[0119] Antigen-binding constructs described herein may be produced using recombinant methods and compositions, e.g., as described in U.S. Pat. No. 4,816,567.

[0120] In one embodiment, isolated nucleic acid encoding an antigen-binding construct described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antigen-binding construct (e.g., the light and/or heavy chains of the antigen-binding construct). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In one embodiment, the nucleic acid is provided in a multicistronic vector. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antigen-binding construct and an amino acid sequence comprising the VH of the antigen-binding polypeptide construct, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antigen-binding polypeptide construct and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antigen-binding polypeptide construct. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster Ovary (CHO) cell, or human embryonic kidney (HEK) cell, or lymphoid cell (e.g., Y0, NS0, Sp20 cell). In one embodiment, a method of making an antigen-binding construct is provided, wherein the method comprises culturing a host cell comprising nucleic acid encoding the antigen-binding construct, as provided above, under conditions suitable for expression of the antigen-binding construct, and optionally recovering the antigen-binding construct from the host cell (or host cell culture medium).

[0121] For recombinant production of the antigen-binding construct, nucleic acid encoding an antigen-binding construct, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antigen-binding construct).

[0122] The term "substantially purified" refers to a construct described herein, or variant thereof that may be substantially or essentially free of components that normally accompany or interact with the protein as found in its naturally occurring environment, i.e. a native cell, or host cell in the case of recombinantly produced heteromultimer that in certain embodiments, is substantially free of cellular material includes preparations of protein having less than about 30%, less than about 25%, less than about 20%, less than about 15%, less than about 10%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% (by dry weight) of contaminating protein. When the heteromultimer or variant thereof is recombinantly produced by the host cells, the protein in certain embodiments is present at about 30%, about 25%, about 20%, about 15%, about 10%, about 5%, about 4%, about 3%, about 2%, or about 1% or less of the dry weight of the cells. When the heteromultimer or variant thereof is recombinantly produced by the host cells, the protein, in certain embodiments, is present in the culture medium at about 5 g/L, about 4 g/L, about 3 g/L, about 2 g/L, about 1 g/L, about 750 mg/L, about 500 mg/L, about 250 mg/L, about 100 mg/L, about 50 mg/L, about 10 mg/L, or about 1 mg/L or less of the dry weight of the cells. In certain embodiments, "substantially purified" heteromultimer produced by the methods described herein, has a purity level of at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, specifically, a purity level of at least about 75%, 80%, 85%, and more specifically, a purity level of at least about 90%, a purity level of at least about 95%, a purity level of at least about 99% or greater as determined by appropriate methods such as SDS/PAGE analysis, RP-HPLC, SEC, and capillary electrophoresis.

[0123] Suitable host cells for cloning or expression of antigen-binding construct-encoding vectors include prokaryotic or eukaryotic cells described herein.

[0124] A "recombinant host cell" or "host cell" refers to a cell that includes an exogenous polynucleotide, regardless of the method used for insertion, for example, direct uptake, transduction, f-mating, or other methods known in the art to create recombinant host cells. The exogenous polynucleotide may be maintained as a nonintegrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

[0125] As used herein, the term "eukaryote" refers to organisms belonging to the phylogenetic domain Eucarya such as animals (including but not limited to, mammals, insects, reptiles, birds, etc.), ciliates, plants (including but not limited to, monocots, dicots, algae, etc.), fungi, yeasts, flagellates, microsporidia, protists, etc.

[0126] As used herein, the term "prokaryote" refers to prokaryotic organisms. For example, a non-eukaryotic organism can belong to the Eubacteria (including but not limited to, Escherichia coli, Thermus thermophilus, Bacillus stearothermophilus, Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas putida, etc.) phylogenetic domain, or the Archaea (including but not limited to, Methanococcus j annaschii, Methanobacterium thermoautotrophicum, Halobacterium such as Haloferax volcanii and Halobacterium species NRC-1, Archaeoglobus fulgidus, Pyrococcus furiosus, Pyrococcus horikoshii, Aeuropyrum pernix, etc.) phylogenetic domain.

[0127] For example, antigen-binding construct may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antigen-binding construct fragments and polypeptides in bacteria, see, e.g., U.S. Pat. Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antigen-binding construct may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0128] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antigen-binding construct-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antigen-binding construct with a partially or fully human glycosylation pattern. See Gerngross, Nat. Biotech. 22:1409-1414 (2004), and Li et al., Nat. Biotech. 24:210-215 (2006).

[0129] Suitable host cells for the expression of glycosylated antigen-binding constructs are also derived from multicellular organisms (invertebrates

and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodopterafrugiperda* cells.

[0130] Plant cell cultures can also be utilized as hosts. See, e.g., U.S. Pat. Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antigen-binding constructs in transgenic plants).

[0131] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, e.g., in Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including $DHFR^-$ CHO cells (Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antigen-binding construct production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, N.J.), pp. 255-268 (2003).

[0132] In one embodiment, the antigen-binding constructs described herein are produced in stable mammalian cells, by a method comprising: transfecting at least one stable mammalian cell with: nucleic acid encoding the antigen-binding construct, in a predetermined ratio; and expressing the nucleic acid in the at least one mammalian cell. In some embodiments, the predetermined ratio of nucleic acid is determined in transient transfection experiments to determine the relative ratio of input nucleic acids that results in the highest percentage of the antigen-binding construct in the expressed product.

[0133] In some embodiments is the method of producing a antigen-binding construct in stable mammalian cells as described herein wherein the expression product of the at least one stable mammalian cell comprises a larger percentage of the desired glycosylated antigen-binding construct as compared to the monomeric heavy or light chain polypeptides, or other antibodies.

[0134] In some embodiments is the method of producing a glycosylated antigen-binding construct in stable mammalian cells described herein, said method comprising identifying and purifying the desired glycosylated antigen-binding construct. In some embodiments, the said identification is by one or both of liquid chromatography and mass spectrometry.

[0135] If required, the antigen-binding constructs can be purified or isolated after expression. Proteins may be isolated or purified in a variety of ways known to those skilled in the art. Standard purification methods include chromatographic techniques, including ion exchange, hydrophobic interaction, affinity, sizing or gel filtration, and reversed-phase, carried out at atmospheric pressure or at high pressure using systems such as FPLC and HPLC. Purification methods also include electrophoretic, immunological, precipitation, dialysis, and chromatofocusing techniques. Ultrafiltration and diafiltration techniques, in conjunction with protein concentration, are also useful. As is well known in the art, a variety of natural proteins bind Fc and antibodies, and these proteins can find use in the present invention for purification of antigen-binding constructs. For example, the bacterial proteins A and G bind to the Fc region. Likewise, the bacterial protein L binds to the Fab region of some antibodies. Purification can often be enabled by a particular fusion partner. For example, antibodies may be purified using glutathione resin if a GST fusion is employed, Ni^{+2} affinity chromatography if a His-tag is employed, or immobilized anti-flag antibody if a flag-tag is used. For general guidance in suitable purification techniques, see, e.g. Protein Purification: Principles and Practice, 3rd Ed., Scopes, Springer-Verlag, NY, 1994. The degree of purification necessary will vary depending on the use of the antigen-binding constructs. In some instances no purification is necessary.

[0136] In certain embodiments the antigen-binding constructs are purified using Anion Exchange Chromatography including, but not limited to, chromatography on Q-sepharose, DEAE sepharose, poros HQ, poros DEAF, Toyopearl Q, Toyopearl QAE, Toyopearl DEAE, Resource/Source Q and DEAE, Fractogel Q and DEAE columns.

[0137] In specific embodiments the proteins described herein are purified using Cation Exchange Chromatography including, but not limited to, SP-sepharose, CM sepharose, poros HS, poros CM, Toyopearl SP, Toyopearl CM, Resource/Source S and CM, Fractogel S and CM columns and their equivalents and comparables.

[0138] In addition, antigen-binding constructs described herein can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W. H. Freeman & Co., N.Y and Hunkapiller et al., Nature, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4diaminobutyric acid, alpha-amino isobutyric acid, 4aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, □-alanine, fluoro-amino acids, designer amino acids such as □-methyl amino acids, CO-methyl amino acids, N□-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

Post-translational modifications:

[0139] In certain embodiments antigen-binding constructs described herein are differentially modified during or after translation.

[0140] The term "modified," as used herein refers to any changes made to a given polypeptide, such as changes to the length of the polypeptide,

the amino acid sequence, chemical structure, co-translational modification, or post-translational modification of a polypeptide. The form "(modified)" term means that the polypeptides being discussed are optionally modified, that is, the polypeptides under discussion can be modified or unmodified.

[0141] The term "post-translationally modified" refers to any modification of a natural or non-natural amino acid that occurs to such an amino acid after it has been incorporated into a polypeptide chain. The term encompasses, by way of example only, co-translational in vivo modifications, co-translational in vitro modifications (such as in a cell-free translation system), post-translational in vivo modifications, and post-translational in vitro modifications.

[0142] In some embodiments, the modification is at least one of: glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage and linkage to an antibody molecule or antigen-binding construct or other cellular ligand. In some embodiments, the antigen-binding construct is chemically modified by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; and metabolic synthesis in the presence of tunicamycin.

[0143] Additional post-translational modifications of antigen-binding constructs described herein include, for example, N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The antigen-binding constructs described herein are modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein. In certain embodiments, examples of suitable enzyme labels include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine, carbon, sulfur, tritium, indium, technetium, thallium, gallium, palladium, molybdenum, xenon, fluorine.

[0144] In specific embodiments, antigen-binding constructs described herein are attached to macrocyclic chelators that associate with radiometal ions.

[0145] In some embodiments, the antigen-binding constructs described herein are modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. In certain embodiments, the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. In certain embodiments, polypeptides from antigen-binding constructs described herein are branched, for example, as a result of ubiquitination, and in some embodiments are cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides are a result from posttranslation natural processes or made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent crosslinks, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS--STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

[0146] In certain embodiments, antigen-binding constructs described herein are attached to solid supports, which are particularly useful for immunoassays or purification of polypeptides that are bound by, that bind to, or associate with proteins described herein. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Pharmaceutical compositions

[0147] Also provided herein are pharmaceutical compositions comprising an antigen-binding construct described herein. Pharmaceutical compositions comprise the construct and a pharmaceutically acceptable carrier.

[0148] The term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. In some aspects, the carrier is a man-made carrier not found in nature. Water can be used as a carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper

administration to the patient. The formulation should suit the mode of administration.

[0149] In certain embodiments, the composition comprising the construct is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0150] In certain embodiments, the compositions described herein are formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxide isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Treatment: Medical uses

[0151] In this section, and throughout, references to treatments and methods of treatment refers to the construct for use in that treatment or method. Methods of treatment are not claimed.

[0152] Provided is a method of treating a disease or disorder comprising administering to a subject in which such treatment, prevention or amelioration is desired, an antigen-binding construct described herein, in an amount effective to treat, prevent or ameliorate the disease or disorder.

[0153] The invention provides the antigen binding construct (i.e. V10000) for use in a method of inhibiting tumor growth in a human subject, wherein the human subject is administered at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 doses of the antigen binding construct, and/or wherein each dose is administered at least every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days. More general description is provided below for reference, but treatments other than to inhibit tumor growth in a human subject with the claimed dose administration are not part of the claimed invention.

[0154] "Disorder" refers to any condition that would benefit from treatment with an antigen-binding construct or method described herein. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. In some embodiments, the disorder is cancer, as described in more detail below.

[0155] The term "subject" refers to an animal, in some embodiments a mammal, which is the object of treatment, observation or experiment. An animal may be a human, a non-human primate, a companion animal (e.g., dogs, cats, and the like), farm animal (e.g., cows, sheep, pigs, horses, and the like) or a laboratory animal (e.g., rats, mice, guinea pigs, and the like).

[0156] The term "mammal" as used herein includes but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.

[0157] "Treatment" refers to clinical intervention in an attempt to alter the natural course of the individual or cell being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include preventing occurrence or recurrence of disease, alleviation of symptoms, diminishing of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antigen-binding constructs described herein are used to delay development of a disease or disorder. In one embodiment, antigen-binding constructs and methods described herein effect tumor regression. In one embodiment, antigen-binding constructs and methods described herein effect inhibition of tumor/cancer growth.

[0158] Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, improved survival, and remission or improved prognosis. In some embodiments, antigen-binding constructs described herein are used to delay development of a disease or to slow the progression of a disease.

[0159] The term "effective amount" as used herein refers to that amount of construct being administered, which will accomplish the goal of the recited method, e.g., relieve to some extent one or more of the symptoms of the disease, condition or disorder being treated. The amount of the composition described herein which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a therapeutic protein can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses are extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0160] The antigen-binding construct is administered to the subject. Various delivery systems are known and can be used to administer an antigen-binding construct formulation described herein, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal,

intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, in certain embodiments, it is desirable to introduce the antigen-binding construct compositions described herein into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0161] In a specific embodiment, it is desirable to administer the antigen-binding constructs, or compositions described herein locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antigen-binding construct, described herein, care must be taken to use materials to which the protein does not absorb.

[0162] In another embodiment, the antigen-binding constructs or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249: 1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

[0163] In yet another embodiment, the antigen-binding constructs or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J. Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, vol. 2, pp. 115-138 (1984)).

[0164] In a specific embodiment comprising a nucleic acid encoding antigen-binding constructs decribed herein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0165] In certain embodiments an antigen-binding construct described herein is administered as a combination with antigen-binding constructs with non-overlapping binding target epitopes.

[0166] The amount of the antigen-binding construct which will be effective in the treatment, inhibition and prevention of a disease or disorder can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses are extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0167] The antigen-binding constructs described herein may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in an embodiment, human antigen-binding constructs, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

Methods of treating cancers

[0168] Described herein are methods of treating a HER2+ cancer or a tumor in a subject, and methods of inhibiting the growth of a HER2+ tumor cell or killing a HER2+ tumor cell using the antigen-binding constructs described herein.

[0169] By a HER2+ cancer is meant a cancer that expresses HER2 such that the antigen-binding constructs described herein are able to bind to the cancer. As is known in the art, HER2+ cancers express HER2 at varying levels. To determine ErbB, e.g. ErbB2 (HER2) expression in the cancer, various diagnostic/prognostic assays are available. In one embodiment, ErbB2 overexpression may be analyzed by IHC, e.g. using the HERCEPTEST® (Dako). Paraffin embedded tissue sections from a tumor biopsy may be subjected to the IHC assay and accorded a ErbB2 protein staining intensity criteria as follows:

[0170] Score 0 no staining is observed or membrane staining is observed in less than 10% of tumor cells.

[0171] Score 1+ a faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane.

[0172] Score 2+ a weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.

[0173] Score 3+ a moderate to strong complete membrane staining is observed in more than 10% of the tumor cells.

[0174] Those tumors with 0 or 1+ scores for ErbB2 overexpression assessment may be characterized as not overexpressing ErbB2, whereas those tumors with 2+ or 3+ scores may be characterized as overexpressing ErbB2.

[0175] Alternatively, or additionally, fluorescence in situ hybridization (FISH) assays such as the INFORM™ (sold by Ventana, Ariz.) or PATHVISION™ (Vysis, Ill.) may be carried out on formalin-fixed, paraffin-embedded tumor tissue to determine the extent (if any) of ErbB2 overexpression in the tumor. In comparison with IHC assay, the FISH assay, which measures HER2 gene amplification, seems to correlate better with response of patients to treatment with HERCEPTIN®, and is currently considered to be the preferred assay to identify patients likely to benefit from HERCEPTIN® treatment.

[0176] Table D describes the expression level of HER2 on several representative breast cancer and other cancer cell lines (Subik et al. (2010) Breast Cancer: Basic Clinical Research:4; 35-41; Prang et a. (2005) British Journal of Cancer Research:92; 342-349). As shown in the table, MCF-7 and MDA-MB-231 cells are considered to be low HER2 expressing cells; JIMT-1, and ZR-75-1 cells are considered to be medium HER2 expressing cells, and SKBR3 and BT-474 cells are considered to be high HER2 expressing cells. SKOV3 (ovarian cancer) cells are considered to be medium HER2 expressing cells.

[0177] Described herein are methods of treating a subject having a HER2+ cancer or a tumor comprising providing to the subject an effective amount of a pharmaceutical composition comprising an antigen-binding construct described herein.

[0178] Also described herein is the use of an HER2 antigen-binding construct described herein for the manufacture of a medicament for treating a cancer or a tumor. Also described herein are HER2 antigen-binding constructs for use in the treatment of cancer or a tumor.

[0179] In some embodiments, the subject being treated has pancreatic cancer, head and neck cancer, gastric cancer, colorectal cancer, breast cancer, renal cancer, cervical cancer, ovarian cancer, brain cancer, endometrial cancer, bladder cancer, non-small cell lung cancer or an epidermal-derived cancer. In some embodiments, the tumor is metastatic.

[0180] In general, the tumor in the subject being treated expresses an average of 10,000 or more copies of HER2 per tumor cell. In certain embodiments the tumor is HER2 0-1+, 1+, HER2 2+ or HER2 3+ as determined by IHC. In some embodiments the tumor is HER2 2+ or lower, or HER2 1+ or lower. In some embodiments, the tumor has an amplified HER2 gene. In some embodiments the HER2 gene is non-amplified.

[0181] In some embodiments, the tumor of the subject being treated with the antigen-binding constructs is a breast cancer. In some embodiments, the breast cancer expresses HER2 at a 3+ level. In some emboidments the breast cancer expresses HER2 at less than a 3+ level. In a specific embodiment, the breast cancer expresses HER2 at a 2+ level or lower. In a specific embodiment, the breast cancer expresses HER2 at a 1+ level or lower. In some embodiments, the breast cancer expresses estrogen receptors (ER+) and/or progesterone receptors (PR+). In some embodiments, the breast cancer is ER- and or PR-. In some embodiments the breast cancer has an amplified HER2 gene. In some embodiments the HER2 gene is non-amplified. In some embodiments, the breast cancer is a HER2 3+ estrogen receptor negative (ER-), progesterone receptor negative (PR-), trastuzumab resistant, chemotherapy resistant invasive ductal breast cancer. In another embodiment, the breast cancer is a HER2 3+ ER-, PR-, trastuzumab resistant inflammatory breast cancer. In another embodiment, the breast cancer is a HER2 3+, ER-, PR-, invasive ductal carcinoma. In another embodiment, the breast cancer is a HER2 2+ HER2 gene amplified trastuzumab and pertuzumab resistant breast cancer. In some embodiments, the breast cancer is triple negative (ER-, PR- and low HER2-expressing). In some embodiments the breast cancer is resistant or refractory to trastuzumab, pertuzumab and/or trastuzumab conjugated to DM1 (ado-trastuzumab emtansine or T-DM1).

[0182] In one embodiment, the tumor is an HER2 2/3+ ovarian epithelial adenocarcinoma having an amplified HER2 gene.

[0183] Provided herein are methods for treating a subject having a HER2+ tumor that is resistant or becomes resistant to other standard-of-care therapies comprising administering to the subject a pharmaceutical composition comprising the antigen-binding constructs described herein. In certain embodiments the antigen-binding constructs described herein are provided to subjects that are unresponsive to current therapies, optionally in combination with one or more current anti-HER2 therapies. In some embodiments the current anti-HER2 therapies include, but are not limited to, anti-HER2 or anti-HER3 monospecific bivalent antibodies, trastuzumab, pertuzumab, T-DM1, a bi-specific HER2/HER3 scFv, or combinations thereof. In some embodiments, the cancer is resistant to various chemotherapeutic agents such as taxanes. In some embodiments the cancer is resistant to trastuzumab. In some embodiment the cancer is resistant to pertuzumab. In one embodiment, the cancer is resistant or refractory to TDM1 (trastuzumab conjugated to DM1). In some embodiments, the subject has previously been treated with an anti-HER2 antibody such as trastuzumab, pertuzumab or DM1. In some embodiments, the subject has not been previously treated with an anti-HER2 antibody. In one embodiment, the antigen-binding construct is provided to a subject for the treatment of metastatic cancer when the patient has progressed on previous anti-HER2 therapy.

[0184] Provided herein are methods of treating a subject having a HER2+ tumor comprising providing an effective amount of a pharmaceutical composition comprising an antigen-binding construct described herein in conjunction with an additional anti-tumor agent. The additional anti-tumor agent may be a therapeutic antibody as noted above, or a chemotherapeutic agent. Chemotherapeutic agents useful for use in combination with the antigen-binding constructs of the invention include cisplatin, carboplatin, paclitaxel, albumin-bound paclitaxel, nab-paclitaxel, docetaxel, gemcitabine, vinorelbine, irinotecan, etoposide, vinblastine, pemetrexed, 5-fluorouracil (with or without folinic acid), capecitabine, carboplatin, epirubicin, oxaliplatin, folfirinox, abraxane, navelbine and cyclophosphamide, capecitabine, gemcitabine, navelbine, paclitaxel, nab-paclitaxel.

[0185] In some embodiments, the tumor is non-small cell lung cancer, and the additional agent is one or more of cisplatin, carboplatin, paclitaxel, albumin-bound paclitaxel, nab-paclitaxel, capecitabine, navelbine, docetaxel, gemcitabine, vinorelbine, irinotecan, etoposide, vinblastine or pemetrexed. In embodiments, the tumor is gastric or stomach cancer, and the additional agent is one or more of 5-fluorouracil (with or without

folinic acid), capecitabine, carboplatin, cisplatin, docetaxel, epirubicin, irinotecan, oxaliplatin, nab-paclitaxel or paclitaxel. In other embodiments the tumor is pancreatic cancer, and the additional agent is one or more of nab-paclitaxel, capecitabine, navelbine, gemcitabine, folfirinox, abraxane, or 5-fluorouracil. In other embodiments the tumor is a estrogen and/or progesterone positive breast cancer, and the additional agent is one or more of paclitaxel, capecitabine, navelbine, gemcitabine, paclitaxel or nab-paclitaxel or a combination of (a) doxorubicin and epirubicin, (b) a combination of paclitaxel and docetaxel, or (c) a combination of 5-fluorouracil, cyclophosphamide and carboplatin. In other embodiments, the tumor is head and neck cancer, and the additional agent is one or more of paclitaxel, capecitabine, navelbine, gemcitabine or nab-paclitaxel carboplatin, doxorubicin or cisplatin. In other embodiments, the tumor is ovarian cancer and the additional agent may be one or more of capecitabine, navelbine, gemcitabine, nab-paclitaxel, cisplatin, carboplatin, or a taxane such as paclitaxel or docetaxel.

[0186] The additional agents may be administered to the subject being treated concurrently with the antigen-binding constructs or sequentially.

[0187] The subject being treated with the antigen-binding constructs may be a human, a non-human primate or other mammal such as a mouse.

[0188] In some embodiments, the result of providing an effective amount of the antigen-binding construct to a subject having a tumor is shrinking the tumor, inhibiting growth of the tumor, increasing time to progression of the tumor, prolonging disease-free survival of the subject, decreasing metastases, increasing the progression-free survival of the subject, or increasing overall survival of the subject or increasing the overall survival of a group of subjects receiving the treatment..

[0189] Also described herein are methods of killing or inhibiting the growth of a HER2-expressing tumor cell comprising contacting the cell with the antigen-binding construct provided herein.

[0190] In various embodiments, a tumor cell may be a HER2 1+ or 2+ human pancreatic carcinoma cell, a HER2 3+ human lung carcinoma cell, a HER2 2+ human Caucasian bronchioaveolar carcinoma cell, a human pharyngeal carcinoma cell, a HER2 2+ human tongue squamous cell carcinoma cell, a HER2 2+ squamous cell carcinoma cell of the pharynx, a HER2 1+ or 2+ human colorectal carcinoma cell, a HER2 3+ human gastric carcinoma cell, a HER2 1+ human breast ductal ER+ (estrogen receptor-positive) carcinoma cell, a HER2 2+/3+ human ER+, HER2-amplified breast carcinoma cell, a HER2 0+/1+ human triple negative breast carcinoma cell, a HER2 2+ human endometrioid carcinoma cell, a HER2 1+ lung-metastatic malignant melanoma cell, a HER2 1+ human cervix carcinoma cell, Her2 1+human renal cell carcinoma cell, or a HER2 1+ human ovary carcinoma cell.

[0191] In embodiments in which the antigen-binding constructs are conjugated to DM1, the tumor cell may be a HER2 1+ or 2+ or 3+ human pancreatic carcinoma cell, a HER2 2+ metastatic pancreatic carcinoma cell, a HER2 0+/1+, +3+ human lung carcinoma cell, a HER2 2+ human Caucasian bronchioaveolar carcinoma cell, a HER2 0+ anaplastic lung carcinoma, a human non-small cell lung carcinoma cell, a human pharyngeal carcinoma cell, a HER2 2+ human tongue squamous cell carcinoma cell, a HER2 2+ squamous cell carcinoma cell of the pharynx, a HER2 1+ or 2+ human colorectal carcinoma cell, a HER2 0+, 1+ or 3+ human gastric carcinoma cell, a HER2 1+ human breast ductal ER+ (estrogen receptor-positive) carcinoma cell, a HER2 2+/3+ human ER+, HER2-amplified breast carcinoma cell, a HER2 0+/1+ human triple negative breast carcinoma cell, a HER2 0+ human breast ductal carcinoma (Basal B , Mesenchymal-like triple negative) cell, a HER2 2+ ER+ breast carcinoma, a HER2 0+ human metastatic breast carcinoma cell (ER-, HER2-amplified, luminal A, TN), a human uterus mesodermal tumor (mixed grade III) cell, a 2+ human endometrioid carcinoma cell, a HER2 1+ human skin epidermoid carcinoma cell, a HER2 1+ lung-metastatic malignant melanoma cell, a HER2 1+ malignant melanoma cell, a human cervix epidermoid carcinoma vcell, a HER2 1+ human urinary bladder carcinoma cell, a HER2 1+ human cervix carcinoma cell, Her2 1+human renal cell carcinoma cell, or a HER2 1+, 2+ or 3+ human ovary carcinoma cell.

[0192] In some embodiments the tumor cell may be one or more of the following cell lines: pancreatic tumor cell lines BxPC3, Capan-1, MiaPaca2; lung tumor cell lines Calu-3, NCI-H322; head and neck tumor cells lines Detroit 562, SCC-25, FaDu; colorectal tumor cell lines HT29, SNU-C2B; gastric tumor cell line NCI-N87; breast tumor cell lines MCF-7, MDA-MB-175, MDA-MB-361, MDA-MB-231,BT-20, JIMT-1, SkBr3, BT-474; uterine tumor cell line TOV-112D; skin tumor cell line Malme-3M; cervical tumor cell lines Caski, MS751; bladder tumor cell line T24, ovarian tumor cell lines CaOV3, and SKOV3.

[0193] In some embodiments in which the antigen-binding constructs are conjugated to DM1, the tumor cell may be one or more of the following cell lines: pancreatic tumor cell lines BxPC3, Capan-1, MiaPaca2, SW 1990, Panel; lung tumor cell lines A549, Calu-3, Calu-6, NCI-H2126, NCI-H322; head and neck tumor cells lines Detroit 562, SCC-15, SCC-25, FaDu; colorectal tumor cell lines Colo201, DLD-1, HCT116, HT29, SNU-C2B; gastric tumor cell lines SNU-1, SNU-16, NCI-N87; breast tumor cell lines SkBr3, MCF-7, MDA-MB-175, MDA-MB-361, MDA-MB-231, ZR-75-1, BT-20, BT549, BT-474, CAMA-1, MDA-MB-453, JIMT-1, T47D; Uterine tumor cell lines SK-UT-1, TOV-112D; skin tumor cell lines A431, Malme-3M, SKEMEL28; cervical tumor cell lines Caski, MS751; bladder tumor cell line T24, renal tumor cell line ACHN; ovarian tumor cell lines CaOV3, Ovar-3, and SKOV3.

[0194] Also described herein are methods of treating a subject having a HER2 expressing (HER2+) tumor such as a HER2+ lung, head and neck, or breast tumor by administering an antigen binding construct disclosed herein. In some aspects, the tumor volume in the subject after receiving at least seven doses of the antigen binding construct is less than the tumor volume of a control subject receiving an equivalent amount of trastuzumab. In some aspects, the survival of the subject receiving the antigen binding construct is increased as compared to a control subject receiving an equivalent amount of a non-specific control antibody or as compared to a control subject not receiving treatment.

[0195] In some aspects, the tumor is a lung tumor, optionally wherein the tumor is a non-squamous non-small cell lung tumor that is HER2-low, non-HER2 gene amplified. In some aspects, the tumor is HER3+. In some aspects, the tumor is EGFR low. In some aspects, the tumor is moderately sensitive to Cisplatin at the MTD.

[0196] In some aspects, the tumor is a head and neck tumor, optionally wherein the tumor is a squamous cell tumor of the head and neck that is HER2 low, non-HER2 gene amplified. In some aspects, the tumor is HER3+ low. In some aspects, the tumor is EGFR+. In some aspects, the tumor is highly sensitive to Cisplatin at the MTD.

[0197] In some aspects, the tumor is a breast tumor, optionally wherein the tumor is a ER+/PR- breast cancer with a luminal B molecular classification.

[0198] According to the claimed invention, the human subject is administered at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 doses; and/or each dose is administered at least every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20, days.

[0199] In some aspects, the amount of at least one of the plurality of doses is at least 0.3, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg/kg. In some aspects, the amount of each of the plurality of doses is at least 0.3, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg/kg.

[0200] In some aspects, each dose is administered at least daily, weekly, or monthly. In some aspects, treatment continues for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or 31 days; at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 weeks; or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 months.

[0201] In some aspects, the mean tumor volume in the subject after receiving at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 doses is less than the mean tumor volume of a control subject receiving an equivalent amount of trastuzumab.

[0202] In some aspects, overall survival of the subject is significantly increased as compared to a control subject receiving an equivalent amount of a non-specific control antibody or as compared to a control subject not receiving treatment. In some aspects, the significance is measured by a log rank test. In some aspects, the p value is less than 0.5, 0.01, or 0.001.

[0203] In some aspects, overall survival of the subject is more significantly increased as compared to a control subject receiving an equivalent amount of trastuzumab. In some aspects, the antigen-binding construct p value is less than 0.001 and wherein the trastuzumab p value is greater than 0.001.

[0204] In some aspects, the p value of the significance of the increase relative to the control subject receiving an equivalent amount of a non-specific control antibody is less than the p value of an increase in survival of a second control receiving an equivalent amount of trastuzumab as compared to the control subject receiving an equivalent amount of a non-specific control antibody. In some aspects, the antigen-binding construct p value is less than 0.001 and wherein the trastuzumab p value is greater than 0.001.

[0205] In some aspects, overall survival of the subject after receiving a combination of the antigen-binding construct and an additional agent is significantly increased as compared to a control subject receiving an equivalent amount of trastuzumab alone.

[0206] In some aspects, overall survival of the subject is significantly increased as compared to a control subject receiving a lesser amount of trastuzumab.

Kits and Articles of Manufacture - Not claimed

[0207] Also described herein (but not part of the claimed invention) are kits comprising one or more antigen-binding construct described herein. Individual components of the kit would be packaged in separate containers and, associated with such containers, can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale. The kit may optionally contain instructions or directions outlining the method of use or administration regimen for the antigen-binding construct.

[0208] When one or more components of the kit are provided as solutions, for example an aqueous solution, or a sterile aqueous solution, the container means may itself be an inhalant, syringe, pipette, eye dropper, or other such like apparatus, from which the solution may be administered to a subject or applied to and mixed with the other components of the kit.

[0209] The components of the kit may also be provided in dried or lyophilized form and the kit can additionally contain a suitable solvent for reconstitution of the lyophilized components. Irrespective of the number or type of containers, the kits described herein also may comprise an instrument for assisting with the administration of the composition to a patient. Such an instrument may be an inhalant, nasal spray device, syringe, pipette, forceps, measured spoon, eye dropper or similar medically approved delivery vehicle.

[0210] In another aspect described herein, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a T cell activating antigen-binding construct described herein. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antigen-binding construct described herein; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment described herein may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), 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, and syringes.

Polypeptides and polynucleotides

[0211] The antigen-binding constructs described herein comprise at least one polypeptide. Also described are polynucleotides encoding the polypeptides described herein. The antigen-binding constructs are typically isolated.

[0212] As used herein, "isolated" means an agent (e.g., a polypeptide or polynucleotide) that has been identified and separated and/or recovered from a component of its natural cell culture environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antigen-binding construct, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. Isolated also refers to an agent that has been synthetically produced, e.g., via human intervention.

[0213] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. That is, a description directed to a polypeptide applies equally to a description of a peptide and a description of a protein, and vice versa. The terms apply to naturally occurring amino acid polymers as well as amino acid polymers in which one or more amino acid residues is a non-naturally encoded amino acid. As used herein, the terms encompass amino acid chains of any length, including full length proteins, wherein the amino acid residues are linked by covalent peptide bonds.

[0214] The term "amino acid" refers to naturally occurring and non-naturally occurring amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally encoded amino acids are the 20 common amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, praline, serine, threonine, tryptophan, tyrosine, and valine) and pyrrolysine and selenocysteine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an a carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, such as, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (such as, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Reference to an amino acid includes, for example, naturally occurring proteogenic L-amino acids; D-amino acids, chemically modified amino acids such as amino acid variants and derivatives; naturally occurring non-proteogenic amino acids such as β-alanine, ornithine, etc.; and chemically synthesized compounds having properties known in the art to be characteristic of amino acids. Examples of non-naturally occurring amino acids include, but are not limited to, α-methyl amino acids (e.g. α-methyl alanine), D-amino acids, histidine-like amino acids (e.g., 2-amino-histidine, β-hydroxy-histidine, homohistidine), amino acids having an extra methylene in the side chain ("homo" amino acids), and amino acids in which a carboxylic acid functional group in the side chain is replaced with a sulfonic acid group (e.g., cysteic acid). The incorporation of non-natural amino acids, including synthetic non-native amino acids, substituted amino acids, or one or more D-amino acids into the proteins of the present invention may be advantageous in a number of different ways. D-amino acid-containing peptides, etc., exhibit increased stability in vitro or in vivo compared to L-amino acid-containing counterparts. Thus, the construction of peptides, etc., incorporating D-amino acids can be particularly useful when greater intracellular stability is desired or required. More specifically, D-peptides, etc., are resistant to endogenous peptidases and proteases, thereby providing improved bioavailability of the molecule, and prolonged lifetimes in vivo when such properties are desirable. Additionally, D-peptides, etc., cannot be processed efficiently for major histocompatibility complex class II-restricted presentation to T helper cells, and are therefore, less likely to induce humoral immune responses in the whole organism.

[0215] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0216] Also included in the invention are polynucleotides encoding polypeptides of the antigen-binding constructs. The term "polynucleotide" or "nucleotide sequence" is intended to indicate a consecutive stretch of two or more nucleotide molecules. The nucleotide sequence may be of genomic, cDNA, RNA, semisynthetic or synthetic origin, or any combination thereof.

[0217] The term "nucleic acid" refers to deoxyribonucleotides, deoxyribonucleosides, ribonucleosides, or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides which have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless specifically limited otherwise, the term also refers to oligonucleotide analogs including PNA (peptidonucleic acid), analogs of DNA used in antisense technology (phosphorothioates, phosphoroamidates, and the like). Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (including but not limited to, degenerate codon substitutions) and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608 (1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)).

[0218] "Conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, "conservatively modified variants" refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of ordinary skill in the art will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0219] As to amino acid sequences, one of ordinary skill in the art will recognize that individual substitutions, deletions or additions to a nucleic acid,

peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the deletion of an amino acid, addition of an amino acid, or substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are known to those of ordinary skill in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles described herein.

[0220] Conservative substitution tables providing functionally similar amino acids are known to those of ordinary skill in the art. The following eight groups each contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and [0139] 8) Cysteine (C), Methionine (M) (see, e.g., Creighton, Proteins: Structures and Molecular Properties (W H Freeman & Co.; 2nd edition (December 1993)

[0221] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same. Sequences are "substantially identical" if they have a percentage of amino acid residues or nucleotides that are the same (i.e., about 60% identity, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, or about 95% identity over a specified region), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms (or other algorithms available to persons of ordinary skill in the art) or by manual alignment and visual inspection. This definition also refers to the complement of a test sequence. The identity can exist over a region that is at least about 50 amino acids or nucleotides in length, or over a region that is 75-100 amino acids or nucleotides in length, or, where not specified, across the entire sequence of a polynucleotide or polypeptide. A polynucleotide encoding a polypeptide of the present invention, including homologs from species other than human, may be obtained by a process comprising the steps of screening a library under stringent hybridization conditions with a labeled probe having a polynucleotide sequence described herein or a fragment thereof, and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to the skilled artisan.

[0222] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0223] A "comparison window", as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are known to those of ordinary skill in the art. Optimal alignment of sequences for comparison can be conducted, including but not limited to, by the local homology algorithm of Smith and Waterman (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman (1988) Proc. Nat'l. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by manual alignment and visual inspection (see, e.g., Ausubel et al., Current Protocols in Molecular Biology (1995 supplement)).

[0224] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1997) Nuc. Acids Res. 25:3389-3402, and Altschul et al. (1990) J. Mol. Biol. 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information available at the World Wide Web at ncbi.nlm.nih.gov. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) or 10, M=5, N=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff (1992) Proc. Natl. Acad. Sci. USA 89:10915) alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The BLAST algorithm is typically performed with the "low complexity" filter turned off.

[0225] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-5787). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, or less than about 0.01, or less than about 0.001.

[0226] The phrase "selectively (or specifically) hybridizes to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent hybridization conditions when that sequence is present in a complex mixture (including but not limited to, total cellular or library DNA or RNA).

[0227] The phrase "stringent hybridization conditions" refers to hybridization of sequences of DNA, RNA, or other nucleic acids, or combinations thereof under conditions of low ionic strength and high temperature as is known in the art. Typically, under stringent conditions a probe will hybridize to its target subsequence in a complex mixture of nucleic acid (including but not limited to, total cellular or library DNA or RNA) but does not hybridize to other sequences in the complex mixture. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993).

[0228] As used herein, the terms "engineer, engineered, engineering", are considered to include any manipulation of the peptide backbone or the post-translational modifications of a naturally occurring or recombinant polypeptide or fragment thereof. Engineering includes modifications of the

amino acid sequence, of the glycosylation pattern, or of the side chain group of individual amino acids, as well as combinations of these approaches. The engineered proteins are expressed and produced by standard molecular biology techniques.

[0229] By "isolated nucleic acid molecule or polynucleotide" is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a polypeptide contained in a vector is considered isolated. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. An isolated polynucleotide includes a polynucleotide molecule contained in cells that ordinarily contain the polynucleotide molecule, but the polynucleotide molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location. Isolated RNA molecules include in vivo or in vitro RNA transcripts, as well as positive and negative strand forms, and double-stranded forms. Isolated polynucleotides or nucleic acids described herein, further include such molecules produced synthetically, e.g., via PCR or chemical synthesis. In addition, a polynucleotide or a nucleic acid, in certain embodiments, include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

[0230] The term "polymerase chain reaction" or "PCR" generally refers to a method for amplification of a desired nucleotide sequence in vitro, as described, for example, in U.S. Pat. No. 4,683,195. In general, the PCR method involves repeated cycles of primer extension synthesis, using oligonucleotide primers capable of hybridising preferentially to a template nucleic acid.

[0231] By a nucleic acid or polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence. As a practical matter, whether any particular polynucleotide sequence is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs, such as the ones discussed above for polypeptides (e.g. ALIGN-2).

[0232] A derivative, or a variant of a polypeptide is said to share "homology" or be "homologous" with the peptide if the amino acid sequences of the derivative or variant has at least 50% identity with a 100 amino acid sequence from the original peptide. In certain embodiments, the derivative or variant is at least 75% the same as that of either the peptide or a fragment of the peptide having the same number of amino acid residues as the derivative.. In certain embodiments, the derivative or variant is at least 85% the same as that of either the peptide or a fragment of the peptide having the same number of amino acid residues as the derivative. In certain embodiments, the amino acid sequence of the derivative is at least 90% the same as the peptide or a fragment of the peptide having the same number of amino acid residues as the derivative. In some embodiments, the amino acid sequence of the derivative is at least 95% the same as the peptide or a fragment of the peptide having the same number of amino acid residues as the derivative. In certain embodiments, the derivative or variant is at least 99% the same as that of either the peptide or a fragment of the peptide having the same number of amino acid residues as the derivative.

[0233] The term "modified," as used herein refers to any changes made to a given polypeptide, such as changes to the length of the polypeptide, the amino acid sequence, chemical structure, co-translational modification, or post-translational modification of a polypeptide. The form "(modified)" term means that the polypeptides being discussed are optionally modified, that is, the polypeptides under discussion can be modified or unmodified.

[0234] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention or for any other reason.

EXAMPLES

[0235] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

[0236] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T.E. Creighton, Proteins: Structures and Molecular Properties (W.H. Freeman and Company, 1993); A.L. Lehninger, Biochemistry (Worth Publishers, Inc., current addition); Sambrook, et al., Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Methods In Enzymology (S. Colowick and N. Kaplan eds., Academic Press, Inc.); Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg Advanced Organic Chemistry 3rd Ed. (Plenum Press) Vols A and B(1992).

[0237] The antigen-binding construct provided for use according to the invention is referred to in the Examples below as the "v10000" construct. References to other constructs in the Examples are for reference and illustration, and do not form part of the claimed invention.

Example 1: Preparation of exemplary Anti-HER2 bispecific antibodies and controls

[0238] A number of exemplary anti-HER2 biparatopic antibodies (or antigen-binding constructs) and controls were prepared as described below. The antibodies and controls have been prepared in different formats, and representations of exemplary biparatopic formats are shown in Figure 1.

In all of the formats shown in Figure 1, the heterodimeric Fc is depicted with one chain (Chain A) shown in black and the other (Chain B) shown in grey, while one antigen-binding domain (1) is shown in hatched fill, while the other antigen-binding domain (2) is shown in white.

[0239] Figure 1A depicts the structure of a biparatopic antibody in a Fab-Fab format. Figures 1B to 1E depict the structure of possible versions of a biparatopic antibody in an scFv-Fab format. In Figure 1B, antigen-binding domain 1 is an scFv, fused to Chain A, while antigen-binding domain 2 is a Fab, fused to Chain B. In Figure 1C, antigen-binding domain 1 is a Fab, fused to Chain A, while antigen-binding domain 2 is an scFv, fused to Chain B. In Figure 1D, antigen-binding domain 2 is a Fab, fused to Chain A, while antigen-binding domain 1 is an scFv, fused to Chain B. In Figure 1E, antigen-binding domain 2 is an scFv, fused to Chain A, while antigen-binding domain 1 is a Fab, fused to Chain B. In Figure 1F, both antigen-binding domains are scFvs..

[0240] The sequences of the following variants are provided in the Sequence Table found after the Examples. CDR regions were identified using a combination of the Kabat and Chothia methods. Regions may vary slightly based on method used for identification.

Exemplary anti-HER2 biparatopic antibodies

[0241] Exemplary anti-HER2 biparatopic antibodies were prepared as shown in Table 1.

Table 1: Exemplary anti-HER2 biparatbopic antibodies

Variant		Chain A	Chain B
5019	domain containing the epitope	ECD2	ECD4
	Format	Fab	scFv
	Antibody name	Pertuzumab	Trastuzumab
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T366I_N390R_K392M_T394W
5020	domain containing the epitope	ECD4	ECD2
	format	scFv	Fab
	Antibody name	Trastuzumab	Pertuzumab
	CH3 sequence substitutions	L351Y_S400E_F405A_Y407V	T350V_T366L_K392L_T394W
7091	domain containing the epitope	ECD2	ECD4
	format	Fab	scFv
	Antibody name	Pertuzumab	Trastuzumab
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T350V_T366L_K392L_T394W
10000	domain containing the epitope	ECD2	ECD4
	format	Fab	scFv
	Antibody name	Pertuzumab - with Y96A in VL region and T30A/A49G/L69F in VH region	Trastuzumab
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T350V_T366L_K392L_T394W
6902	domain containing the epitope	ECD2	ECD4
	format	Fab	Fab
	Antibody name	Trastuzumab	Pertuzumab
	Fab substitutions	HC: L143E_K145T	HC: D146G_Q179K
		LC: Q124R	LC: Q124E_Q160E_T180E
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T350V_T366L_K392L_T394W
6903	domain containing the epitope	ECD2	ECD4
	format	Fab	Fab
	Fab substitutions	HC: L143E_K145T	HC: D146G Q179K
		LC: Q124R_Q1160K_T178R	LC: Q124E_Q160E_T180E
	Antibody name	Trastuzumab	Pertuzumab
	CH3 sequence substitutions	T350V_L351Y_F405 A_Y407V	T350V_T366L_K392L_T394W
6717	domain containing the epitope	ECD4	ECD2
	format	scFv	scFv

Variant		Chain A	Chain B
	Antibody name	Pertuzumab	Trastuzumab
	CH3 sequence substitutions	T350V_L351Y_F405A_Y407V	T366l_N390R_K392M_T394W

Notes:
• CH3 numbering according to EU index as in Kabat referring to the numbering of the EU antibody (Edelman et al., 1969, Proc Natl Acad Sci USA 63:78-85);
• Fab or variable domain numbering according to Kabat (Kabat and Wu, 1991; Kabat et al, Sequences of proteins of immunological interest. 5th Edition - US Department of Health and Human Services, NIH publication n° 91-3242, p 647 (1991))
• "domain containing the epitope"=domain of HER2 to which antigen-binding moiety binds;
• "Antibody name"=antibody from which antigen-binding moiety is derived, includes substitutions compared to wild-type when present;
• "Fab substitutions"=substitutions in Fab that promote correct light chain pairing;
• "CH3 sequence substitutions"=substitutions in CH3 domain that promote formation of heterodimeric Fc

Exemplary anti-HER2 monovalent control antibodies

[0242] v1040: a monovalent anti-HER2 antibody, where the HER2 binding domain is a Fab derived from trastuzumab on chain A, and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A_Y407V in Chain A, T350V_T366L_K392L_T394W in Chain B, and the hinge region of Chain B having the mutation C226S; the antigen-binding domain binds to domain 4 of HER2.

[0243] v630 - a monovalent anti-HER2 antibody, where the HER2 binding domain is an scFv derived from trastuzumab on Chain A, and the Fc region is a heterodimer having the mutations L351Y_S400E_F405A_Y407V in Chain A, T366l_N390R_K392M_T394W in Chain B; and the hinge region having the mutation C226S (EU numbering) in both chains; the antigen-binding domain binds to domain 4 of HER2.

[0244] v4182: a monovalent anti-HER2 antibody, where the HER2 binding domain is a Fab derived from pertuzumab on chain A, and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A_Y407V in Chain A, T350V_T366L_K392L_T394W in Chain B, and the hinge region of Chain B having the mutation C226S; the antigen-binding domain binds to domain 2 of HER2.

Exemplary anti-HER2 monospecific bivalent antibody controls (full-sized antibodies, FSAs)

[0245] v506 is a wild-type anti HER2 produced in-house in Chinese Hamster Ovary (CHO) cells, as a control. Both HER2 binding domains are derived from trastuzumab in the Fab format and the Fc is a wild type homodimer; the antigen-binding domain binds to domain 4 of HER2. This antibody is also referred to as a trastuzumab analog.

[0246] v792, is wild-type trastuzumab with a IgG1 hinge, where both HER2 binding domains are derived from trastuzumab in the Fab format, and the and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A_Y407V in Chain A, and T350V_T366L_K392L_T394W Chain B; the antigen-binding domain binds to domain 4 of HER2. This antibody is also referred to as a trastuzumab analog.

[0247] v4184, a bivalent anti-HER2 antibody, where both HER2 binding domains are derived from pertuzumab in the Fab format, and the Fc region is a heterodimer having the mutations T350V _L351Y _F405A _Y407V in Chain A, and T350V_T366L_K392L_T394W Chain B. The antigen-binding domain binds to domain 2 of HER2. This antibody is also referred to as a pertuzumab analog.

[0248] hIgG, is a commercial non-specific polyclonal antibody control (Jackson ImmunoResearch, # 009-000-003).

[0249] These antibodies and controls (other than human IgG) were cloned and expressed as follows. The genes encoding the antibody heavy and light chains were constructed via gene synthesis using codons optimized for human/mammalian expression. The Trastuzumab Fab sequence was generated from a known HER2/neu domain 4 binding antibody (Carter P. et al. (1992) Humanization of an anti p185 HER2 antibody for human cancer therapy. Proc Natl Acad Sci 89, 4285.) And the Fc was an IgG1 isotype. The scFv sequence was generated from the VH and VL domains of Trastuzumab using a glycine-serine linker (Carter P. et al. (1992) Humanization of an anti p185 her2 antibody for human cancer therapy. Proc Natl Acad Sci 89, 4285.). The Pertuzumab Fab sequence was generated from a known HER2/neu domain 2 binding Ab (Adams CW et al. (2006) Humanization of a recombinant monoclonal antibody to produce a therapeutic her dimerization inhibitor, Pertuzumab. Cancer Immunol Immunother. 2006;55(6):717-27).

[0250] The final gene products were sub-cloned into the mammalian expression vector PTT5 (NRC-BRI, Canada) and expressed in CHO cells (Durocher, Y., Perret, S. & Kamen, A. High-level and high-throughput recombinant protein production by transient transfection of suspension-growing CHO cells. Nucleic acids research 30, e9 (2002)).

[0251] The CHO cells were transfected in exponential growth phase (1.5 to 2 million cells/ml) with aqueous 1mg/ml 25 kDa polyethylenimine (PEI, polysciences) at a PEI:DNA ratio of 2.5:1.(Raymond C. et al. A simplified polyethylenimine-mediated transfection process for large-scale and high-throughput applications. Methods. 55(1):44-51 (2011)). To determine the optimal concentration range for forming heterodimers, the DNA was transfected in optimal DNA ratios of the heavy chain a (HC-A), light chain (LC), and heavy chain B (HC-B) that allow for heterodimer formation (e.g. HC-A/HC-B/LC ratios = 30:30:40 (v5019). Transfected cells were harvested after 5-6 days with the culture medium collected after centrifugation at 4000rpm and clarified using a 0.45µm filter.

[0252] The clarified culture medium was loaded onto a MabSelect SuRe (GE Healthcare) protein-A column and washed with 10 column volumes of PBS buffer at pH 7.2. The antibody was eluted with 10 column volumes of citrate buffer at pH 3.6 with the pooled fractions containing the antibody neutralized with TRIS at pH 11.

[0253] The protein-A antibody eluate was further purified by gel filtration (SEC). For gel filtration, 3.5 mg of the antibody mixture was concentrated to 1.5mL and loaded onto a Sephadex 200 HiLoad 16/600 200 pg column (GE Healthcare) via an AKTA Express FPLC at a flow-rate of 1mL/min. PBS buffer at pH 7.4 was used at a flow-rate of 1mL/min. Fractions corresponding to the purified antibody were collected, concentrated to ~1mg/mL.

[0254] Exemplary anti-HER2 ECD2 x ECD4 biparatopic antibodies with different molecular formats (e.g. v6717, scFv-scFv IgG1; v6903 and v6902 Fab-Fab IgG1; v5019, v7091 and v10000 Fab-scFv IgG1) were cloned, expressed and purified as described above.

[0255] To quantify antibody purity and to determine the amount of target heterodimer protein and possible homodimer and/or half antibody and/or mispaired light chain contaminant, LC-MS intact mass analysis was performed. The LC-MS intact mass analysis was performed as described in Example 2, excluding DAR analysis calculations used for ADC molecules.

[0256] The data is shown in Table 2. Table 2 shows that expression and purification of these biparatopic antibodies resulted in 100% of the desired product for v6717, 91% of the desired heterodimeric product for v6903, and 62% of the desired product for v6902. The numbers in brackets indicate the quantities of the main peak plus a side peak of + 81Da. This side peak is typically detected with variants that contain C-terminal HA tags (such of v6903 and v6902). Adding the main and side peaks yields heterodimer purities of approximately 98% and 67% for v6903 and v6903. Based on the high heterodimer purity, v6903 was identified as the representative Fab-Fab anti-HER2 biparatopic variant for direct comparison to the scFv-scFv and Fab-scFv formats. v6903 was included in all format comparison assays.

Table 2: Expression and purification of antibodies

Variant	Desired heterodimer species (+side peak)
6717	100.0
6903	90.9 (97.7)
6902	62.4 (67.4)

Example 2: Preparation of exemplary anti-HER2 biparatopic antibody drug conjugates (ADCs)

[0257] The following anti-HER2 biparatopic antibody drug conjugates (anti-HER2 biparatopic-ADCs) were prepared. ADCs of variants 5019, 7091, 10000 and 506 were prepared. These ADCs are identified as follows:

v6363 (v5019 conjugated to DM1)

v7148 (v7091 conjugated to DM1)

v10553 (v10000 conjugated to DM1)

v6246 (v506 conjugated to DM1, analogous to T-DM1, trastuzumab-emtansine)

v6249 (human IgG conjugated to DM1)

[0258] The ADCs were prepared via direct coupling to maytansine. Antibodies purified by Protein A and SEC, as described in Example 1 (>95% purity), were used in the preparation of the ADC molecules. ADCs were conjugated following the method described in Kovtun YV, Audette CA, Ye Y, et al. Antibody-drug conjugates designed to eradicate tumors with homogeneous and heterogeneous expression of the target antigen. Cancer Res 2006;66:3214-21. The ADCs had an average molar ratio of 3.0 maytansinoid molecules per antibody as determined by LC/MS and described below.

[0259] Details of the reagents used in the ADC conjugation reaction are as follows: Conjugation Buffer 1: 50 mM Potassium Phosphate/50 mM Sodium Chloride, pH 6.5, 2 mM EDTA. Conjugation Buffer 2: 50 mM Sodium Succinate, pH 5.0. ADC formulation buffer: 20 mM Sodium Succinate, 6% (w/v) Trehalose, 0.02% polysorbate 20, pH 5.0. Dimethylacetamide (DMA); 10 mM SMCC in DMA (prepared before conjugation), 10 mM DM1-SH in DMA (prepared before conjugation),! mM DTNB in PBS, 1 mM Cysteine in buffer, 20 mM Sodium Succinate, pH 5.0. UV-VIS spectrophotometer (Nano drop 100 from Fisher Scientific), PD-10 columns (GE Healthcare).

[0260] The ADCs were prepared as follows. The starting antibody solution was loaded onto the PD-10 column, previously equilibrated with 25 mL of Conjugation Buffer 1, followed by 0.5 ml Conjugation Buffer 1. The antibody eluate was collect and the concentration measured at A_{280} and the concentration was adjusted to 20 mg/mL. The 10 mM SMCC-DM1 solution in DMA was prepared. A 7.5 molar equivalent of SMCC-DM1 to antibody was added to the antibody solution and DMA was added to a final DMA volume of 10% v/v. The reaction was briefly mixed and incubated at RT for 2 h. A second PD-10 column was equilibrated with 25 ml of Conjugation Buffer 1 and the antibody-MCC-DM1 solution was added to the column follow by 0.5 ml of Buffer 1. The antibody-MCC-DM1 eluate was collected and the A_{252} and A_{280} of antibody solution was measured. The Antibody-MCC-DM1 concentration was calculated (□=1.45 $mg^{-1}cm^{-1}$, or 217500 $M^{-1}cm^{-1}$). The ADCs were analyzed on a SEC-HPLC column for high MW analysis (SEC-HPLC column TOSOH, G3000-SWXL, 7.8 mmx30 cm, Buffer, 100 mM Sodium phosphate, 300 mM Sodium Chloride, pH 7.0, flow rate: 1 ml/min).

[0261] ADC drug to antibody ratio (DAR) was analysed by HIC-HPLC_using the Tosoh TSK gel Butyl-NPR column (4.6 mm × 3.5 mm × 2.5 mm). Elution was performed at 1 ml/min using a gradient of 10-90% buffer B over 25 min followed by 100% buffer B for 4 min. Buffer A comprises 20 mM sodium phosphate, 1.5 M ammonium sulphate, pH 7.0. Buffer B comprises 20 mM sodium phosphate, 25% v/v isopropanol, pH 7.0.

[0262] ADC drug to antibody ratio (DAR) was determined by LC-MS by the following method. The antibodies were deglycosylated with PNGase F prior to loading on the LC-MS. Liquid chromatography was carried out on an Agilent 1100 Series HPLC under the following conditions:

[0263] Flow rate: 1mL/min split post column to 100uL/min to MS. Solvents: A = 0.1% formic acid in ddH2O, B = 65% acetonitrile, 25% THF, 9.9% ddH20, 0.1% formic acid. Column: 2.1 × 30mm PorosR2. Column Temperature: 80°C ; solvent also pre-heated. Gradient: 20% B (0-3min), 20-90% B (3-6min), 90-20% B (6-7min), 20%B (7-9min).

[0264] Mass Spectrometry (MS) was subsequently carried out on an LTQ-Orbitrap XL mass spectrometer under the following conditions: Ionization method using Ion Max Electrospray. Calibration and Tuning Method: 2mg/mL solution of CsI is infused at a flowrate of 10µL/min. The Orbitrap was tuned on m/z 2211 using the Automatic Tune feature (overall CsI ion range observed: 1690 to 2800). Cone Voltage: 40V; Tube Lens: 115V; FT Resolution: 7,500; Scan range m/z 400-4000; Scan Delay: 1.5 min. A molecular weight profile of the data was generated using Thermo's Promass deconvolution software. Average DAR of the sample was determined as a function of DAR observed at each fractional peak (using the calculation: Σ (DAR × fractional peak intensity)).

[0265] Table 3 summarizes the average DAR for the ADC molecules. The average DAR for the exemplary anti-HER2 biparatopic antibody and control was approximately 3.

Table 3: Average DAR for ADCs

	DAR (LC-MS)	DAR (HIC)	n
v6246	2.9	3.0	5
v6363	2.6	3.3	5
v7148	3.4	3.9	1
v10553	4.0	4.0	1

Example 3: Expression and bench-scale purification of anti-HER2 biparatopic antibody

[0266] The anti-HER2 biparatopic antibodies (v5019, v7091 and v10000) described in Example 1 were expressed in 10 and/or 25 L volumes and purified by protein A and size exclusion chromatography (SEC) as follows.

[0267] The clarified culture medium was loaded onto a MabSelect SuRe (GE Healthcare) protein-A column and washed with 10 column volumes of PBS buffer at pH 7.2. The antibody was eluted with 10 column volumes of citrate buffer at pH 3.6 with the pooled fractions containing the antibody neutralized with Tris at pH 11.

[0268] The protein-A antibody eluate was further purified by gel filtration (SEC). For gel filtration, 3.5 mg of the antibody mixture was concentrated to 1.5mL and loaded onto a Sephadex 200 HiLoad 16/600 200 pg column (GE Healthcare) via an AKTA Express FPLC at a flow-rate of 1mL/min. PBS buffer at pH 7.4 was used at a flow-rate of 1mL/min. Fractions corresponding to the purified antibody were collected, concentrated to ~1mg/mL. The purified proteins were analyzed by LC-MS as described in Example 2.

[0269] The results of the 10L expression and bench-scale protein A and SEC purification are shown in Figure 2A and 2B. Figure 2A shows the SEC chromatograph of the protein A purified v5019 and Figure 2B shows the non-reducing SDS-PAGE gel that compares the relative purity of a protein A pooled fraction as well as SEC fractions 15 and 19 and pooled SEC fractions 16-18. These results show that the anti-HER2 biparatopic antibody was expressed and that purification by protein A and SEC yielded a pure protein sample. Further quantification was performed by UPLC-SEC and LC-MS analysis and is described in Example 4.

[0270] The results of the 25 L expression and bench-scale protein A purification is shown in Figure 2C. Figure 2C shows SDS-PAGE gel that compares the relative purity of a protein A purified v10000. Lane M contains: protein marker; lane 1 contains: v10000 under reducing conditions; lane 2 contains v10000 under non-reducing conditions. The SDS-PAGE gel shows that v10000 is pure and runs at the correct predicted MW of approximately 125 kDa under non-reducing conditions. Under reducing conditions two heavy chains bands are visible corresponding to the CH-A heavy chain (approximately 49 kDa) and the CH-B heavy chain (approximately 52.5 kDa); the CH-A light chain is visible and runs at the correct predicted mass of approximately 23.5 kDa. These results show that the anti-HER2 biparatopic antibody was expressed and that one-step purification by protein A yielded a pure protein sample. Further quantification was performed by UPLC-SEC and LC-MS analysis and is described in Example 4.

Example 4: Analysis of biparatopic anti-HER2 antibody purity by UPLC-SEC and LC-MS

[0271] The purity and percent aggregation of exemplary protein A and SEC purified biparatopic anti-HER2 heteromultimers was determined by UPLC-SEC by the method described.

[0272] UPLC-SEC analysis was performed using a Waters BEH200 SEC column set to 30°C (2.5 mL, 4.6 × 150 mm, stainless steel, 1.7 µm particles) at 0.4 ml/min. Run times consisted of 7 min and a total volume per injection of 2.8 mL with running buffers of 25 mM sodium phosphate,

150 mM sodium acetate, pH 7.1; and, 150 mM sodium phosphate, pH 6.4-7.1. Detection by absorbance was facilitated at190-400 nm and by fluorescence with excitation at 280 nm and emission collected from 300-360 nm. Peak integration was analyzed by Empower 3 software.

[0273] UPLC-SEC results of the pooled v5019 SEC fractions are shown in Figure 3A. These results indicate that the exemplary anti-HER2 biparatopic antibody was purified to >99% purity with less than 1% HMW species by protein A and SEC chromatography.

[0274] UPLC-SEC results of the v10000 pooled Protein A fractions are shown in Figure 3B. These results indicate that the exemplary anti-HER2 biparatopic antibody was purified to >96% purity with less than 1% HMW species by protein A chromatography.

[0275] The purity of exemplary biparatopic anti-HER2 antibodies was determined using LC-MS under standard conditions by the method described in Example 2. Results from LC-MS analysis of the pooled SEC fractions of v5019 are shown in Figure 4A. This data shows that the exemplary biparatopic anti-HER2 heterodimer has a heterodimer purity of 100%. Results from LC-MS analysis of the pooled protein A fractions of v10000 are shown in Figure 4B. This data shows that the exemplary biparatopic anti-HER2 heterodimer has a heterodimer purity of 98% following a one-step protein A purification.

[0276] Antibodies purified by protein A chromatography and/or protein A and SEC were used for the assays described in the following Examples.

Example 5. Large-scale expression and manufacturability assessment of biparatopic anti-HER2 antibody purified by protein A and CEX chromatography

[0277] The exemplary anti-HER2 biparatopic antibody v5019 described in Example 1 was expressed in a 25 L scale and purified as follows.

[0278] Antibody was obtained from supernatant followed by a two-step purification method that consisted of Protein A purification (MabSelect™ resin; GE Healthcare) followed by cation exchange chromatography (HiTrap™ SP FF resin; GE Healthcare) by the protocol described.

[0279] CHO-3E7 cells were maintained in serum-free Freestyle CHO expression medium (Invitrogen, Carlsbad, CA, USA) in Erlenmeyer Flasks at 37°C with 5% CO2 (Corning Inc., Acton, MA) on an orbital shaker (VWR Scientific, Chester, PA). Two days before transfection, the cells were seeded at an appropriate density in a 50 L CellBag with a volume of 25 L using the Wave Bioreactor System 20/50 (GE Healthcare Bio-Science Corp). On the day of transfection, DNA and PEI (Polysciences, Eppelheim, Germany) were mixed at an optimal ratio and added to the cells using the method described in Example 1. Cell supernatants collected on day 6 was used for further purification.

[0280] Cell culture broth was centrifuged and filtered before loading onto 30 mL Mabselect™ resin packed in XK26/20 (GE Healthcare, Uppsala, Sweden) at 10.0 mL/min. After washing and elution with appropriate buffer, the fractions were collected and neutralized with 1 M Tris-HCl, pH 9.0. The target protein was further purified via 20 mL SP FF resin packed in XK16/20 (GE Healthcare, Uppsala, Sweden). MabSelect™ purified sample was diluted with 20 mM NaAC, pH5.5 to adjust the conductivity to < 5 ms/cm and 50mM citrate acid (pH3.0) was added adjust the sample pH value to 5.5. Sample was loaded at a 1 mL/min onto the HiTrap™ SP FF resin (GE Healthcare) and washed with 20 mM NaAC. Protein was eluted using a gradient elution 0-100% of 20 mM NaAC, 1 M NaCl, pH5.5, 10 CV at 1 mL/min.

[0281] The purified protein was analyzed by SDS-PAGE as described in Example 1, and LC-MS for heterodimer purity by the method described in example 4. The results are shown in Figure 5A and 5B. Figure 5A shows the SDS-PAGE results of v5019 following MabSelect™ and HiTrap™ SP FF purification; lane M contains: protein marker; lane 1: v5019 under reducing conditions (3 µg); Lane 2: v5019 under non-reducing conditions (2.5 µg). The SDS-PAGE gel shows that v5019 is relatively pure following MabSelect™ and HiTrap™ SP FF purification and, under non-reducing conditions, runs at the correct predicted MW of approximately 125 kDa. Under reducing conditions two heavy chains bands are visible corresponding to the CH-A heavy chain (approximately 49 kDa) and the CH-B heavy chain (approximately 52.5 kDa); the CH-A light chain is visible and runs at the correct predicted mass of approximately 23.5 kDa.

[0282] LC-MS analysis of the MabSelect™ and HiTrap™ SP FF purified v5019 was performed to determine heterodimer purity using the method described in Example 4. Results from the LC-MS analysis are shown in Figure 5B. These results show that v5019 purification using MabSelect™ and HiTrap™ SP FF yields protein with > 99% heterodimer purity and with little (<1%) or undetectable homodimer or half antibody contamination.

Example 6: Comparison of Bmax of a biparatopic anti-HER2 antibody against Bmax of controls in cell lines expressing low to high levels of HER2

[0283] The following experiment was performed to measure the ability of an exemplary biparatopic anti-HER2 antibody to bind to cells expressing varying levels of HER2 in comparison to controls. The cell lines used were SKOV3 (HER2 2+/3+), JIMT-1 (HER2 2+), MDA-MB-231 (HER2 0/1+), and MCF7 (HER2 1+). The biparatopic anti-HER2 antibodies tested include v5019, v7091 and v10000. The ability of the biparatopic anti-HER2 antibodies to bind to the HER2 expressing (HER2+) cells was determined as described below, with specific measurement of B_{max} and apparent K_D (equilibrium dissociation constant).

[0284] Binding of the test antibodies to the surface of HER2+ cells was determined by flow cytometry. Cells were washed with PBS and resuspended in DMEM at 1×10^5 cells/ 100 µl. 100 µl cell suspension was added into each microcentrifuge tube, followed by 10 µl/ tube of the antibody variants. The tubes were incubated for 2hr 4°C on a rotator. The microcentrifuge tubes were centrifuged for 2 min 2000 RPM at room temperature and the cell pellets washed with 500 µl media. Each cell pellet was resuspended 100µl of fluorochrome- labelled secondary antibody

diluted in media to 2 µg/sample. The samples were then incubated for 1hr at 4°C on a rotator. After incubation, the cells were centrifuged for 2 min at 2000 rpm and washed in media. The cells were resuspended in 500 µl media, filtered in tube containing 5 µl propidium iodide (PI) and analyzed on a BD LSR II flow cytometer according to the manufacturer's instructions. The K_D of exemplary biparatopic anti-HER2 heterodimer antibody and control antibodies were assessed by FACS with data analysis and curve fitting performed in GraphPad Prism.

[0285] The results are shown in Figures 6A-6G. These results demonstrate that exemplary biparatopic anti-HER2 antibodies (v5019, v7091 and v10000) can bind to HER2+ cells with approximately a 1.5-fold higher Bmax compared to an anti-HER2 FSA (v506). The results in Figure 6A-6G also show that biparatopic anti-HER2 antibodies (v5019, v7091 and v10000) can bind to HER2+ cells with a similar Bmax compared to a combination of two anti-HER2 FSAs (v506 + v4184).

[0286] The binding results for HER2+ SKOV3 cells (HER2 2/3+) are shown in Figures 6A, 6E and Table 4 and Table 5. The results in Figure 6A and Table 4 show that exemplary biparatopic anti-HER2 antibody (v5019) displays approximately a 1.5-fold higher Bmax in binding to SKOV3 cells compared to two different anti-HER2 FSAs (v506 or v4184). The results also show that exemplary biparatopic anti-HER2 antibody (v5019) displays equivalent Bmax compared to the combination of two anti-HER2 FSAs (v506 + v4184). The apparent K_D of v5019 for binding to SKOV3 was approximately 2 to 4-fold higher compared to either anti-HER2 FSA alone (v506 or v4184), or the combination of two anti-HER2 FSAs (v506 + v4184).

Table 4: Binding to SKOV3 cells

Antibody variant	K_D (nM)	Bmax
v506	2.713	29190
v4184	4.108	29204
v5019	8.084	47401
v506 + v4184	4.414	49062

[0287] The results in Figure 6E and Table 5 show that exemplary biparatopic anti-HER2 antibodies (v5019, 7091 and v10000) display approximately a 1.5 to 1.6-fold higher Bmax in binding to SKOV3 cells compared to two different anti-HER2 FSAs (v506 or v4184). The results also show that exemplary biparatopic anti-HER2 antibodies (v5019, 7091 and v10000) display equivalent Bmax compared to the combination of two anti-HER2 FSAs (v506 + v4184). The apparent K_D of v5019, v7091, v10000 and the combination of two anti-HER2 FSAs (v506 + v4184) for binding to SKOV3 was approximately 2 to 3-fold higher compared to either anti-HER2 FSA alone (v506 or v4184).

Table 5: Binding to SKOV3

Antibody Variant	K_D (nM)	Bmax
v506	4.8	30007
v4184	5.6	27628
v506 + v4184	10.0	49014
v5019	13.6	47693
v7091	14.5	44737
v10000	10.3	48054

[0288] Binding curves in the JIMT-1 cell line (HER2 2+) are shown in Figure 6B and Table 6. These results show that exemplary biparatopic anti-HER2 antibody (v5019) displays approximately a 1.5-fold higher Bmax in binding to JIMT-1 cells compared to an anti-HER2 FSAs (v506). The results also show that exemplary biparatopic anti-HER2 antibody (v5019) displays equivalent Bmax compared to the combination of two anti-HER2 FSAs (v506 + v4184). The apparent K_D of v5019 for binding to JIMT-1 was approximately 2-fold higher compared to the anti-HER2 FSA (v506), and was similar (approximately 1.2 fold greater) compared to the combination of two anti-HER2 FSAs (v506 + v4184).

Table 6: Binding to JIMT-1 cells

Antibody variant	K_D (nM)	Bmax
v506	1.875	4905
v5019	4.317	7203
v506 + v4184	5.057	7200

[0289] Binding curves in the MCF7 cell line (HER2 1+) are shown in Figure 6C, 6F and Tables 7 and 8. These results show that exemplary biparatopic anti-HER2 antibodies (v5019, 7091 and v10000) display approximately a 1.5-fold higher Bmax in binding to MCF7 cells compared to an anti-HER2 FSAs (v506). The results in Figure 6C also show that exemplary biparatopic anti-HER2 antibody (v5019) displays equivalent Bmax compared to the combination of two anti-HER2 FSAs (v506 + v4184). The apparent K_D of v5019 for binding to MCF7 was similar to the anti-HER2 FSA (v506) and the combination of two anti-HER2 FSAs (v506 + v4184).

Table 7: Binding to MCF7 cells

Antibody variant	K_D (nM)	Bmax
v506	1.301	542
v5019	1.506	872
v506 + v4184	2.095	903

The results in Figure 6F and Table 8 show that exemplary biparatopic anti-HER2 antibodies (v5019, v7091 and v10000) display approximately 1.6 to 1.7-fold greater Bmax compared to the FSA monospecific v506. The apparent K_D of v5019, v7091 and v10000 was similar to the anti-HER2 FSA (v506).

Table 8: Binding to MCF7 cells

Antibody Variant	K_D (nM)	Bmax
v506	3.5	571
v5019	5.6	968
v7091	6.5	918
v10000	3.7	915

[0290] Binding curves in the MDA-MB-231 cell line (HER2 0/1+) are shown in Figure 6D and Table 9. These results show that exemplary biparatopic anti-HER2 antibody (v5019) displays approximately a 1.5-fold higher Bmax in binding to MDA-MB-231 cells compared to an anti-HER2 FSA (v506). The results also show that exemplary biparatopic anti-HER2 antibody (v5019) displays equivalent Bmax compared to the combination of two anti-HER2 FSAs (v506 + v4184). The apparent K_D of v5019 for binding to MDA-MB-231 was approximately 2.4-fold lower compared to the anti-HER2 FSA (v506) and was approximately 1.7-fold higher compared to the combination of two anti-HER2 FSAs (v506 + v4184).

Table 9: Binding to MDA-MB-231 cells

Antibody Variant	K_D (nM)	Bmax
v506	8.364	0.9521
v5019	3.543	1.411
v506 + v4184	2.040	1.542

[0291] Binding curves in the WI-38 lung fibroblast cell line are shown in Figure 6G and Table 10. The WI-38 cell line is a normal lung epithelium that expresses basal levels (HER2 0+ , ~10,000 receptors/cell) of HER2 (Carter et al. 1992, PNAS, 89:4285-4289; Yarden 2000, HER2: Basic Research, Prognosis and Therapy). These results show that exemplary biparatopic anti-HER2 antibodies (v5019, v7091, v10000) displays equivalent cell surface decoration (Bmax) in binding to WI-38 cells compared to an anti-HER2 FSAs (v506); however, note that binding for v506 did not appear to reach saturation, and thus KD could not be determined. The apparent K_D among the exemplary biparatopic anti-HER2 antibodies was equivalent.

Table 10: Binding to WI-38 cells

Antibody Variant	K_D (nM)	Bmax
v506	Not determined	~366
v5019	7.0	380
v7091	8.3	371
v10000	8.4	418

[0292] These results show that an exemplary biparatopic anti-HER2 antibody can bind to HER2 1+, 2+ and 3+ tumor cells to levels that are approximately 1.5 to 1.6-fold greater than an anti-HER2 monospecific FSA, and that exemplary biparatopic anti-HER2 antibodies can bind to HER2 1+, 2+ and 3+ tumor cells to equivalent levels compared to the combination of two unique monospecific anti-HER2 FSAs with different epitope specificities. These results also show that the biparatopic anti-HER2 antibodies do not show increased binding (i.e. compared to monospecific anti-HER2 antibody, v506) to basal HER2 expressing cells that express approximately 10,000 HER2 receptors/cell or less, and that a threshold for increased cell surface binding to the biparatopic anti-HER2 antibodies occurs when the HER2 receptor level is approximately >10,000 receptors/cell. Based on this data it would be expected that the exemplary biparatopic anti-HER2 antibodies would have increased cell surface binding to HER2 3+, 2+ and 1+ tumor cells but would not have increased cell surface binding to non-tumor cells that express basal levels of the HER2 receptor at approximately 10,000 receptors or less.

Example 7: Ability of biparatopic anti-HER2 antibody to inhibit growth of HER2+ cells

[0293] The ability of an exemplary biparatopic anti-HER2 antibody to inhibit growth of cells expressing HER2 at the 3+ and 2+ level was measured. The experiment was carried out in the HER2 3+ cell lines BT-474, SKBr3, SKOV3, and HER2 2+ JIMT-1. The biparatopic anti-HER2 antibodies v5019, v7091 and v10000 were tested. The ability of the biparatopic anti-HER2 antibodies to inhibit the growth of BT-474 cells (200 nM antibody); SKOV3, SKBr3 and JIMT-1 cells (300 nM antibody) was measured as described below.

[0294] Test antibodies were diluted in media and added to the cells at 10 µl/well in triplicate. The plates were incubated for 3 days 37°C. Cell viability was measured using either AlamarBlue™ (Biosource # dal1100), or Celltiter-Glo® and absorance read as per the manufacturer's instructions. Data was normalized to untreated control and analysis was performed in GraphPad prism.

[0295] The growth inhibition results are shown in Figure 7A-E. A summary of the results is provided in Tables 11A and 11B. The results Figures 7A-B and Table 11A indicate that exemplary anti-HER2 biparatopic (v5019) is capable of growth inhibition of HER2+ SKOV3 and BT-474 cell lines. Figure 10A shows that anti-HER2 biparatopic antibody mediated the greatest growth inhibition of SKOV3 when compared to anti-HER2 FSA (v506) and when compared to the combination of two anti-HER2 FSA antibodies (v506 + v4184).

Table 11A: Growth Inhibition of HER2 3+ Cancer Cells

Treatment	% Survival	
	SKOV3 HER2 2+/3+	BT-474 HER2 3+
v506	88	37
v506 + v4184	96	32

Treatment	% Survival	
	SKOV3 HER2 2+/3+	BT-474 HER2 3+
v5019	77	43

[0296] The results in Figures 7C-E and Table 11B indicate that exemplary anti-HER2 biparatopic antibodies (v5019, v7091 and v10000) can inhibit growth of HER2 3+ SKBR3, HER2 2+/3+ SKOV3, and HER2 2+ JIMT-1 tumor cell lines. Figure 7C shows that anti-HER2 biparatopic antibodies v7091 and v10000 mediated the greatest growth inhibition of HER2 3+ SKBr3 breast tumor cells. Figure 7D shows that anti-HER2 biparatopic antibodies (v7091 and v10000) mediated the greatest growth inhibition of HER2 3+ SKOV3 ovarian tumor cells. Figure 7E shows that anti-HER2 biparatopic antibodies (v7091 and v10000) mediated the greatest growth inhibition of HER2 2+ Herceptin-resistant JIMT-1 tumor cells. In all cell lines tested, exemplary anti-HER2 biparatopic antibodies (v7091 and v10000) mediated greater growth inhibition compared to the anti-HER2 FSA monospecific antibody (v506).

Table 11B: Growth inhibition of HER2 3+ Cancer Cells

Treatment	% Survival		
	SKBr3 HER2 3+	SKOV3 HER2 2+/3+	JIMT-1 HER2 2+
v506	52	107	107
v5019	59	83	106
v7091	35	79	85
v10000	34	73	84

[0297] These results show that exemplary saturating concentrations of biparatopic anti-HER2 antibodies can growth inhibit HER2 3+ and 2+ breast and ovarian and HER2 2+ Trastuzumab resistant tumor cells approximately 20% greater than a FSA anti-HER2 monospecific antibody.

Example 8: Preferential binding of paratopes of biparatopic anti-HER2 antibodies to dimeric HER2 compared to HER2 ECD

[0298] This experiment was performed to determine the ability of the individual paratopes of exemplary biparatopic anti-HER2 antibodies to bind to dimeric HER2 and the HER2 ECD as a surrogate for differential binding between membrane bound HER2 (HER2-Fc) and the shed HER2 ECD. The experiment was carried out as follows.

[0299] Surface plasmon resonance (SPR) analysis: affinity of monovalent anti-HER2 antibodies (v1040 or v4182) for binding to the HER2 extracellular domain (sHER-2, Ebioscience BMS362,_encoding amino acid 23 - 652 of the full length protein) and HER2-Fc (dimeric HER2-Fc fusion encoding the amino acid 1 - 652 of the extracellular domain; Sino Biological Inc., 10004-H02H) was measured by SPR using the T200 system from Biacore (GE Healthcare). Binding to the HER2 ECD was determined by the following method. HER2 ECD in 10 mm Hepes pH 6.8, was immobilized on CM5 chip through amine coupling to a level of 44 RU (response units). Monovalent anti-HER2 antibodies were passed over the surface of the HER2 immobilized chip at concentrations ranging from 0.76-60 nM. Binding to the HER2-Fc was determined by the following method. HER2-Fc in 10 mm Hepes pH 6.8, was immobilized on CM5 chip through amine coupling to a level of 43 RU. Monovalent anti-HER2 antibodies were passed over the surface of the HER2 immobilized chip at concentrations ranging from 0.76-60 nM. Antibody concentrations were analyzed for binding in triplicate. Equilibrium dissociation binding constants (K_D) and kinetics (ka and kd) were determined using the single cycle kinetics method. Sensograms were fit globally to a 1: 1 Langmuir binding model. All experiments were conducted at room temperature.

[0300] Results are shown in Figure 8A, Figure 8B, Table 11C and Table 11D. The results in Figure 8A and Table 11C show SPR binding data of the monovalent anti-HER2 antibody (v1040; representing the antigen-binding domain on CH-B of exemplary anti-HER2 biparatopic antibody). Figure 8A illustrates the K_D values (nM) of v1040 binding to immobilized HER2 ECD or HER2-Fc and shows that monovalent anti-HER2 antibody has a lower K_D for binding to the HER2-Fc compared to the HER2 ECD. Table 11C shows the ka (1/M s) and kd (1/s) values of the monovalent anti-HER2 antibody (OA) compared to the full-sized anti-HER2 antibody (FSA) in binding to the HER2 ECD and HER2-FC ('HER2 mem'). This data shows comparable on (ka) and off (kd) rates of the OA and FSA for binding to the HER2 ECD and HER2-Fc.

Table 11C: ka (1/M s) and kd (1/s) values of the monovalent anti-HER2 antibody (OA) compared to the full-sized anti-HER2 antibody (FSA) in binding to the HER2 ECD and HER2-FC ('HER2 mem'

	ka (1/Ms)	kd (1/s)
OA vs. HER2 ECD	2.00E+05	6.15E-05
FSA vs. HER2 ECD	4.14E+05	2.01E-05
OA vs. HER2 mem	1.88E+05	4.38E-05
FSA vs. HER2 mem	3.41E+05	4.94E-06*

[0301] Results in Figure 8B and Table 11D show the SPR binding data of the monovalent anti-HER2 antibody (v4182; representing the antigen-binding domain on CH-A of exemplary anti-HER2 biparatopic antibody). Figure 8B illustrates the K_D values (nM) of v4182 binding to immobilized HER2 ECD or HER2-Fc and shows that monovalent anti-HER2 antibody has a lower K_D for binding to the HER2-Fc compared to the HER2 ECD. Table 11D shows the ka (1/M s) and kd (1/s) values of the monovalent anti-HER2 antibody (OA) compared to the full-sized anti-HER2 antibody (FSA) in binding to the HER2 ECD and HER2-FC (`HER2 mem'). This data shows comparable on rates (ka) and off rates (kd) of the OA and FSA for binding to the HER2 ECD and HER2-Fc.

Table 11D:

	ka (1/Ms)	kd (1/s)
OA vs. HER2 ECD	9.08E+04	6.17E-04
FSA vs. HER2 ECD	9.55E+04	3.93E-04
OA vs. HER2 mem	1.39E+05	2.04E-04
FSA vs. HER2 mem	1.77E+05	6.84E-05

[0302] These data show that each of the paratopes of the exemplary anti-HER2 biparatopic antibody have lower K_D values for binding to the dimeric HER2 antigen, a representative of membrane bound HER2, as compared to the HER2 ECD. Based on this data it would be expected that the exemplary anti-HER2 antibody would have a higher binding affinity for the membrane bound HER2 antigen as compared to the shed HER2 ECD that is present in the serum of diseased patients and can act as a sink for the therapeutic antibody (Brodowicz T, et al. Soluble HER-2/neu neutralizes biologic effects of anti-HER-2/neu antibody on breast cancer cells in vitro. Int J Cancer. 1997; 73:875-879). For example, baseline HER2 ECD levels ≤ 15 ng/mL; whereas patients with progressive disease have HER2 ECD ≥ 38 ng/mL.

Example 9: Whole cell loading and internalization of biparatopic anti-HER2 antibody in HER2 + cells

[0303] This experiment was performed to assess the ability of an exemplary biparatopic anti-HER2 antibody to be internalized in HER2 2+ cells. The direct internalization method was followed according to the protocol detailed in Schmidt, M. et al., Kinetics of anti-carcinoembryonic antigen antibody internalization: effects of affinity, bivalency, and stability. Cancer Immunol Immunother (2008) 57:1879-1890. Specifically, the antibodies were directly labeled using the AlexaFluor® 488 Protein Labeling Kit (Invitrogen, cat. no. A10235), according to the manufacturer's instructions.

[0304] For the internalization assay, 12 well plates were seeded with 1 × 10^5 cells / well and incubated overnight at 37°C + 5% CO2. The following day, the labeled antibodies were added at 200 nM in DMEM + 10% FBS and incubated 24 hours at 37°C + 5% CO2. Under dark conditions, media was aspirated and wells were washed 2 × 500 µL PBS. To harvest cells, cell dissociation buffer was added (250 µL) at 37°C. Cells were pelleted and resuspended in 100 µL DMEM + 10% FBS without or with anti-Alexa Fluor 488, rabbit IgG fraction (Molecular Probes, A11094) at 50 µg/mL, and incubated on ice for 30 min. Prior to analysis 300 µL DMEM + 10% FBS the samples filtered 4 µl propidium iodide was added. Samples were analyzed using the LSRII flow cytometer.

[0305] The ability of exemplary anti-HER2 biparatopic antibody to internalize in HER2+ cells is shown in Figure 9A and Figure 9B. Figure 9A shows the results of detectable surface and internal antibody in BT-474 cells following 24 h incubation with the exemplary anti-HER2 biparatopic antibody and anti-HER2 FSA control. These results show that incubation with exemplary anti-HER2 biparatopic antibody (v5019) results in approximately 2-fold more internalized antibody in BT-474 cells compared to the anti-HER2 FSA control. Figure 9B shows the results of detectable surface and internal antibody in JIMT-1 cells following 24 h incubation with the exemplary anti-HER2 biparatopic antibody and anti-HER2 FSA control. These results show that incubation with exemplary anti-HER2 biparatopic antibody (v5019) results in approximately 2-fold more internalized antibody in JIMT-1 cells compared to the anti-HER2 FSA control. The amount of surface staining post 24 h was comparable among the biparatopic anti-HER2 and anti-HER2 FSA in both BT-474 and JIMT-1 cells.

[0306] The results in Figure 10A-F show a comparison of detectable antibody bound to the surface of whole cells after 2 h at 4°C, compared to antibody bound to the surface following incubation for 24 h at 37°C; in addition to the amount of internalized antibody following 24 h at 37°C. Figure 10A shows the results in BT-474 cells following incubation with the exemplary anti-HER2 biparatopic antibody and anti-HER2 FSA control. These results show that incubation of exemplary anti-HER2 biparatopic antibody with BT-474 cells for 24 h results in approximately a 15% reduction of antibody detected on the surface of whole cells. Figure 10A also shows that incubation with exemplary anti-HER2 biparatopic antibody (v5019) results in approximately 2-fold more internalized antibody in BT-474 cells compared to the anti-HER2 FSA control.

[0307] Figure 10B shows the results in JIMT-1 cells following incubation with the exemplary anti-HER2 biparatopic antibody and anti-HER2 FSA control. Figure 10B is a repeat of the experiment shown in Figure 9B with the addition of surface staining following 2 h at 4°C. These results show that incubation of exemplary anti-HER2 biparatopic antibody with JIMT-1 cells for 24 h results in approximately a 57% reduction of antibody detected on the surface of whole cells. Figure 10B also shows that incubation with exemplary anti-HER2 biparatopic antibody (v5019) results more internalized antibody in BT-474 cells following 24 incubation at 37°C, compared to the anti-HER2 FSA control.

[0308] Figure 10C shows the results in SKOV3 cells following incubation with the exemplary anti-HER2 biparatopic antibody. These results show that incubation of exemplary anti-HER2 biparatopic antibody with SKOV3 cells for 24 h results in approximately a 32% reduction of antibody detected on the surface of whole cells.

[0309] Figure 10D shows the results in MCF7 cells following incubation with the exemplary anti-HER2 biparatopic antibody. These results show that incubation of exemplary anti-HER2 biparatopic antibody with MCF7 cells for 24 h results in approximately a 45% reduction of antibody detected on the surface of whole cells.

[0310] Figure 10E shows the results in SKOV3 cells following incubation with the exemplary anti-HER2 biparatopic antibodies, v5019, v7091 and v10000. These results show that incubation of exemplary anti-HER2 biparatopic antibodies results in 1.5 to 1.8-fold more internalized antibody with SKOV3 cells compared to the anti-HER2 FSA control. Incubation with the anti-HER2 FSA control for 24 h resulted in the greatest reduction (-77%) of antibody detected on the surface of whole cells.

[0311] Figure 10F shows the results in JIMT-1 cells following incubation with the exemplary anti-HER2 biparatopic antibodies, v5019, v7091 and v10000. These results show that incubation of exemplary anti-HER2 biparatopic antibodies results in 1.4 to 1.8-fold more internalized antibody with

JIMT-1 cells compared to the anti-HER2 FSA control. Incubation with the anti-HER2 biparatopic antibodies (v5019 and v10000) for 24 h resulted in the greatest reduction (-64%) of antibody detected on the surface of whole cells.

[0312] These results show that exemplary anti-HER2 biparatopic antibodies have superior internalization properties in HER2+ cells compared to a monospecific anti-HER2 FSA. The reduction of surface antibody detected following 24 h incubation at 37°C shows that an exemplary anti-HER2 biparatopic antibody is capable of reducing the amount of cell surface HER2 receptor following incubation in HER2+ cells and that surface HER2 reduction post incubation is greatest in HER2 2+ tumor cells.

Example 10: Cellular staining and location of an anti-HER2 biparatopic antibody following incubation with HER2+ cells at 1, 3 and 16 hours

[0313] This experiment was performed to analyze internalization of the exemplary anti-HER2 biparatopic antibody in HER2+ JIMT-1 cells at different time points and as an orthogonal method to that presented in Example 9 to analyze whole cell loading and internalization.

[0314] JIMT-1 cells were incubated with the antibody (v506, v4184, v5019, or a combination of v506 and v4184) at 200 nM in serum-free DMEM, 37 °C + 5% CO_2 for 1h, 3h and 16h. Cells were gently washed two times with warmed sterile PBS (500 ml/well). Cells were fixed with 250 ml of 10% formalin/PBS solution for 10 min at RT. The fixed cells were washed three times with PBS (500 µl/well), permeabilized with 250 µl/well of PBS containing 0.2%Triton X-100 for 5 min, and washed three times with 500 µl/well PBS. Cells were blocked with 500 µl/well of PBS + 5% goat serum for 1 h at RT. Blocking buffer was removed, and 300 µl/well secondary antibody (Alexa Fluor 488-conjugated AffiniPure Fab Fragment Goat anti-Human IgG (H+L); Jackson ImmunoResearch Laboritories, Inc.;109-547-003) was incubated for 1 h at RT. Cells were washed three times with 500 µl/well of PBS and the coverslips containing fixed cells were then mounted on a slide using Prolong gold anti-fade with DAPI (Life Technologies; #P36931). 60X single images were acquired using Olympus FV1000 Confocal microscope.

[0315] The results indicated that the exemplary anti-HER2 biparatopic antibody (v5019) was internalized into JIMT-1 cells at 3 h and was primarily located close to the nuclei. Comparing images at the 3h incubation showed a greater amount of internal staining associated with the anti-HER2 biparatopic antibody compared to the combination of two anti-HER2 FSAs (v506 +v4184) and compared to the individual anti-HER2 FSA (v506 or v4184). Differences in the cellular location of antibody staining were seen when the anti-HER2 biparatopic antibody (v5019) results were compared with the anti-HER2 FSA (v4184); where the anti-HER2 FSA (v4184) showed pronounced plasma membrane staining at the 1, 3 and 16 h time points. The amount of detectable antibody was reduced at the 16 h for the anti-HER2 FSA (v506), the combination of two anti-HER2 FSAs (v506 + v4184) and anti-HER2 biparatopic antibody treatments (data not shown).

[0316] These results show that the exemplary anti-HER2 biparatopic antibody v5019 was internalized in HER2+ cells and the internalized antibody was detectable after 3 h incubation. These results are consistent with the results presented in Example 9 that show exemplary anti-HER2 biparatopic antibody can internalize to greater amounts in HER2+ cells compared to an anti-HER2 FSA.

Example 11: ADCC of HER2+ cells mediated by biparatopic anti-HER2 antibody compared to controls

[0317] This experiment was performed in order to measure the ability of an exemplary biparatopic anti-HER2 antibody to mediate ADCC in SKOV3 cells (ovarian cancer, HER2 2+/3+).

[0318] Target cells were pre-incubated with test antibodies (10-fold descending concentrations from 45 µg/ml) for 30 min followed by adding effector cells with effector/target cell ratio of 5:1 and the incubation continued for 6 hours at 37°C + 5% CO_2. Samples were tested with 8 concentrations, 10 fold descending from 45 µg/ml. LDH release was measured using LDH assay kit.

[0319] Dose-response studies were performed with various concentrations of the samples with a effector/target (E/T) ratios of 5:1. 3:1 and 1:1. Half maximal effective concentration (EC_{50}) values were analyzed with the sigmoidal dose-response non-linear regression fit using GraphPad prism.

[0320] Cells were maintained in McCoy's 5a complete medium at 37 °C / 5% CO_2 and regularly sub-cultured with suitable medium supplemented with 10% FBS according to protocol from ATCC. Cells with passage number fewer than p10 were used in the assays. The samples were diluted to concentrations between 0.3-300 nM with phenol red free DMEM medium supplemented with 1% FBS and 1% pen/strep prior to use in the assay.

[0321] The ADCC results in HER2+ SKOV3 cells at an effector to target cell ratio of 5:1 are shown in Figure 11A and Table 12. These results show that the exemplary biparatopic anti-HER2 antibody (v5019) mediated the greatest percentage of maximum target cell lysis by ADCC when compared to the anti-HER2 FSA (v792) and combination of two different anti-HER2 FSAs (v792+v4184). The difference in maximum cell lysis mediated by the exemplary biparatopic anti-HER2 antibody was approximately 1.6-fold greater compared to the anti-HER2 FSA, and approximately 1.2-fold greater compared to a combination of two different anti-HER2 FSAs (v792 + v4184).

Table 12:

Antibody variant	EC_{50} (nM)	% Max cell Lysis
v792	~0.032	17.82
v5019	~0.164	28.57
v792 + v4184	~0.042	23.85

[0322] The ADCC results in HER2+ SKOV3 cells at an effector to target cell ratio of 3:1 are shown in Figure 11B and Table 13. These results show

that the exemplary biparatopic anti-HER2 antibody (v5019) mediated the greatest percentage of maximum target cell lysis by ADCC when compared to the anti-HER2 FSA (v792) and combination of two different anti-HER2 FSAs (v792+v4184). The difference in maximum cell lysis mediated by the exemplary biparatopic anti-HER2 antibody was approximately 1.3-fold greater compared to the anti-HER2 FSA, and approximately 1.8-fold greater compared to a combination of two different anti-HER2 FSAs (v792 + v4184).

Table 13:

Antibody Variant	EC_{50} (nM)	% Max Cell Lysis
v792	1.064	16.9
v5019	~0.4608	22.3
v792 + v4184	~1.078	12.3

[0323] The ADCC results in HER2+ SKOV3 cells at an effector to target cell ratio of 1:1 are shown in Figure 11C and Table 14. These results show that the exemplary biparatopic anti-HER2 antibody (v5019) mediated the greatest percentage of maximum target cell lysis by ADCC when to compared to the anti-HER2 FSA (v792) and combination of two different anti-HER2 FSAs (v792+v4184). The difference in maximum cell lysis mediated by the exemplary biparatopic anti-HER2 antibody was approximately 1.8-fold greater compared to the anti-HER2 FSA, and approximately 1.13-fold greater compared to a combination of two different anti-HER2 FSAs (v792 + v4184).

Table 14:

Antibody variant	EC_{50} (nM)	% Max Cell Lysis
v792	1.429	7.529
v5019	~1.075	13.29
v792 + v4184	~0.1121	11.73

[0324] The results in Figure 11 and Tables 12-14 show that the exemplary biparatopic HER2 antibody mediates the greatest ADCC of SKOV3 cells at different E:T ratios when compared to an anti-HER2 FSA and combination of two anti-HER2 FSAs. The observation of increased ADCC mediated by the anti-HER2 biparatopic antibody would be expected in HER2+ diseased patients who express variable and/or reduced circulating effector cells following chemotherapy (Suzuki E. et al. Clin Cancer Res 2007;13:1875-1882).The observations in Figure 11 are consistent with the whole cell binding Bmax data presented in Example 6, that shows an approximate 1.5-fold increase in cell binding to the exemplary anti-HER2 biparatopic antibody compared to the anti-HER2 FSA.

Example 12: Ability of exemplary anti-HER2 antibody to bind to HER2 ECD

[0325] An SPR assay was used to evaluate the mechanism by which an exemplary anti-HER2 biparatopic antibody binds to HER2 ECD; specifically, to understand whether both paratopes of one biparatopic antibody molecule can bind to one HER2 ECD (Cis binding; 1:1 antibody to HER2 molecules) or if each paratope of one biparatopic antibody can bind two different HER2 ECDs (Trans binding; 1:2 antibody to HER2 molecules). A representation of cis vs. trans binding is illustrated in Figure 14. The correlation between a reduced (slower) off-rate with increasing antibody capture levels (surface density) is an indication of Trans binding (i.e. one antibody molecule binding to two HER2 molecules.

[0326] Affinity and binding kinetics of the exemplary biparatopic anti-HER2 antibody (v5019) to recombinant human HER2 were measured and compared to that of monovalent anti-HER2 antibodies (v630 or v4182; comprising the individual paratopes of v5019) was measured by SPR using the T200 system from Biacore (GE Healthcare). Between 2000 and 4000 RU of anti-human Fc injected at concentration between 5 and 10 µg/ml was immobilized on a CM5 chip using standard amine coupling. Monovalent anti-HER2 antibody (v630 or v4182) and exemplary biparatopic anti-HER2 antibody (v5019) were captured on the anti-human Fc (injected at concentration ranging 0.08 to 8 µg/ml in PBST, 1 min at 10ul/min) at response levels ranging from 350 - 15 RU. Recombinant human HER2 was diluted in PBST and injected at starting concentration of either 120 nM, 200 nM or 300 nM with 3-fold dilutions and injected at a flow rate of 50 µl /min for 3 minutes, followed by dissociation for another 30 minutes at the end of the last injection. HER2 dilutions were analyzed in duplicate. Sensograms were fit globally to a 1: 1 Langmuir binding model. All experiments were conducted at 25°C.

[0327] The results are shown in Figure 12 and Figure 13.

[0328] The results in Figure 12A show the ka (1/Ms) of monovalent anti-HER2 (v630 and v4182) and exemplary biparatopic anti-HER2 antibody (v5019) for binding to recombinant human HER2 over a range of injected and captured antibody concentrations on the surface of the chip. These results show that ka does not change when for v630, v4182 and v5019 at different antibody capture levels.

[0329] The results in Figure 12B show the kd (1/s) of monovalent anti-HER2 (v630 and v4182) and exemplary biparatopic anti-HER2 antibody (v5019) for binding to recombinant human HER2 over a range of injected and captured antibody concentrations on the surface of the chip. These results show that kd decreased only for the exemplary anti-HER2 biparatopic antibody (v5019) at increasing antibody capture levels.

[0330] The results in Figure 12C show the K_D (M) of monovalent anti-HER2 (v630 and v4182) and exemplary biparatopic anti-HER2 antibody (v5019) for binding to recombinant human HER2 over a range of injected and captured antibody concentrations on the surface of the chip. These results show that K_D decreased only for the exemplary anti-HER2 biparatopic antibody (v5019) at increasing antibody capture levels. This result correlated to the decreasing kd values shown in Figure 15B.

[0331] The results in Figure 13A show the kd (1/s) of exemplary biparatopic anti-HER2 antibody (v5019) for binding to recombinant human HER2 over a range of antibody capture levels. These results show kd values are inversely proportional to higher RUs of antibody captured on the surface

of the chip (i.e slower off-rates at higher antibody capture levels). The results indicate that exemplary biparatopic anti-HER2 antibody (v5019) is capable of binding HER2 ECD2 and HER2 ECD4 on two separate HER2 molecules (i.e. trans binding) as is evidenced by the reduction in off-rate at higher antibody capture levels. This data is supported by a similar experiment presented in Figure 47 and discussed in Example 43, where bivalent monospecific anti-HER2 FSA (v506) demonstrated Cis binding (1:1 antibody to HER2) where the kd (1/s) and K_D (M) values remained constant at increasing antibody capture levels as is expected for this molecule.

[0332] The results in Figure 13B show the kd (1/s) of monovalent anti-HER2 antibody (v4182) for binding to recombinant human HER2 over a range of antibody capture levels. These results show no change in kd values over the range of different antibody RUs captured on the surface of the chip. These results show that monovalent anti-HER2 antibody (v4182) is binding monovalently 1:1 (cis binding).

[0333] The results in Figure 13C show the kd (1/s) of monovalent anti-HER2 antibody (v630) for binding to recombinant human HER2 over a range of antibody capture levels. These results show no change in kd values over the range of different antibody RUs captured on the surface of the chip. These results show that monovalent anti-HER2 antibody (v630) is binding monovalently 1:1 (cis binding). This data is supported by the experiment presented in Figure 47 and discussed in Example 43X, where the bivalent monospecific anti-HER2 FSA (v506) showed no change in kd (1/s).

[0334] The results in Figures 12, and Figure 13 indicate that exemplary biparatopic anti-HER2 antibody (v5019) is capable of simultaneously binding to two HER2 molecules in trans (antibody to HER2 ratio 1:2). The trans mechanism of binding detected by SPR is consistent with the higher cell surface saturation binding data (Bmax), presented in Example 6, in combination with the internalization data presented in Examples 9 and 10.

Example 13: Effect of exemplary biparatopic anti-HER2 antibody incubation on AKT phosphorylation in BT-474 cells

[0335] The ability of an exemplary anti-HER2 biparatopic antibody to reduce pAKT signaling in BT-474 cells was tested using the AKT Colorimetric In-Cell ELISA Kit (Thermo Scientifiic; cat no. 62215) according to the manufacturer's instructions with the following modifications. Cells were seeded at 5×10^3/well and incubated 24 h at 37°C + 5% CO_2. Cells were incubated with 100 nM antibody for with 30 min followed by a 15 min incubation with rhHRG-β1. Cells were washed, fixed, and permeabilized according to the instructions. Secondary antibodies (1:5000; Jackson ImmunoReasearch, HRP-donkey anti-mouse IgG, JIR, Cat#715-036-150, HRP-donkey anti-rabbit IgG, JIR, Cat#711-036-452) were added and the assay processed according to the manufacturer's instructions.

[0336] The results in Figure 15 show that incubation with exemplary anti-HER2 biparatopic antibody mediated an approximate 1.2-fold reduction in p-Akt levels in the presence of HRGβ1 relative to the human IgG control (CTL). The combination of two anti-HER2 FSAs (v506 + v4184) mediated the greatest reduction in p-Akt levels in the presence HRGβ1 that was approximately 1.5-fold less compared to the human IgG control. A modest reduction in p-Akt was detected with the exemplary anti-HER2 biparatopic antibody in the absence of ligand (HRGβ1) compared to the human IgG control antibody.

[0337] These data show that exemplary anti-HER2 biparatopic antibody can block ligand-activated signaling in HER2+ cells.

Example 14: Effect of biparatopic anti-HER2 antibody on cardiomyocyte viability

[0338] The effect of exemplary biparatopic anti-HER2 antibodies and ADCs on cardiomyocyte viability was measured in order to obtain a preliminary indication of potentially cardiotoxic effects.

[0339] iCell cardiomyocytes (Cellular Dynamics International, CMC-100-010), that express basal levels of the HER2 receptor, were grown according the manufacturer's instructions and used as target cells to assess cardiomyocyte health following antibody treatment. The assay was performed as follows. Cells were seeded in 96-well plates (15,000 cells/well) and maintained for 48 h. The cell medium was replaced with maintenance media and cells were maintained for 72h. To access the effects of antibody-induced cardiotoxicity, cells were treated for 72 h with 10 and 100 nM of, variants alone or in combinations. To access the effects of anthracycline-induced cardiotoxicity (alone or in combination with the exemplary biparatopic anti-HER2 antibodies), cells were treated with 3 uM (~IC_{20}) of doxorubicin for 1 hr followed by 72 h with 10 and 100 nM of, antibody variants alone or in combinations. Cell viability was assessed by quantitating cellular ATP levels with the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, G7570) and/or Sulphorhodamine (Sigma 230162-5G) as per the manufacturer's instructions.

[0340] The results are shown in Figure 16A-C. The results in Figure 16A show that incubation of the cardiomyocytes with therapeutically relevant concentrations of exemplary anti-HER2 biparatopic antibody (v5019) and exemplary anti-HER2 biparatopic-ADC (v6363), did not affect cardiomyocyte viability relative to the untreated control ('mock').

[0341] The results in Figure 16B show that incubation of the cardiomyocytes with therapeutically relevant concentrations of exemplary anti-HER2 biparatopic antibodies (v5019, v7091 and v10000), and exemplary anti-HER2 biparatopic-ADCs (v6363, v7148 and v10553), had no effect on cardiomyocyte viability relative to the untreated control ('mock'). Based on the results in Figure 16A and 16B it is expected that exemplary anti-HER2 biparatopic antibodies and exemplary anti-HER2 biparatopic-ADCs should not induce cardiomyopathy, for example through mitochondrial dysfunction, as is reported with other anti-HER2 targeting antibodies (Grazette L.P. et al. Inhibition of ErbB2 Causes Mitochondrial Dysfunction in Cardiomyocytes; Journal of the American College of Cardiology: 2004; 44:11).

[0342] The results in Figure 16C show that pretreatment of the cardiomyocytes with doxorubicin followed by incubation with therapeutically relevant concentrations of exemplary anti-HER2 biparatopic antibodies (v5019, v7091 and v10000) and exemplary anti-HER2 biparatopic-ADCs (v6363, v7148 and v10553), had no effect on cardiomyocyte viability relative to the untreated control + doxorubicin ('Mock + Dox'). Based on the results in Figure 16C it is expected that exemplary anti-HER2 biparatopic antibodies and exemplary anti-HER2 biparatopic-ADCs should not result in an

increased risk of cardiac dysfunction in patients receiving concurrent anthracycline treatment (Seidman A, Hudis C, Pierri MK, et al. Cardiac dysfunction in the trastuzumab clinical trials experience. J Clin Oncol (2002) 20:1215-1221).

[0343] Figures 16A-C show that incubation of cardiomyocytes with the anti-HER2 biparatopic antibodies and ADCs had equivalent effects compared to monospecific anti-HER2 FSA antibody (v506), anti-HER2 FSA combination (v506 + v4184) and ADC (v6246) when treated either alone, or in combination with doxorubicin. Based on these results, it is expected that exemplary anti-HER2 biparatopic antibodies and ADCs would not have greater cardiotoxic effects compared to anti-monospecific anti-HER2 FSA, trastuzumab or ADC, T-DM1.

Example 15: Cytotoxicity of exemplary biparatopic anti-HER2-ADCs in HER2+ cells

[0344] The ability of exemplary biparatopic anti-HER2-ADC antibodies (v6363, v7148 and v10553) to mediate cellular cytotoxicity in HER2+ cells was measured. Human IgG conjugated to DM1 (v6249) was used as a control in some cases. The experiment was carried out in HER2+ breast tumor cell lines JIMT-1, MCF7, MDA-MB-231, the HER2+ ovarian tumor cell line SKOV3, and HER2+ gastric cell line NCI-N87. The cytotoxicity of exemplary biparatopic anti-HER2-ADC antibodies in HER2+ cells was evaluated and compared to the monospecific anti-HER2 FSA-ADC (v6246) and anti-HER2-FSA-ADC + anti-HER2-FSA controls (v6246 + v4184). The method was conducted as described in Example 7 with the following modifications. The anti-HER2 ADCs were incubated with the target SKOV3 and JIMT-1 (Figure 17A and B) cells for 24 h, cells washed, media replaced and cell survival was evaluated after 5 day incubation at 37°C. The anti-HER2 ADCs were incubated with target MCF7 and MDA-MB-231 target cells for 6 h (Figure 17C and D), cells washed media replaced and cell survival was evaluated at 5 days incubation at 37°C. In Figure 17E-G, anti-HER2 ADCs were incubated continuously with target SKOV3, JIMT-1, NCI-N87 cells for 5 days. Cell viability was measured as described in Example 7 using either AlamarBlue™ (Figures 17A-D) or Celltiter-Glo® (Figures 17E-G).

[0345] The results are shown in Figure 17A-G and the data is summarized in Tables 15 and 16.

[0346] The results in Figure 17A and Table 15 and 16 show that exemplary anti-HER2 biparatopic-ADC (v6363) is more cytotoxic in JIMT-1 compared to the anti-HER2-FSA-ADC (v6246) and the combination of anti-HER2-FSA-ADC + anti-HER2 FSA (v6246 +v4184). The exemplary anti-HER2 biparatopic-ADC had a superior EC_{50} that was approximately 13-fold lower compared to the anti-HER2 FSA-ADC control.

[0347] The results in Figure 17B and Table 15 show that exemplary anti-HER2 biparatopic-ADC (v6363) is more cytotoxic in SKOV3 compared to the anti-HER2-FSA-ADC (v6246) and the combination of anti-HER2-FSA-ADC + anti-HER2 FSA (v6246 +v4184). The exemplary anti-HER2 biparatopic-ADC had a superior EC_{50} that was approximately 5-fold lower compared to the anti-HER2 FSA-ADC control.

[0348] The results in Figure 17C and Table 15 show that exemplary anti-HER2 biparatopic-ADC (v6363) is more cytotoxic in MCF7 compared to the anti-HER2-FSA-ADC (v6246) and the combination of anti-HER2-FSA-ADC + anti-HER2 FSA (v6246 +v4184). The exemplary anti-HER2 biparatopic-ADC had a superior EC_{50} that was approximately 2-fold lower compared to the anti-HER2 FSA-ADC control.

[0349] The results in Figure 17D and Table 15 show that exemplary anti-HER2 biparatopic-ADC (v6363) is more cytotoxic in MDA-MB-231 compared to the anti-HER2-FSA-ADC (v6246) and the combination of anti-HER2-FSA-ADC + anti-HER2 FSA (v6246 +v4184). The exemplary anti-HER2 biparatopic-ADC had a superior EC_{50} that was approximately 2-fold lower compared to the anti-HER2 FSA-ADC control.

Table 15:

Antibody Variant	EC_{50} (nM)			
	SKOV3	JIMT-1	MCF7	MDA-MB-231
v6246	0.9225	5.942	122.0	~1075
v6246 + 4184	3.146	12.68	~24432	136.4
v6363	0.1776	0.4443	58.55	141.0

[0350] The results in Figure 17E and Table 16 show that exemplary anti-HER2 biparatopic-ADCs (v6363, v7148 and v10553) are more cytotoxic in SKOV3 ovarian tumor cells compared to the anti-HER2-FSA-ADC (v6246). The exemplary anti-HER2 biparatopic-ADCs had a superior EC_{50} values that were approximately 2 to 7-fold lower compared to the anti-HER2 FSA-ADC control.

[0351] The results in Figure 17F and Table 16 show that exemplary anti-HER2 biparatopic-ADCs (v6363, v7148 and v10553) are more cytotoxic in JIMT-1 breast tumor cells compared to the anti-HER2-FSA-ADC (v6246). The exemplary anti-HER2 biparatopic-ADCs had a superior EC_{50} values were approximately 6 to 9-fold lower compared to the anti-HER2 FSA-ADC control.

[0352] The results in Figure 17G and Table 16 show that exemplary anti-HER2 biparatopic-ADCs (v6363, v7148 and v10553) are cytotoxic in NCI-N87 gastric tumor cells. The exemplary anti-HER2 biparatopic-ADCs had has approximately equivalent EC_{50} values compared to the anti-HER2 FSA-ADC control.

Table 16:

Antibody variant	EC_{50} (nM)		
	SKOV3	JIMT-1	NCI-N87
v6246	0.22	3.52	1.04
v6363	0.03	0.56	1.33
v7148	0.06	0.56	2.74
v10553	0.09	0.39	1.69

These results show that exemplary anti-HER2 biparatopic-ADCs (v6363, v7148 and v10553) are more cytotoxic compared to anti-HER-FSA-ADC control in HER2 3+, 2+, and 1+ breast tumor cells. These results also show that exemplary anti-HER2 biparatopic-ADCs (v6363, v7148 and v10553) are cytotoxic in HER2 2/3+ gastric tumor cells. These results are consistent with the internalization results presented in Example 9.

Example 16: Effect of a biparatopic anti-HER2 antibody in a human ovarian cancer cell xenograft model

[0353] The established human ovarian cancer cell derived xenograft model SKOV3 was used to assess the anti-tumor efficacy of an exemplary biparatopic anti-HER2 antibody.

[0354] Female athymic nude mice were inoculated with the tumor via the insertion of a $1mm^3$ tumor fragment subcutaneously. Tumors were monitored until they reached an average volume of $220mm^3$; animals were then randomized into 3 treatment groups: IgG control, anti-HER2 FSA (v506), and biparatopic anti-HER2 antibody (v5019).

[0355] Fifteen animals were included in each group. Dosing for each group is as follows:

[0356] A) IgG control was dosed intravenously with a loading dose of 30mg/kg on study day 1 then with maintenance doses of 20 mg/kg twice per week to study day 39.

[0357] B) Anti-HER2 FSA (v506) was dosed intravenously with a loading dose of 15 mg/kg on study day 1 then with maintenance doses of 10 mg/kg twice per week to study day 18. On days 22 through 39, 5 mg/kg anti-HER2 FSA was dosed intravenously twice per week. Anti-HER2 FSA (v4184) was dosed simultaneously at 5 mg/kg intraperitoneally twice per week.

[0358] C) Biparatopic anti-HER2 antibody was dosed intravenously with a loading dose of 15mg/kg on study day 1 then with maintenance doses of 10 mg/kg twice per week to study day 39.

[0359] Tumor volume was measured twice weekly over the course of the study, number of responders and median survival was assessed at day 22. The results are shown in Figure 18 and Table 17.

[0360] The biparatopic anti-HER2 and anti-HER2 FSA demonstrated superior tumor growth inhibition compared to IgG control. The biparatopic anti-HER2 antibody induced superior tumor growth inhibition compared to anti-HER2 FSA combination (Figure 18A). The biparatopic anti-HER2 antibody was associated with an increase in the number of responding tumors compared to anti-HER2 FSA v506 at day 22 (11 and 5, respectively) (Table 17). The exemplary biparatopic anti-HER2 antibody and anti-HER2 FSA demonstrated superior survival compared to IgG control. The biparatopic anti-HER2 antibody had a superior median survival (61 days) compared to anti-HER2 FSA (36 days)(Figure 18B and Table 17). On study day 22 a second anti-HER2 FSA (v4184) was added in combination to the anti-HER2 FSA (v506). The combination of two anti-HER2 FSAs induced a further tumour growth inhibition compared to anti-HER2 FSA (v506) alone.

Table 17:

n=15, Day 22	IgG	v506	v5019
Mean TV (mm3) (% change from Baseline)	1908 (+766%)	1291 (+486%)	697 (+217%)
% TGI	0	32	63
Responders (TV <50% of control)	0/15	5/15	11/15
Median Survival (days)	22	36	61

Example 17: Effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) in a human ovarian cancer cell line xenograft model

[0361] The established human ovarian cancer cell derived xenograft model SKOV3 was used to assess the anti-tumor efficacy of an exemplary biparatopic anti-HER2 antibody conjugated to DM1 (v6363).

[0362] Female athymic nude mice were inoculated with the tumor via the insertion of a $1mm^3$ tumor fragment subcutaneously. Tumors were monitored until they reached an average volume of $220mm^3$; animals were then randomized into 3 treatment groups: IgG control, anti-HER2 FSA-ADC, and a biparatopic anti-HER2-ADC.

[0363] Fifteen animals were included in each group. Dosing for each group is as follows:

[0364] A) IgG control was dosed intravenously with a loading dose of 30mg/kg on study day 1 then with maintenance doses of 20mg/kg twice per week to study day 39.

[0365] B) Anti-HER2 FSA-ADC (v6246) was dosed intravenously with a loading dose of 10 mg/kg on study day 1 then with a maintenance dose of 5 mg/kg on day 15 and 29.

[0366] C) Biparatopic anti-HER2 antibody-ADC (v6363) was dosed intravenously with a loading dose of 10 mg/kg on study day 1 then with a maintenance dose of 5 mg/kg on day 15 and 29.

[0367] Tumor volume was measured throughout the study, and the number of responders and median survival was assessed at day 22. The

results are shown in Figure 19. A summary of the results is shown in Table 18.

[0368] The biparatopic anti-HER2-ADC and anti-HER2 FSA-ADC inhibited tumor growth better than IgG control (Figure 19A and Table 18). The biparatopic anti-HER2-ADC inhibited tumor growth to a greater degree than did the anti-HER2 FSA-ADC. The biparatopic anti-HER2-ADC group was associated with an increase in the number of responding tumors compared to anti-HER2 FSA-ADC (11 and 9, respectively). The biparatopic anti-HER2-ADC and anti-HER2 FSA-ADC groups demonstrated superior survival compared to IgG control (Figure 19B and Table 18). The biparatopic anti-HER2 antibody group demonstrated median survival of 61 days compared to the anti-HER2 FSA-ADC which had a median survival of 36 days (Figure 19B and Table 18).

Table 18:

n=15, Day 22	IgG	v6246	v6363
Mean TV (mm3) (% change from Baseline)	1908 (+766%)	873 (+297%)	632 (+187%)
% TGI	0	54%	67%
Responders (TV <50% of control)	0/15	9/15	11/15
Median survival (days)	22	36	61

Example 18: Effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) in a human primary cell xenograft model (HBCx-13b)

[0369] The trastuzumab resistant patient derived xenograft model from human breast cancer, HBCx-13B, was used to assess the anti-tumor efficacy of an exemplary biparatopic anti-HER2 antibody conjugated to DM1.

[0370] Female athymic nude mice were inoculated with the tumor via the insertion of a 20mm^3 tumor fragment subcutaneously. Tumors were monitored until they reached an average volume of 100mm^3; animals were then randomized into 3 treatment groups: anti-HER2 FSA (v506), anti-HER2 FSA-ADC (v6246), and the biparatopic anti-HER2-ADC (v6363). Seven animals were included in each group. Dosing for each group was as follows:

[0371] A) Anti-HER2 FSA was dosed intravenously with a loading dose of 15mg/kg on study day 1 and maintenance doses of 10mg/kg administered on study days 4, 8, 11, 15, 18, 22, and 25.

[0372] B) Anti-HER2 FSA-ADC was dosed intravenously with a loading dose of 10 mg/kg on study day 1 then with a maintenance dose of 5 mg/kg on day 22.

[0373] C) Biparatopic anti-HER2 antibody-ADC was dosed intravenously with a loading dose of 10 mg/kg on study day 1 then with a maintenance dose of 5 mg/kg on day 22.

[0374] Tumor volume was measured throughout the study, and mean tumor volume, complete response, and zero residual disease parameters were assessed at Day 50. The results are shown in Figure 20. A summary of the results is shown in Table 19.

[0375] The biparatopic anti-HER2-ADC and anti-HER2 FSA-ADC demonstrated greater tumor growth inhibition compared to an anti-HER2 FSA (v506). The biparatopic anti-HER2-ADC inhibited tumor growth better than the anti-HER2 FSA-ADC. The biparatopic anti-HER2-ADC group as compared to the anti-HER2 FSA-ADC group was associated with an increase in the number of tumors showing complete responses (more than a 10% decrease below baseline), 7 and 4 respectively, and showing zero residual disease, 5 and 2 respectively.

Table 19:

n=7, Day 50	v506	v6246	v6363
Mean TV (mm3) (% change from Baseline)	1149 (+1018%)	262 (+153%)	26 (-75%)
% TGI	0%	77%	98%
Complete response (>10% baseline regression)	0	4/7	7/7
Zero residual disease (TV<20mm3)	0	2/7	5/7

Example 19: Effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) in a human primary cell xenograft model (T226)

[0376] The patient derived trastuzumab resistant xenograft model from human breast cancer, T226, was used to assess the anti-tumor efficacy of an exemplary biparatopic anti-HER2-ADC.

[0377] Female athymic nude mice were inoculated with the tumor via the insertion of a 20mm^3 tumor fragment subcutaneously. Tumors were monitored until they reached an average volume of 100mm^3; animals were then randomized into 4 treatment groups: IgG control (n=15), anti-HER2 FSA (v506; n=15), anti-HER2 FSA-ADC (v6246; n=16), and the biparatopic anti-HER2-ADC conjugate (v6363; n=16). Dosing for each group was as follows:

[0378] A) IgG control was dosed intravenously with a loading dose of 15 mg/kg on study day 1 and maintenance doses of 10 mg/kg administered on study days 4, 8, 11, 15, 18, 22, and 25

[0379] B) Anti-HER2 FSA was dosed intravenously with a loading dose of 15 mg/kg on study day 1 and maintenance doses of 10 mg/kg administered on study days 4, 8, 11, 15, 18, 22, and 25

[0380] C) Anti-HER2 FSA-ADC was dosed intravenously with 5 mg/kg on study days 1 and 15

[0381] D) Biparatopic anti-HER2-ADC conjugate was dosed intravenously with 5 mg/kg on study days 1 and 15.

[0382] Tumor volume was measured throughout the course of the study, and mean tumor volume and complete response parameters were assessed at day 31. The results are shown in Figure 21. A summary of the results is shown in Table 20.

[0383] The biparatopic anti-HER2-ADC and anti-HER2 FSA-ADC demonstrated better tumor growth inhibition compared to the anti-HER2 FSA (v506) and IgG control. The exemplary biparatopic anti-HER2-ADC induced equivalent tumor growth inhibition and complete baseline regression compared to anti-HER2 FSA-ADC (Figure 21 and Table 20) in this model.

Table 20:

Day 31	IgG (n=13)	v506 (n=13)	v6246 (n=16)	v6363 (n=16)
Mean TV (mm3) (% change from Baseline)	1797 (+1728%)	1611 (+1573)	422 (+332%)	572 (+483%)
% TGI (vs. hIgG)	0%	11%	77%	68%
Complete response (>10% baseline regression)	0/13	0/14	1/16	1/16

Example 20: Effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) in a human primary cell xenograft model (HBCx-5)

[0384] The patient derived trastuzumab resistant xenograft model from human breast cancer, HBCx-5 (invasive ductal carcinoma, luminal B), was used to assess the anti-tumor efficacy of an exemplary biparatopic anti-HER2-ADC.

[0385] Female athymic nude mice were inoculated with the tumor via the insertion of a 20mm^3 tumor fragment subcutaneously. Tumors were monitored until they reached an average volume of 100 mm^3; animals were then randomized into 4 treatment groups: IgG control (n=15), anti-HER2 FSA (v506; n=15), anti-HER2 FSA-ADC (v6246; n=16), and the biparatopic anti-HER2-ADC (v6363; n=16). Dosing for each group was as follows:

[0386] A) IgG control was dosed intravenously with a loading dose of 15 mg/kg on study day 1 and maintenance doses of 10 mg/kg administered on study days 4, 8, 11, 15, 18, 22, and 25

[0387] B) Anti-HER2 FSA was dosed intravenously with a loading dose of 15 mg/kg on study day 1 and maintenance doses of 10 mg/kg administered on study days 4, 8, 11, 15, 18, 22, and 25

[0388] C) Anti-HER2 FSA-ADC was dosed intravenously with 10 mg/kg on study days 1 and 15, 22, 29, 36

[0389] D) Biparatopic anti-HER2-ADC was dosed intravenously with 10 mg/kg on study days 1 and 15, 22, 29, 36.

[0390] Tumor volume was measured throughout the course of the study, and the mean tumor volume, T/C ratio, number of responders, complete response, and zero residual disease parameters were assessed at day 43. The results are shown in Figure 22. A summary of the results is shown in Table 21.

[0391] The biparatopic anti-HER2-ADC and anti-HER2 FSA-ADC demonstrated better tumor growth inhibition compared to an anti-HER2 FSA (v506) and IgG control. The exemplary biparatopic anti-HER2-ADC induced equivalent tumor growth inhibition and had an increased number of responders compared to anti-HER2 FSA-ADC (Figure 22 and Table 21) in the trastuzumab resistant HBCx-5 human breast cancer xenograft model.

Table 21:

Day 43	IgG (n=4)	Herceptin (n=5)	T-DM1 (n=7)	6363 (n=7)
Mean TV (mm3) (% change from Baseline)	922 (+693%)	815 (+598%)	193 (+65%)	241 (+106%)
T/C (IgG) ratio	1	0.88	0.21	0.26
Responders (TV<50% of control)	0/4	1/5	6/7	7/7
Complete response (>10% baseline regression)	0/4	0/5	1/7	0/7
Zero residual disease (TV<20mm3)	0/4	0/5	0/7	0/7

Example 21: Effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) to anti-HER2 treatment resistant tumors in a human cell line xenograft model (SKOV3)

[0392] The established human ovarian cancer cell derived xenograft model SKOV3, described in Example 17, was used to assess the anti-tumor efficacy of an exemplary biparatopic anti-HER2-ADC in anti-HER2 treatment resistant tumors.

[0393] The methods were followed as described in Example 17 with the following modifications. A cohort of animals was dosed with an anti-HER2 antibody intravenously with 15 mg/kg on study day 1 and with 10 mg/kg on day 4, 8, 15; however, this treatment failed to demonstrate an efficacious response by day 15 in this model. This treatment group was then converted to treatment with the exemplary biparatopic anti-HER2 antibody drug conjugate (v6363) and was dosed with 5 mg/kg and on study day 19 and 27 and 15 mg/kg on study day 34, 41 and 48.

[0394] Tumor volume was measured twice weekly throughout the course of the experiment.

[0395] The results are shown in Figure 23 and indicate that the group treated with exemplary biparatopic anti-HER2-ADC (v6363) showed tumor regression to a mean tumor volume less than the initial mean starting volume of 220mm^3.

Example 22: Effect of a biparatopic anti-HER2 antibody drug conjugate (ADC) on anti-HER2 treatment resistant tumors in human primary cell xenograft model (HBCx-13b)

[0396] The trastuzumab resistant patient derived xenograft model from human breast cancer, HBCx-13B, was used to assess the anti-tumor efficacy of an exemplary biparatopic anti-HER2 antibody conjugated to DM1.

[0397] The methods were followed as described in Example 18 with the following modifications. A cohort of animals was dosed with a bi-specific anti-ErbB family targeting antibody intravenously with 15 mg/kg on study day 1 and with 10 mg/kg on day 4, 8, 15, 18, 22, and 25; however, this treatment failed to demonstrate an efficacious response. This treatment group was then converted to treatment with the exemplary biparatopic anti-HER2 antibody drug conjugate (v6363) and was dosed with 10 mg/kg on days 31, 52 and with 5 mg/kg on day 45. Tumor volume was measured throughout the duration of the study.

[0398] The results are shown in Figure 24. These results show that the exemplary biparatopic anti-HER2-ADC (v6363) prevented tumour progression. From the first dose to day 57 the tumour volume of the v6363 treated group increased by less than 2% while in the same interval the v506 treated group grew by more than 110%.

Example 23: Analysis of fucose content of an exemplary biparatopic anti- HER2 antibody

[0399] Glycopeptide analysis was performed to quantify the fucose content of the N-linked glycan of the exemplary biparatopic anti-HER2 antibodies (v5019, v7091 and v10000).

[0400] The glycopeptide analysis was performed as follows. Antibody samples were reduced with 10 mM DTT at 56°C 1 h and alkylated with 55 mM iodoacetamide at RT 1 h and digested in-solution with trypsin in 50 mM ammonium bicarbonate overnight at 37° C. Tryptic digests were analyzed by nanoLC-MS/MS on a QTof-Ultima. The NCBI database was searched with Mascot to identify protein sequences. MaxEnt3 (MassLynx) was used to deconvolute the glycopeptide ions and to quantify the different glycoforms.

[0401] A summary of the glycopeptide analysis results is in Table 22. The N-linked glycans of exemplary biparatopic anti-HER2 antibodies (v5019, v7091 and v10000) are, approximately 90% fucosylated (10% N-linked glycans without fucose). The N-linked glycans of monospecific anti-HER2 FSA (v506) are, approximately 96% fucosylated (4% N-linked glycans without fucose) and Herceptin® is approximately 87% fucosylated (4% N-linked glycans without fucose).

Table 22: Fc N-linked Glycopeptide Analysis

Antibody Variant	Average % of Glycopeptides Observed With Fucose	Average % of Glycopeptides Observed Without Fucose	n
v506	96.4	3.6	5
Herceptin®	86.5	13.4	4
v5019	90.5	9.4	6
v7091	89.9	26.9	3
v10000	89.2	10.7	5

[0402] These results show that biparatopic anti-HER2 antibodies (with a heterodimeric Fc), expressed transiently in CHO cells, have approximately 3% higher fucose content in the N-glycan compared to commercial Herceptin®. The homodimeric anti-HER2 FSA (v506), expressed transiently in CHO cells, has the highest fucose content of approximately 96%.

Example 24: Thermal Stability of an exemplary biparatopic anti-HER2 antibody

[0403] Thermal stability of exemplary biparatopic anti-HER2 antibodies (v5019, v7091 and v10000) and ADCs (v6363, v7148 and v10533) was measured by DSC as described below.

[0404] DSC was performed in the MicroCal™ VP-Capillary DSC (GE Healthcare) using a purified protein sample (anti-HER2 biparatopic antibodies and anti-HER2 biparatopic-ADCs) adjusted to about 0.3 mg/ml in PBS. The sample was scanned from 20 to 100°C at a 60°C/hr rate, with low

feedback, 8 sec filter, 5 min preTstat, and 70 psi nitrogen pressure. The resulting thermogram was analyzed using Origin 7 software.

[0405] The thermal stability results of exemplary biparatopic anti-HER2 antibodies (v5019, v7091 and v10000) are shown in Figure 25A-C. Figure 25A shows the thermogram for v5019; the Fc and chain A Fab of each have a T_m of 75° Celsius and the chain B scFv of 5019 has a T_m of 69° Celsius. Figure 25B shows the thermogram for v10000; the Fc CH3 domain has a T_m 82° Celsius, Fab chain A has T_m of 76.5° Celsius and the chain B scFv has a T_m of 69.5° Celsius. Figure 25C shows the thermogram for v7091; the Fc CH3 domain has a T_m 82° Celsius, Fab chain A has T_m of 76.7° Celsius and the chain B scFv has a T_m of 69.5° Celsius.

[0406] The thermal stability results of exemplary biparatopic anti-HER2 ADCs (v6363, v7148 and v10533) are shown in Figure 26A-C. Figure 26A shows the thermogram for v6363; the Fc has a T_m of 75° Celsius and the chain A Fab and Fc CH3 domain have a T_m of 75° Celsius. The chain B scFv of 6363 has a T_m of 69° Celsius. Figure 26B shows the thermogram for v10553; the Fc CH3 domain has a T_m of 83° Celsius, the chain A Fab has a T_m of 75.7° Celsius and the chain B scFv has a T_m of 66.2° Celsius. Figure 26C shows the thermogram for v7148; the Fc CH3 domain has a T_m of 82.6° Celsius, the chain A Fab has a T_m of 74.8° Celsius and the chain B scFv has a T_m of 66.6° Celsius.

[0407] The exemplary biparatopic antibodies and ADCs have thermal stability comparable to wildtype IgG.

Example 25: Ability of an exemplary Biparatopic anti-HER2 antibody to elicit ADCC of breast tumor cells expressing varying levels of HER2

[0408] The ability of exemplary biparatopic antibody (v5019) to elicit dose-dependent ADCC of HER2 positive 3+, 2+, and 0/1+ HER2 expressing (triple-negative) breast cancer cell lines was examined. The ADCC experiments were performed as described in Example 11 with the exception that NK effector cell to target cell ratio remained constant at 5: 1 .

[0409] The ADCC results are shown in Figure 27 and Table 23. The results in Figure27A-C show that exemplary biparatopic antibody (v5019) elicits approximately 1.2 to 1.3-fold greater maximum cell lysis of HER2 positive 3+, 2+ and 0/1+ HER2 expressing breast cancer cells compared to Herceptin®. The results also show that v5019 (90% N-glycans with fucose) more effectively mediates ADCC of HER2 positive 3+, 2+ and 0/1+ HER2 expressing breast cancer despite having approximately a 4% higher fucose content in the N-glycan (resulting in lower binding affinity to CD16 on NK cells) compared to Herceptin® (86% N-glycans with fucose; Example 23). The higher target cell killing elicited by v5019 is presumably due to increased tumor cell decoration as described in Example 6.

Table 23: ADCC of HER2 3+, 2+ and 0/1+ HER2 expressing breast cancer cells

Treatment	SKBr3 HER2 3+		JIMT-1 HER2 2+		MDA-MB-231 HER2 0/1+	
	Max % Target Cell Lysis	EC_{50} (nM)	Max % Target Cell Lysis	EC_{50} (nM)	Max % Target Cell Lysis	EC_{50} (nM)
v5019	30	~0.9	60	0.001	53	0.9
Herceptin®	23	~0.9	51	0.002	44	0.9

[0410] The ADCC results in Figure 27D show that exemplary biparatopic antibodies (v7091 and v10000) elicit similar maximal cell lysis compared to Herceptin® in the basal HER2 expressing WI-38 cell line. The ADCC results support the cell binding data (Example 6), showing that a threshold for increased binding and ADCC occurs when the HER2 receptor levels are greater than 10,000 HER2/cell. Based on this data it would be expected that the exemplary biparatopic anti-HER2 antibodies would have increased cell surface binding and ADCC of HER2 3+, 2+ and 1+ tumor cells but would not have increase cell surface binding and ADCC of non-tumor cells that express basal levels of the HER2 receptor at approximately 10,000 receptors or less.

Example 26: Effect of Antibody Afucosylation on ADCC

[0411] The ability of afucosylated exemplary biparatopic antibodies (v5019-afuco, 10000-afuco) to elicit dose-dependent ADCC of HER2 positive 2/3+, 2+ and 0/1+ HER2 expressing (triple-negative) breast cancer cell lines, was examined. ADCC experiments were performed as described in Example 11, in SKOV3 cells, MDA-MB-231 cells and ZR75-1 cells with the exception that a constant NK effector cell or PBMC effector to target (E:T) cell ratio of 5:1 was used. Afucosylated exemplary biparatopic antibodies were produced transiently in CHO cells as described in Example 1, using the transiently expressed RMD enzyme as described in von Horsten et al. 2010 Glycobiology 20:1607-1618. The fucose content of v5019-afuco and v10000-afuco were measured as described in Example 23 and determined to be less < 2% fucosylated (data not shown). Data using NK effector cells is shown in Figure 28A-B, while data using PBMCs is shown in Figure 28C.

[0412] Figure 28A, Figure 28B and Table 24 show that afucosylated v5019 (v5019-afuco) elicits ADCC of HER 2/3+ and 0/1+ HER2 expressing breast cancer cells with approximately 1.5 to 1.7-fold higher maximum cell lysis than Herceptin®.

Table 24: ADCC of HER2 2/3+ and basal HER2 expressing (triple-negative) breast cancer cells

Treatment	SKOV3 HER2 2+/3+		MDA-MD-231 HER2 0/1+	
	Max % Target Cell Lysis	EC_{50} (nM)	Max % Target Cell Lysis	EC_{50} (nM)
v5019-afucosylated	24	~0.6	58	-0.6
Herceptin®	14	~0.6	40	-0.3

[0413] The results in Figure 28C and Table 25 show that v10000 elicits ADCC of HER2 2+ ZR-75-1 breast cancer cells with approximately 1.3-fold greater maximal cell lysis than Herceptin®, and v10000-afuco elicits approximately 1.5-fold greater maximal cell lysis than Herceptin®.

Table 25: ADCC of HER2 2/3+ breast cancer cells

Treatment	ZR-751 HER2 2+	
	Max % Target Cell Lysis	EC_{50} (nM)
v10000	28	~0.06
v10000-afucosylated	32	~0.7
Herceptin®	21	~0.5

[0414] The ADCC results show that the exemplary afucosylated biparatopic antibodies (v5019-afuco, v10000-afuco) elicit approximately 15-25% greater maximum cell lysis compared to the fucosylated antibodies (v5019 Example 25, v10000) when Herceptin® is used as a benchmark. These results show that reducing the fucose content of the Fc N-glycan results in increased maximal cell lysis by ADCC.

Example 27: Ability of exemplary Biparatopic anti-HER2 antibody to inhibit growth of HER2 3+ breast cancer cells in the presence of exogenous growth-stimulatory ligands (EGF and HRG)

[0415] The ability of 5019 to inhibit growth of HER2 3+ breast cancer cells in the presence of exogenous growth-stimulatory ligands (EGF and HRG) was examined.

[0416] Test antibodies and exogenous ligand (10 ng/mL HRG or 50 ng/mL EGF) were added to the target BT-474 HER2 3+ cells in triplicate and incubated for 5 days at 37°C. Cell viability was measured using AlamarBlue™ (37°C for 2hr), absorbance read at 530/ 580 nm. Data was normalised to untreated control and analysis was performed using GraphPad Prism.

[0417] The results are shown in Figure 29 and Table 26. The results show that exemplary biparatopic antibody v5019 inhibits the growth of HER2 3+ breast cancer cells in the absence of growth stimulatory ligand (70% inhibition), as well as in the presence of EGF (40% inhibition) or HRG (~10% inhibition). The anti-HER2 monospecific FSA (v506) does not block EGF or HRG induced tumor cell growth via other erbB receptors EGFR and HER3. v5019 is superior to v506 in inhibiting HER2 and ligand-dependent dimerization and growth via other companion erbB receptors.

Table 26: Growth Inhibition of HER2 3+ Cancer Cells

Treatment	% Survival		
	Antibody only	+ EGF	+ HRG
Mock	100	122	110
v506	41	114	129
v5019	31	56	92

[0418] These results show that exemplary biparatopic antibody is capable of reducing ligand-dependent growth of HER2+ cells, presumably due binding of the anti-ECD2 chain A Fab arm and subsequent blocking of ligand stimulated receptor homo- and heterodimerization, and erbB signaling.

Example 28: Effect of a Biparatopic anti HER2 antibody in a Trastuzumab-resistant and chemotherapy resistant HER2 3+ patient-derived (PDX) metastatic breast cancer xenograft model of invasive ductal breast carcinoma

[0419] The HER2 3+ (ER-PR negative) patient derived xenograft model from invasive ductal human breast cancer, HBCx-13B, was used to assess the anti-tumor efficacy of an exemplary biparatopic anti-HER2 antibody, v7187. v7187 is an afucosylated version of v5019. The model is resistant to single agent trastuzumab, the combination of trastuzumab and pertuzumab (see example 31), capecitabine, docetaxel, and adriamycin/cyclophosphamide.

[0420] Female athymic nude mice were inoculated subcutaneously with a 20 mm^3 tumor fragment. Tumors were then monitored until reaching an average volume of 140 mm3. Animals were then randomized into 2 treatment groups: vehicle control and v7187 with eight animals in each group. IV Dosing was as follows. Vehicle control was dosed intravenously with 5 ml/kg of formulation buffer twice per week to study day 43. v7187 was dosed intravenously with 10 mg/kg twice per week to study day 43. Tumor volume was measured throughout the study, and other parameters assessed at day 43 as shown in Table 27.

[0421] The results are shown in Figure 30 and Table 27. The results show that tumors treated with vehicle control showed continual progression and exceeded 1600 mm^3 by study day 43. Mice treated with v7187 showed significantly greater tumor growth inhibition (T/C - 0.44) with a mean tumor volume of 740 mm^3 on day 43. v7187 induced responses in 5/8 tumors with a single tumor showing complete regression with zero residual disease on study day 43. Animals treated with v7187 had a superior response rate with 5/8 tumors responding to therapy compared to 0/8 mice treated with vehicle control. In addition, treatment with v7187 significantly delayed tumor progression compared to vehicle control with doubling times of 19 and 11 days respectively.

Table 27:

Tumour Response		Vehicle	V7087
Day 43	Mean TV (mm3) (% Change from Baseline)	1683 (+1079%)	740 (+422%)
	T/C ratio	1	0.44
	Responders (TV<50% of control)	0/8	5/8
	PR (>10% baseline regression)	0/8	1/8
	ZRD (TV<20mm3)	0/8	1/8
Time to progression	Doubling time (days)	11	19

[0422] These data show that the exemplary anti-HER2 biparatopic (v7187) is efficacious in a Trastuzumab+Pertuzumab resistant HER2 3+ metastatic breast cancer tumor xenograft model. V7187 treatment has a high response rate and can significantly impair tumor progression of standard of care treatment resistant HER2 3+ breast cancers.

Example 29: Assessment of Biparatopic anti-HER2 ADC binding to HER2+ tumor cell lines

[0423] The ability of exemplary biparatopic anti-HER2 ADCs to bind and saturate HER2 positive 3+, 2+, breast and ovarian tumor cell lines was analyzed by FACS as described in Example 6.

[0424] The data is shown in Figure 31. Figure 31A shows v6363 binding to SKOV3 tumor cell lines with approximately a 2.0-fold greater Bmax (MFI) than T-DM1 (v6246) at saturating concentrations. Figure 31B shows v6363 binds to JIMT-1 tumor cell lines with approximately a 1.6-fold greater Bmax (MFI) than T-DM1 (v6246) at saturating concentrations. These data show that v6363 (ADC) has similar tumor cell binding properties of increased cell surface binding compared to the parent unconjugated v5019 antibody (Example 6). Conjugation of v5019 with SMCC-DM1 (v6363) does not alter the antigen-binding properties of the antibody.

[0425] The FACS binding assay was repeated to include direct comparison to the exemplary biparatopic antibodies (v5019, v7091 and v10000) and ADCs (v6363, v7148 and v10553). The data is shown in Figure 31C and Figure 31D. The exemplary biparatopic anti-HER2 ADCs (v6363, v7148 and v10553) have equivalent cell surface saturation (Bmax) compared to the unlabeled biparatopic antibodies (v5019, v7091 and v10000).

[0426] These data show that conjugation of exemplary biparatopic antibodies (v5019, v7091 and v10000) with SMCC-DM1 does not alter the binding properties. The exemplary anti-HER2 biparatopic anti-HER2 ADCs (v6363, v7148 and v10553) have approximately 1.5-fold (or greater) increased cell surface binding compared to a monospecific anti-HER2 ADC (v6246, T-DM1).

Example 30: Dose-Dependent Tumour Growth Inhibition of an exemplary anti-HER2 biparatopic-ADC in a HER2 3+ (ER-PR negative) patient derived xenograft model

[0427] The HER2 3+ (ER-PR negative) patient derived xenograft model from invasive ductal human breast cancer, HBCx-13B, was used to assess the anti-tumor efficacy of an exemplary biparatopic anti-HER2 ADC, v6363. The model is resistant to single agent trastuzumab, the combination of trastuzumab and pertuzumab (see example 31), capecitabine, docetaxel, and adriamycin/cyclophosphamide.

[0428] Female athymic nude mice were inoculated with the tumor via the subcutaneous insertion of a 20 mm^3 tumor fragment. Tumors were monitored until they reached an average volume of 160 mm^3; animals were then randomized into 5 treatment groups: non-specific human IgG control, and 4 escalating doses of v6363. 8-10 animals were included in each group. Dosing for each group was as follows. IgG control was dosed intravenously with 10 mg/kg twice per week to study day 29. v6363 was dosed intravenously with 0.3, 1, 3, or 10 mg/kg on study days 1, 15, and 29. Tumor volume was assessed throughout the study and parameters assessed as indicated in Table 29.

[0429] The results are shown in Figure 32 and Table 28. These results show that the exemplary anti-HER2 biparatopic ADC (v6363) mediated dose-dependent tumor growth inhibition in the Trastuzumab-resistant HBCx-13b PDX model (Figure 32A). In addition, v6363 improved overall survival in a dose-dependent manner, with median survival time of more than 63 days for 3 mg/kg and 10 mg/kg doses compared to 43 days for IgG control (Figure 32B and Table 28). The 3 mg/kg dose was associated with an increased response rate (5/10) compared to control (0/8). All mice treated with v6363 at 10 mg/kg dose not only responded to therapy (9/9) but also showed prevention of tumor progression. Moreover, the majority of tumors had objective partial responses (7/9) and, at the end of the study, many had zero residual disease (6/9). v6363 was well tolerated at all doses, no adverse events were observed and no body weight loss was observed.

Table 28:

Tumour Response		IgG	6363 0.3 mg/kg	6363 1 mg/kg	6363 3 mg/kg	6363 10 mg/kg
Day 43	Mean TV (mm3) (% change from Baseline)	1963 (+1119%)	1916 (+1073%)	1613 (+895%)	1268 (+682%)	84 (-49%)
	T/C (IgG) ratio	1	0.97	0.82	0.64	0.04
	Responders (TV<50% of control)	0/8	0/10	2/10	5/10	9/9
	PR (> 10% baseline regression)	0/8	0/10	0/10	0/10	7/9

Tumour Response		IgG	6363 0.3 mg/kg	6363 1 mg/kg	6363 3 mg/kg	6363 10 mg/kg
	ZRD (TV<20mm3)	0/8	0/10	0/10	0/10	6/9
Time to progression	Tumor doubling time (days)	9	9	14	17	52
Survival Response	Median Survival (Days)	43	41	50	>63	>63
Body Weight	% Change from Baseline	+10%	+10%	+9%	+5%	+0%

[0430] These data show that the exemplary anti-HER2 biparatopic ADC (v6363) is efficacious in a Trastuzumab+Pertuzumab resistant HER2 3+ metastatic breast cancer tumor xenograft model. v6363 treatment is associated with a high response rate, significantly impairs tumor progression, and prolongs survival in a standard of care resistant HER2 3+ breast cancers.

Example 31: Biparatopic anti-HER2-ADC Compared to Standard of Care Combinations in the Trastuzumab Resistant PDX HBCx-13b

[0431] The efficacy of v6363 in a HER2 3+, ER-PR negative Trastuzumab resistant patient-derived breast cancer xenograft model (HBCx-13b), was evaluated and compared to to the combination of: Herceptin™ + Peijeta™ ; and Herceptin™ + Docetaxel.

[0432] Female athymic nude mice were inoculated with the tumor via the subcutaneous insertion of a 20 mm3 tumor fragment. Tumors were monitored until they reached an average volume of 100 mm3; animals were then randomized into 4 treatment groups (8-10 animals/group): non-specific human IgG control, Herceptin™ +Docetaxel, Herceptin™ +Perjeta™ , and v6363. Dosing for each group was as follow. IgG control was dosed intravenously with 10 mg/kg twice per week to study day 29. Herceptin™ +Docetaxel combination Herceptin™ was dosed intravenously with 10 mg/kg IV twice weekly to study day 29 and Docetaxel was dosed intraperitoneally with 20 mg/kg on study day 1 and 22. Herceptin™ +Perjeta™ combination Herceptin was dosed intravenously with 5 mg/kg twice per week to study day 29 and Peijeta™ was dosed intravenously with 5 mg/kg twice per week to study day 29. The dosing of Herceptin™ and Perjeta™ was concurrent. v6363 was dosed intravenously with 10 mg/kg on study day 1, 15, and 29.

[0433] The results are shown in Figure 33 and Table 29. Figure 33A shows tumor volume over time, and Figure 33B shows a survival plot. These results show that the combination of Herceptin™ + Perjeta™ did not produce any tumor growth inhibition compared to control IgG and exceeded 1800 mm^3 on day 39. The combination of Herceptin™ + Docetaxel did not significantly reduce tumor growth but did prolong median survival to 53 days compared to 43 days for IgG control. v6363 produced significant tumor growth inhibition (T/C - 0.04), where, all tumors responded to therapy and 7/10 tumors experienced complete regressions (zero residual disease). v6363 significantly prolonged survival compared to both combination therapies. Body weights across cohorts were not significantly affected by treatments.

Table 29:

Tumour Response		IgG	HerceptinTM + PerjetaTM	HerceptinTM + Docetaxel	v6363 10mg/kg
Day 39	Mean TV (mm3) (% change from Baseline)	1809 (+1023%)	1975 (+1085%)	1328 (+714%)	76 (-54%)
	T/C (IgG) ratio	1.0	1.10	0.73	0.04
	Responders (TV<50% of control)	0/8	0/8	1/10	9/9
	PR (>10% baseline regression)	0/8	0/8	0/10	8/9
	ZRD (TV<20mm3)	0/8	0/8	0/10	6/9
Survival Response	Median Survival (days)	43	39	53	>63
Body Weight	% Change from Baseline	+10%	+7%	+3%	-2%

[0434] These results show that exemplary anti-HER2 biparatopic ADC (v6363) is superior to standard of care combinations with respect to all parameters tested in this xenograft model.

Example 32: Efficacy of a Biparatopic anti-HER2-ADC in HER2+ Trastuzumab-Resistant Breast Cancer Cell Derived Tumour Xenograft Model

[0435] The efficacy of v6363 in a HER2 3+ Trastuzumab resistant breast cancer cell-derived (JIMT-1, HER2 2+) xenograft model was evaluated (Tanner et al. 2004. Molecular Cancer Therapeutics 3: 1585-1592).

[0436] Female RAG2 mice were inoculated with the tumor subcutaneously. Tumors were monitored until they reached an average volume of 115 mm^3; animals were then randomized into 2 treatment groups: Trastuzumab (n=10) and v6363. Dosing for each group was as follows. Trastuzumab was dosed intravenously with 15 mg/kg on study day 1 and 10 mg/kg twice per week to study day 26. v6363 was dosed intravenously with 5 mg/kg on study days 1 and 15 and with 10 mg/kg on day 23 and 30 and 9 mg/kg on day 37 and 44.

[0437] The results are shown in Figure 34 and Table 30. These results show that v6363 significantly inhibited tumor growth (T/C - 0.74) compared to Trastuzumab on study day 36. v6363 and Trastuzumab treatment did not significantly change body weight. v6363 serum exposure was 17.9 µg/ml 7 days after the first 10 mg/kg dose.

Table 30:

Tumour Response		Trastuzumab	6363
Day 36	Mean TV (mm3) (% change from Baseline)	718 (+541)	532 (+335%)
	T/C (Tras) ratio	1	0.74
	Responders (TV<50% of control)	1/10	2/13
	PR (>10% baseline regression)	0/10	0/13
	ZRD (TV<20mm3)	0/10	0/13
Body Weight	% Change from Baseline	+5.8%	+3.1%
Drug Exposure (day 7)	Mean Serum Concentration (ug/ml)	187.2	17.9

[0438] These results show that exemplary anti-HER2 biparatopic ADC (v6363) is efficacious in a Trastuzumab-resistant breast cancer and has a potential utility in treating breast cancers that are resistant to current standards of care.

Example 33: FcγR binding to heterodimeric Fc of anti-HER2 biparatopic antibodies and anti-HER2 biparatopic-ADCs

[0439] The binding of anti-HER2 biparatopic antibody (v5019, v7019 v10000) and ADC (v6363, v7148 and v10553) having a heterodimeric Fc, to human FcγRs was assessed and compared to anti-HER2 FSA (v506) and ADC (v6246) having a homodimeric Fc.

[0440] Affinity of FcγR to antibody Fc region was measured by SPR using a ProteOn XPR36 (BIO-RAD). HER2 was immobilized (3000 RU) on CM5 chip by standard amine coupling. Antibodies were antigen captured on the HER2 surface. Purified FcγR was injected various concentration (20-30 µl/min) for 2 minutes, followed by 4 minute dissociation. Sensograms were fit globally to a 1: 1 Langmuir binding model. Experiments were conducted at 25°C.

[0441] The results are shown in Table 31. The exemplary heterodimeric anti-HER2 biparatopic antibodies and ADCs bound to CD16aF, CD16aV158, CD32aH, CD32aR131, CD32bY163 and CD64A with comparable affinities. Conjugation of the antibodies with SMCC-DM1 does not negatively affect FcγR binding. The heterodimeric anti-HER2 biparatopic antibodies have approximately 1.3 to 2-fold higher affinity to CD16aF, CD32aR131, CD32aH compared to homodimeric anti-HER2 FSA (v506) and ADC (v6246). These results show that the heterodimeric anti-HER2 biparatopic antibodies and ADCs bind different polymorphic forms of FcγRs on immune effector cells with similar or greater affinity than a WT homodimeric IgG1.

Table 31: Human FcγR Binding by SPR

Variant	10uM CD16a v158		10uM CD16aF		11uM CD32aR131		10uM CD32aH		10uM CD32b Y163		100nM CD64A	
	KD Ave	SD	KD Ave	SD	KD Ave	SD	KD Ave	SD	KD Ave	SD	KD Ave	SD
v506	**1.5E-07**	2E-08	**7.1E-07**	1.E-08	**7.6E-07**	1.E-07	**6.3E-07**	2E-08	**2.4E-06**	1.E-07	**8.64E-10**	4.33E-10
v6246	**1.6E-07**	2E-08	**7.0E-07**	9.E-09	**7.4E-07**	7.E-08	**6.3E-07**	2E-08	**2.1E-06**	7.E-08	**1.08E-09**	5.13E-10
v10000	**1.2E-07**	1E-08	**4.8E-07**	2.E-08	**5.1E-07**	9.E-08	**4.6E-07**	2E-08	**1.5E-06**	7.E-08	**8.41E-10**	4.74E-10
v10553	**1.2E-07**	2E-08	**4.9E-07**	2.E-07	**3.5E-07**	1.E-07	**3.6E-07**	4E-09	**1.2E-06**	7E-08	**4.95E-10**	1.41E-10
v7091	**1.2E-07**	1E-08	**5.1E-07**	2.E-08	**5.6E-07**	9.E-08	**5.0E-07**	3E-08	**1.7E-06**	8E-08	**9.68E-10**	5.05E-10
v7148	**1.2E-07**	2E-08	**5.4E-07**	2.E-07	**3.7E-07**	1.E-07	**4.2E-07**	1E-08	**1.5E-06**	1.E-07	**5.77E-10**	2.02E-10
v5019	**1.3E-07**	1E-08	**5.2E-07**	1.E-08	**5.6E-07**	6.E-08	**4.7E-07**	2E-08	**1.6E-06**	2.E-07	**8.44E-10**	4.88E-10
v6363	**1.2E-07**	2E-08	**4.SE-07**	1.E-07	**3.5E-07**	1.E-07	**3.4E-07**	1E-08	**1.2E-06**	5.E-08	**4.58E-10**	1.13E-10

Example 34: Efficacy of exemplary anti-HER2 biparatopic antibodies in vivo in a trastuzumab sensitive ovarian cancer cell derived tumour xenograft model

[0442] The established human ovarian cancer cell derived xenograft model SKOV3, described in Example 17, was used to assess the anti-tumor efficacy of the exemplary biparatopic anti-HER2 antibodies, v5019, v7091 and v10000.

[0443] Female athymic nude mice were inoculated with a tumor suspension of 325,000 cells in HBSS subcutaneously on the left flank. Tumors were monitored until they reached an average volume of 190 mm^3 and enrolled in a randomized and staggered fashion into 4 treatment groups: non-specific human IgG control, v5019, v7091, and v10000. Dosing for each group was as follows. Non-specific human IgG was dosed intravenously with 10 mg/kg starting on study day 1 twice per week to study day 26. V5019, v7091, and v10000 were dosed intravenously with 3 mg/kg starting on study day 1 twice per week to study day 26. Tumor volume was measured throughout the study, and the parameters listed in Table 32 were measured at day 29.

[0444] The data are presented in Figure 35A (tumor growth), Figure 35B (survival plot) and Table 32 and show that treatment with v5019, v7091

and v10000 resulted in comparable tumor growth inhibition (T/C: 0.53-0.71), number of responding tumors, time to progression, and survival on study day 29 compared to IgG control. The serum exposure of v5019, v7091, and v10000 was similar (31-41 microg/ml) on study day 7.

Table 32:

Tumour Response		IgG (n=8)	v5019 (n=11)	V7091 (n=11)	V10000 (n=11)
Day 29	Mean TV (mm3) (% change from Baseline)	1903 (+899%)	1001 (+416%)	1354 (+618%)	1114 (+503%)
	T/C (Tras) ratio	1	0.53	0.71	0.58
	Responders (TV<50% of control)	1/8	5/11	4/11	6/11
	PR (>10% baseline regression)	0/8	1/11	0/11	0/11
	ZRD (TV<20mm3)	0/8	0/11	0/11	0/11
Time to progression	Tumor doubling time (days)	12	15	16	15
Survival	Median survival (days)	29	Na	37	41
Drug Exposure (day 7)	Mean Serum Concentration (ug/ml)	na	31.2	41.0	31.2

[0445] These results show that the exemplary anti-HER2 biparatopic antibodies, v5019, v7091, and v10000) have potential utility in treating moderately Trastuzumab sensitive HER2 overexpressing ovarian cancers.

Example 35: Exemplary biparatopic anti-her2 antibodies dose-dependently inhibit tumour growth in the trastuzumab-sensitive ovarian cancer cell derived tumour xenograft

[0446] The established human ovarian cancer cell derived xenograft model SKOV3, described in Example 17, was used to assess the dose-dependent efficacy of an exemplary biparatopic anti-HER2 antibody, v10000.

[0447] Female athymic nude mice were inoculated with a tumor suspension of 325,000 cells in HBSS subcutaneously on the left flank. Tumors were monitored until they reached an average volume of 190 mm^3 and enrolled in a randomized and staggered fashion into 6 treatment groups: non-specific human IgG control and 5 escalating doses of v10000. 9-13 animals were included in each group. Dosing for each group was as follows. IgG control was dosed intravenously with 10 mg/kg twice per week to study day 26. V10000 was dosed intravenously with 0.1, 0.3, 1, 3, or 10 mg/kg twice per week.

[0448] The data are presented in Figure 36 and Table 33 and show that treatment with v10000 dose dependently induces tumor growth inhibition (T/C: 0.28-0.73) compared to control IgG. In addition, v10000 was dose-dependently associated with responding tumors (7/9 at 10 mg/kg and 3/11 at 0.1 mg/kg) increased time to progression (24 days at 10 mg/kg and 12 days at 0.1 mg/kg) on study day 29. The serum exposure of v10000 on day 7 was dose dependent and increased from 0.46 microg/ml with a 0.1 mg/kg dose to 79.3 microg/ml with a 10 mg/kg dose.

Table 33:

Tumor Response		IgG (n=8)	V10000, 10 mg/kg (n=9)	V10000, 3 mg/kg (n=ll)	V10000, 1 mg/kg (n=ll)	V10000, 0.3 mg/kg (n=13)	V10000, 0.1 mg/kg (n=ll)
Day 29	Mean TV (mm3) (% change from Baseline)	1903 (+899%)	543 (+281%)	1114 (+503%)	1534 (+688%)	1535 (+694%)	1385 (+643%)
	T/C ratio	1	0.28	0.58	0.81	0.81	0.73
	Responders (TV<50% of control)	1/8	7/9	6/11	2/11	3/13	3/11
	PR (>10% baseline regression)	0/8	1/9	0/11	0/11	0/13	0/11
	ZRD (TV<20mm3)	0/8	0/9	0/11	0/11	0/13	0/11
Time to Progression	Tumor doubling time (days)	12	24	15	14	12	12
Drug Exposure (D ay 7)	Mean Serum Concentration (ug/ml)	na	79.3	31.2	4.7	1.5	0.46

[0449] These results show that the exemplary anti-HER2 biparatopic antibody, v10000, inhibits tumor progression in a dose-dependent manner.

Example 36: Ability of anti-HER2 biparatopic antibody and anti-HER2 biparatopic-ADC to inhibit growth of cell lines expressing HER2, and EGFR and/or HER3 at the 3+, 2+ or 1+ levels

[0450] The following experiment was performed to measure the ability of an exemplary biparatopic anti-HER2 antibody (v10000) and corresponding biparatopic anti-HER2 ADC (v10553) to inhibit growth of a selection of breast, colorectal, gastric, lung, skin, ovarian, renal, pancreatic, head and neck, uterine and bladder tumor cell lines that express HER2, and EGFR and/or HER3 at the 3+, 2+, 1+ or 0+ level as defined

by IHC.

[0451] The experiment was conducted as follows. The optimal seeding density for each cell line was uniquely determined to identify a seeding density that yielded approximately 60-90% confluency after the 72 hr duration of the assay. Each cell line was seeded at the optimal seeding density, in the appropriate growth medium per cell line, in a 96-well plate and incubated for 24°C at 36°C and 5% CO_2. Antibodies were added at three concentrations (v10000 at 300, 30 and 0.3 nM; v10553 at 300, 1, 0.1 nM), along with the positive and vehicle controls. The positive control chemococktail drug combination of 5-FU (5-fluorouracil), paclitaxel, cisplatin, etoposide (25 microM), the vehicle control consisted of PBS. The antibody treatments and controls were incubated with the cells for 72 h in a cell culture incubator at 36°C and 5% CO_2. The plates were centrifuged at 1200 RPM for 10 min and culture medium completely removed by aspiration. RPMI SFM medium (200 microL) and MTS (20 microL) was added to each well and incubated at 36°C and 5% CO_2 for 3 h. Optical density was read at 490 nM and percent growth inhibition was determined relative to the vehicle control.

[0452] The results are shown in Figure 37 and a summary of all test results are shown in Figure 38. Figure 37A shows the growth inhibition results of v10000. These results show that v 10000 can inhibit growth of breast, colorectal, gastric, lung, skin, ovarian, renal, pancreatic, head and neck, uterine, and endometrial tumor cell lines that express HER2 and coexpress EGFR and/or HER3 at the 3+, 2+, 1+ or 0+ level. The activity of v10000 and v10553 at 300 nM is summarized in Figure 38, where '+' indicates cell lines that showed a reduction in cell viability at 300 nM that was > 5% of the vehicle control, and '-' indicates ≤ 5% viability of the vehicle control.

[0453] Figure 37B shows the growth inhibition results of v10553. These results show that v10553 can inhibit growth of breast, colorectal, gastric, lung, skin, ovarian, renal, pancreatic, head and neck, uterine and bladder tumor cell lines that express HER2 and coexpress EGFR and/or HER3 at the 3+, 2+, 1+ or 0+ level (see also Figure 38). The results plotted in Figure 37B are defined by cell lines that showed a minimum of dose-dependent growth inhibition at 300 and 1 nM, and where the growth inhibition at 1 nM is equal or greater than 5% (Figure 37B).

[0454] These results show that exemplary biparatopic antibody v10000 and ADC v10553 can inhibit growth of tumor cells originating from breast, colorectal, gastric, lung, skin, ovarian, renal, pancreatic, head and neck, uterine and bladder histologies that express HER2 at the 3+, 2/3+, 2+, 1+ and 0/1+ levels and that coexpress EGFR and/or HER3 at the 2+, 1+ levels.

Example 37: Ability of anti-HER2 biparatopic antibodies to mediate ADCC of HER2 2+, 1+ and 0/1+ cancer cells

[0455] The following experiment was conducted to determine the ability of anti-HER2 biparatopic antibodies to mediate ADCC of tumor cells that express HER2 at the 2+, 1+ and/or 0/1+ levels and that coexpress EGFR and/or HER3 at the 2+ or 1+ level. The anti-HER2 biparatopic antibodies tested were 5019, 10000, and 10154 (an afucosylated version of v10000), with Herceptin™ and v506 as controls.

[0456] The ADCC experiment was conducted as described in Example 11 and Example 25 with E/T: 5:1 with NK-92 effector cells (Figure 39), and as described in Example 26 with E/T 30:1 with PBMC effector cells.

[0457] The results are shown in Figure 39 (NK-92 effector cells) and Figure 40 (PBMC effector cells). Figure 39A shows the ADCC results of the HER2 2+ head and neck tumor cell line (hypopharyngeal carcinoma), FaDu, where the anti-HER2 biparatopic elicits approximately 15% maximal cell lysis. Figure 39C shows the ADCC results of the HER2 1+ BxPC3 pancreatic tumor cell line, and Figure 39D the results of the HER2 2+ MiaPaca2 pancreatic tumor cell line. Figure 39B shows the ADCC results of the HER2 0/1+ A549 NSCLC (non-small cell lung cancer) tumor cell line. In the BxPC3, MiaPaca2 and A549 tumor cell lines, v10000 mediated approximately 5% maximal tumor cell lysis.

[0458] Figure 40 shows the ADCC results in A549, NCI-N87, and HCT-116 cells, where PBMCs were used as the effector cells. Figure 40A shows the ADCC results of the HER2 0/1+ A549 NSCLC tumor cell line, where v10000 elicited ~ 28% maximum cell lysis and this was comparable to Herceptin™ that has equivalent level of fucose content in the N-linked glycan. The exemplary 100% afucosylated (0% fucose) biparatopic v10154 shows an increase in maximal cell lysis (40% maximum cell lysis) and increased potency compared to v10000 and Herceptin that have approximately 88% fucose in the N-linked glycan.

[0459] Figure 40B shows the ADCC results of the HER2 3+ gastric tumor cell line, NCI-N87. Figure 40B shows that exemplary biparatopic v5019 (approximately 88% fucosylated) mediates approximately 23% maximal cell lysis and has a lower EC50 compared to Trastuzumab v506 (approximately 98% fucosylated).

[0460] Figure 40C shows the ADCC results of the HER2 1+ HCT-116 colorectal tumor cell line. Figure 40C shows that exemplary biparatopic v5019 (approximately 88% fucosylated) mediates approximately 25% maximal cell lysis and is more potent compared to Trastuzumab v506 (approximately 98% fucosylated).

[0461] These results show that exemplary anti-HER2 biparatopic antibodies can elicit ADCC of HER2 01/+, 2+ and 3+ tumor cells that originate from head and neck, gastric, NSCLC, and pancreatic tumor histologies. ADCC in the presence of NK-92 cells as the effector cells had an apparent HER2 2+ receptor level requirement (i.e. 2+ or greater) to show higher (> 5%) percentage of maximum cell lysis. However, when PBMC cells were used as effector cells higher levels of maximum cell lysis were achieved (>5% and up to 28% or 40%; v10000 and v10154, respectively) and were independent of HER2 receptor density as ADCC >5% was seen at the 0/1+, 1+ and 3+ HER2 receptor density levels.

Example 38: HER2 binding affinity and kinetics as measured by SPR

[0462] As indicated in Example 1, anti-HER2 biparatopic antibodies having different antigen-binding moiety formats were constructed, as described

in Table 1. The formats included scFv-scFv format (v6717), Fab-Fab format (v6902 and v6903), along with Fab-scFv format (v5019, v7091, and v10000). The following experiment was conducted to compare HER2 binding affinity and kinetics of these exemplary anti-HER2 biparatopic antibody formats.

[0463] Affinity and binding kinetics to murine HER2 ECD (Sino Biological 50714-M08H) was measured by single cycle kinetics with the T200 SPR system from Biacore (GE Healthcare). Between 2000-4000 RU of anti-human Fc was immobilized on a CM5 chip using standard amine coupling. 5019 was captured on the anti-human Fc surface at 50 RU. Recombinant HER2 ECD (1.8-120 nM) was injected at 50 µl/min for 3 minutes, followed by a 30 minute dissociation after the last injection. HER2 dilutions were analyzed in duplicate. Sensorgrams were fit globally to a 1:1 Langmuir binding model. All experiments were conducted at room temperature, 25°C.

[0464] The results in Table 34 show that Fab-scFv biparatopic antibodies (v5019 and v7091), Fab-Fab variants (v6902 and v6903) and the scFv-scFv variant (v6717) have comparable binding affinity (1-4 nM). The Fab-scFv variant v10000 had higher binding affinity (lower KD) of approximately 0.6 nM. The monspecific anti-HER2 ECD4 antibody (v506) and anti-HER2 ECD2 antibody (v4184) were included in the assay as controls. These results indicate that the molecular formats including v6717, v6902, v6903, v5019 and/or v7091 have equivalent binding affinities, and thus differences in function between these antibodies may be considered to result from differences in format.

Table 34:

	AVERAGE			STD DEV		
Antibody Variant	Ka (1/Ms)	Kd (1/s)	KD (M)	Ka (1/Ms)	Kd (1/s)	KD (M)
v506	7.34E+04	4.08E-05	5.56E-10	1.13.E+03	3.04E-06	3.28E-11
v4184	3.61E+04	5.46E-04	1.56E-08	7.78.E+03	2.80E-05	4.12E-09
v5019	6.01E+04	7.77E-05	1.29E-09	1.30.E+03	8.56E-07	4.74E-11
v7091	5.17E+04	1.19E-04	2.31E-09	2.70.E+03	1.49E-05	4.09E-10
v10000	6.44E+04	3.69E-05	5.79E-10	6.18.E+03	6.72.E-06	1.42.E-10
v6902	6.83E+04	1.72E-04	2.72E-09	1.93E+04	4.49E-05	1.43E-09
v6903	7.10E+04	1.71E-04	2.75E-09	3.60E+04	3.96E-06	1.34E-09
v6717	1.50E+05	5.33E-04	4.45E-09	1.28E+05	2.58E-04	2.11E-09

Example 39: Effect of anti-HER2 biparatopic antibody format on binding to HER2+ tumor cells

[0465] The following experiment was conducted to compare the whole cell binding properties (Bmax and apparent K_D) of exemplary anti-HER2 ECD2 × ECD4 biparatopic antibodies that have different molecular formats (e.g. v6717, scFv-scFv IgG1; v6903 and v6902 Fab-Fab IgG1; v5019, v7091 and v10000 Fab-scFv IgG1).

[0466] The experiment was conducted as described in Example 6. The results are shown in Figure 41 and Tables 35-38. Figure 41A and Table 35 shows the FACS binding results of the exemplary biparatopic antibodies to the BT474 HER2 3+ breast tumor cell line. The results show that all anti-HER2 antibodies have a higher Bmax (1.5 to 1.7-fold greater) when compared to the monospecific bivalent anti-HER2 antibody v506. The Fab-scFv (v5019, v7091 and v10000) and the Fab-Fab (v6903) formats had approximately a 1.7-fold increased Bmax and the scFv-scFv format (v6717) had a 1.5-fold increased Bmax compared to v506. An equimolar combination of FSAs v506 and v4184 resulted in a 1.7-fold increase in Bmax. The apparent K_D of the exemplary anti-HER2 biparatopic antibodies was approximately 2 to 3-fold higher compared to the monospecific v506.

Table 35: FACS binding BT-474

Antibody Variant	K_D(nM)	Bmax
v506	9.0	23536
v10000	16	39665
v506+ v4184	16	40320
v5019	21	39727
v7091	22	36718
v6717	30	36392
v6903	31	40321

[0467] Figure 41B and Table 36 shows the FACS binding results to the JIMT-1 HER2 2+ breast tumor cell line. The results show that all anti-HER2 antibodies have a higher Bmax (1.5 to 1.8-fold greater) when compared to the monospecific bivalent anti-HER2 antibody v506. The Fab-scFv (v7091 and v10000) and the Fab-Fab (v6903) formats had approximately a 1.7-fold increased Bmax, the scFv-scFv format (v6717) had a 1.5-fold increased Bmax and the Fab-scFv (v5019) and FSA combination (v506 + v4184) had a 1.8-fold increased Bmax compared to v506. The apparent K_D of the exemplary anti-HER2 biparatopic Fab-scFv antibodies was approximately 2 to 4-fold higher compared to the monospecific v506; whereas the K_D of the Fab-Fab (v6903) and scFv-scFv (v6717) were approximately 8-fold higher compared to v506.

Table 36: FACS Binding JIMT-1

Antibody Variant	K_D (nM)	Bmax
v506	3.5	2574

Antibody Variant	K_D (nM)	Bmax
v10000	7.6	4435
v506+ v4184	8.0	4617
v5019	12	4690
v7091	14	4456
v6717	26	3769
v6903	28	4452

[0468] Figure 41C and Table 37 shows the FACS binding results of the exemplary biparatopic antibodies to the HER2 1+ MCF7 breast tumor cell line. The results show that anti-HER2 antibody v10000 and FSA combination (v506 + v4184) have a 1.6-fold higher Bmax compared to the monospecific bivalent anti-HER2 antibody v506. The Fab-scFv (v5019, v7091) had approximately a 1.4-fold; the scFv-scFv format (v6717) a 1.3-fold, and the Fab-Fab format (v6903) had a 1.2-fold increased Bmax compared to v506. The apparent K_D of the exemplary anti-HER2 biparatopic Fab-scFv, Fab-Fab (v6903) and FSA combination (v506 + v4184) was approximately 2 to 3-fold lower compared to v506; whereas the K_D of the scFv-scFv (v6717) was approximately 3-fold higher compared to v506.

Table 37: FACS Binding MCF7

Antibody Variant	K_D (nM)	Bmax
v506+ v4184	4.5	1410
v7091	6.1	1216
v5019	6.3	1201
v10000	6.8	1381
v6903	7.1	1105
v506	12	889
v6717	32	1167

[0469] Figure 41D and Table 38 shows the FACS binding results of the exemplary biparatopic antibodies to the HER2 0/1+ MDA-MD-231 breast tumor cell line. The results show that exemplary biparatopic anti-HER2 antibodies had approximately 1.3 to 1.4-fold increased Bmax compared to the monospecific bivalent anti-HER2 antibody v506. The FSA combination (v506 + v4184) had a 1.7-fold increased Bmax The apparent K_D of the exemplary anti-HER2 biparatopic Fab-scFv antibodies (v5019, v7091, v10000) and FSA combination (v506 + v4184) had an approximate equivalent KD compared to v506; whereas Fab-Fab (v6903) and scFv-scFv (v6717) was approximately 4 and 16-fold higher K_D respectively, compared to v506.

Table 38: FACS Binding MDA-MB-231

Antibody Variant	K_D (nM)	Bmax
v506	4.8	395
v10000	5.6	558
v506+ v4184	7.3	662
v7091	7.9	525
v5019	8.7	548
v6903	17	534
v6717	77	524

[0470] The tumor cell binding results show that anti-HER2 biparatopic antibodies with different molecular formats have an increased Bmax on HER2 3+, 2+, 1+ and 0/1+ tumor cells compared to a bivalent monospecific anti-HER2 antibody. Of the different anti-HER2 biparatopic antibodies, the scFv-scFv format had the lowest Bmax gain relative to v506 on HER2 3+, 2+, 1+ and 0/1+ tumor cellsThese results also show that scFv-scFv and Fab-Fab formats have the greatest increase in K_D on HER2 3+, 2+, 1+ and 0/1+ tumor cells compared monospecific v506 (3 to 16-fold increase) and the biparatopic Fab-scFv formats (approximately 2-fold or greater). The increase in K_D is an indication of a reduction in avid binding and suggests that different biparatopic formats have unique mechanisms of binding to HER2 on the cell surface.

Example 40: Effect of anti-HER2 biparatopic antibody format on internalization in HER2+ cells

[0471] The following experiment was conducted to compare the ability of exemplary anti-HER2 ECD2 x ECD4 biparatopic antibodies that have different molecular formats (e.g. v6717, scFv-scFv IgG1; v6903 and v6902 Fab-Fab IgG1; v5019, v7091 and v10000 Fab-scFv IgG1) to internalize in HER2+ cells expressing HER2 at varying levels.

[0472] The experiment was conducted as detailed in Example 9. The results are shown in Figure 42 and Tables 39-41. Figure 42A and Table 39 show the internalization results in HER2 3+ BT-474. These results show that the Fab-scFv format (v10000) and the FSA combination (v506 + v4184) have 2.2-fold greater quantities of intracellular antibody, compared to the monospecific anti-HER2 v506. The scFv-scFv format (v6717) had 1.9-fold greater; the Fab-scFv formats (v5019 and v7091) had 1.5 to 1.7-fold greater; and the Fab-Fab formats (v6902 and v6903) had 1.2 to 1.3-

fold greater quantities of intracellular antibody accumulation compared to v506.

Table 39: Internalization BT-474

Antibody Variant	Surface 4°C	Surface37°C	Internal 37°C
v506	2156	1590	3453
v6902	2407	2077	4035
v6903	2717	986	4573
v7091	2759	2227	5111
v5019	2867	2675	5710
v6717	2006	1212	6498
v10000	3355	2851	7528
v506 + v4184	3998	2326	7569

[0473] Figure 42B and Table 40 show the internalization results in HER2 2+ JIMT-1. These results show that the Fab-scFv format (v10000) and the FSA combination (v506 + v4184) have respectively 1.8 and 1.9-fold greater quantities of intracellular antibody, compared to the monospecific anti-HER2 v506. The scFv-scFv (v6717) and the Fab-scFv formats (v5019) have 1.4-fold greater; and the Fab-scFv (v7091) and Fab-Fab formats (v6902 and v6903) had 1.2-fold greater quantities of intracellular antibody accumulation compared to v506.

Table 40: Internalization JIMT-1

Antibody Variant	Surface 4°C	Surface 37°C	Internal 37°C
v506	337	-7.1	759
v6902	389	152	926
v7091	426	102	935
v6903	392	130	945
v5019	437	5.2	1035
v6717	247	31	1082
v10000	474	103	1375
v506 + v4184	583	89	1449

[0474] Figure 42C and Table 41 show the internalization results in HER2 1+ MCF7. These results show that the scFv-scFv format and Fab-scFv formats have 3.0 and 2.8-fold greater quantities of intracellular antibody, compared to the monospecific anti-HER2 v506. The Fab-scFv format (v10000) and the FSA combination (v506 + v4184) have approximately 2.0-fold; the Fab-scFv (v7091) and Fab-Fab (v6903) formats have 1.8-fold greater quantities of intracellular antibody accumulation compared to v506.

Table 41: Internalization MCF7

Antibody Variant	Surface 4°C	Surface 37°C	Internal 37°C
v506	48	10	48
v7091	77	27	87
v6903	81	35	89
v10000	78	20	96
v506 + v4184	87	19	103
v5019	81	17	134
v6717	48	31	145

[0475] These results show that anti-HER2 biparatopic antibodies with different molecular formats have unique degrees of internalization in HER2 3+, 2+ and 1+ tumor cells that varies with respect to the structure and format of the antigen-binding domains. In general, the monospecific FSA combination of v506 and v4184, the Fab-scFv (v10000, v7091 and v5019) and the scFv-scFv (v6717) biparatopic formats had the higher internalization values in the HER2 3+, 2+ and 1+ tumor cells. Whereas, the Fab-Fab biparatopic formats (v6902 and v6903) had the lowest internalization values in the HER2 3+, 2+ and 1+ tumor cells. These data suggest that the molecular format and geometric spacing of the antigen-binding domains has an influence on the ability of the biparatopic antibodies to cross-link HER2 receptors, and subsequently to internalize in HER2 + tumor cells. The Fab-Fab biparatopic format, having the greatest distance between the two antigen-binding domains, resulted in the lowest degree of internalization, whereas the Fab-scFv and scFv-scFv formats, having shorter distances between the antigen-binding domains, had greater internalization in HER2 + cells. This is consistent with the correlation of potency and shorter linker length as described in Jost et al 2013, Structure 21, 1979-1991).

Example 41: Effect of anti-HER2 biparatopic antibody format on ADCC in HER2+ cells

[0476] The following experiment was conducted to compare the ability of exemplary anti-HER2 ECD2 x ECD4 biparatopic antibodies that have different molecular formats (e.g. v6717, scFv-scFv IgG1; v6903 and v6902 Fab-Fab IgG1; v5019, v7091 and v10000 Fab-scFv IgG1) to mediate ADCC in HER2+ cells expressing HER2 at varying levels.

[0477] Prior to performing the ADCC assay, glycopeptide analysis was performed on the antibody samples to quantify the fucose content in the N-linked glycopeptide. The method was followed as described in Example 23. The results are shown in Table 42; the data shows that exemplary biparatopic variants v5019, v6717, v6903 have equivalent fucose content in the N-linked glycan (91-93%). Antibody samples with equivalent levels of fucose in the N-glycan were selected for the ADCC assay to normalize for fucose content in the interpretation of the ADCC assay results.

Table 42: LC-MS Tryptic peptide analysis

Variant	Percentage of Glycopeptides Observed WITH Fucose	Percentage of Glycopeptides Observed WITHOUT Fucose
v6903	90.7	9.3
v6717	92.8	7.2
v5019	91.3	8.7

[0478] The ADCC experiment was conducted as described in Example 11 with E/T: 5:1 with NK-92 effector cells. The ADCC results are shown in Figure 43 and Tables 43-45. Figure 43A and Table 43 show the ADCC results in HER2 2+ JIMT-1 breast tumor cells. These data show that v5019, v6717 and v6903 elicit similar levels of maximum cell lysis and that the scFv-scFv format (v6717) is less potent compared to v5019 and v6903 when HER2 2+ tumor cells are targets.

Table 43: JIMT-1 ADCC

Antibody variant	EC50 (nM)	% Max Cell Lysis
v6903	~ 0.03	48
v5019	~ 0.16	47
v6717	~ 0.72	51

[0479] Figure 43B and Table 44 show the ADCC results in HER2 1+ MCF7 breast tumor cells. These data show that v5019 and v6717 have slightly higher maximum cell lysis (27-30%) compared to v6903 (24%). These data also show that v6717 is the least potent, followed by v6903 and v5019, which have lower EC50 values.

Table 44: MCF7 ADCC

Antibody variant	EC_{50} (nM)	% Max Cell Lysis
v5019	~ 0.69	27
v6717	109	30
v6903	0.94	24

[0480] Figure 43C and Table 45 show the ADCC results in HER2 0/1+ MDA-MB-231 breast tumor cells. These data show that v5019 shows slightly higher maximum cell lysis (77%) compared to v6903 (62%) and v6717 (63%). These data also show that v6717 is the least potent, followed by v6903 and v5019, which have lower EC_{50} values.

Table 45: MDA-MB-231 ADCC

Antibody variant	EC_{50} (nM)	% Max Cell Lysis(top only)
v5019	0.20	71
v6717	10	63
v6903	0.79	62

[0481] These data show that exemplary anti-HER2 ECD2 x ECD4 biparatopic antibodies elicit similar levels of maximum cell lysis by ADCC in HER2 2+ and 1+ tumor cells. Despite similarities in maximal cell lysis, these data also show that the different molecular formats have unique ADCC potencies. The scFv-scFv was the least potent (greatest EC_{50} values) in the HER2 2+ and HER2 1+. Differential potencies among the three formats was seen in the ADCC data targeting HER2 1+ cells, where the EC50 values for v6717 > v6903 > v5019. These data are consistent with the observations presented in Example 40 (FACS binding), where an increase in K_D (reduced affinity) was seen with the Fab-Fab and scFv-scFv formats.

Example 42: Effect of anti-HER2 biparatopic antibody format on growth of HER2 + tumor cells

[0482] The following experiment was conducted to compare the effect of anti-HER2 biparatopic antibody format on growth of HER2 3+, 2+ and 1+ tumor cells, either basal growth or ligand-stimulated. Basal growth was measured as described in Example 15, while ligand-stimulated growth was measured as described in Example 27. In both types of experiments, growth was measured as % survival with respect to control treatment.

[0483] Figure 44 and Table 46 show the effect of exemplary anti-HER2 ECD2 x ECD4 biparatopic antibodies on growth of HER2 3+ breast cancer cells (BT-474) in the presence of exogenous growth-stimulatory ligands (EGF and HRG). In the absence of EGF or HRG, the anti-HER2 biparatopic antibodies were able to inhibit growth of BT-474 cells, where % survival of each treatment group ranked as follows: v6903 < v506 + v4184 < 506 < v7091 < v5019 < v10000 < v6717. In the presence of HRG, growth inhibition relative to the mock control was achieved only with the FSA combination of v506 + v4184. In the presence of EGF, growth inhibition relative to the mock control was achieved, where % survival of each treatment group ranked as follows: v6903 < v506 + v4184 < 7091< v10000 < 5019.

Table 46

Treatment	% Survival		
	Antibody only	+ HRG	+ EGF
Mock	100	143	131
v6717	113	126	129
v10000	70	118	78
v5019	67	133	81
v7091	61	119	61
v506	53	141	118
v506 + v4184	43	89	45
v6903	32	120	39

[0484] Figure 45 shows the dose-dependent effect of the anti-HER2 biparatopic antibody formats on growth inhibition of the SKBr3 HER2 3+ cell line. The data is consistent with the results presented in Figure 44, where the rank order potency/efficacy of the biparatopic formats is as follows Fab-Fab > Fab-scFv > scFv-scFv in HER2 3+ tumor cells.

[0485] The effect of anti-HER2 biparatopic antibody formats on survival of HER2+ cells is shown in Figure 46, where Figure 46A shows the result in the Trastuzumab sensitive SKOV3 HER2 2+/3+ cell line at 300 nM; Figure 46B shows the result in JIMT-1 HER2 2+ (Trastuzumab resistant) cells at 300nM, and Figure 46C shows the result in MCF7 HER2 1+ cell line at 300 nM. In the SKOV3 cell line, little difference was observed among the biparatopic formats in the extent of growth inhibition, and no growth inhibition was observed by any of the test antibodies in JIMT-1 and MCF7 cells.

[0486] The data in Figure 44 and Figure 45 show that anti-HER2 ECD2 x ECD4 biparatopic antibodies with the Fab-scFv and Fab-Fab formats (v5019, v7091, v10000, v6903) are capable of growth inhibition HER2 3+ tumor cells in the absence, and presence of EGF or HRG. In the HER2 3+ cell lines BT-474 and SKBR3, growth inhibition relative to the mock control rank ordered as follows, where v506 + v4184 > v6903 > v7091 > v10000 > v5019 > v506 > v6717. The distance between antigen-binding domains (Fab-Fab > Fab-scFv > scFv-scFv) correlates with the rank order of growth inhibition in the HER2 3+ tumor cells. Based on the data in trastuzumab-sensitive tumor cells, BT-474, and SKBr3, it may be expected that the growth inhibition difference among formats is significant at the HER2 3+ level but less so at the HER2 2+ or HER2 1+ levels.

Example 43: Evaluation of HER2 binding affinity and kinetic at varying antibody capture levels

[0487] The following experiment was conducted to compare HER2 binding kinetics (kd, off-rate) of exemplary anti-HER2 ECD2 x ECD4 biparatopic antibodies when captured at varying surface densities by SPR. The correlation between a reduced (slower) off-rate with increasing antibody capture levels (surface density) is an indication of Trans binding (i.e. one antibody molecule binding to two HER2 molecules, described in Example 12). In this experiment the Fab-Fab format (v6903) was compared to the Fab-scFv format (v7091) to determine potential difference in Trans binding among the variants. Due to the larger spatial distance between antigen-binding domains, it is hypothesized that the Fab-Fab format may be capable of Cis binding (engaging ECD 2 and 4 on one HER2 molecule); whereas, the Fab-scFv would not capable of Cis binding due to the shorter distance between the it's antigen-binding domains. The anti-HER2 monospecific v506 was included as a control.

[0488] The experiment was conducted by SPR as described in Example 12. The data are shown in Figure 47. Figure 47A shows the plot and linear regression analysis for the kd (1/s) at different antibody capture levels with v6903 and v7091. Both v7091 and v6093 show a trend for decreasing off-rate with increasing surface capture levels; however, the correlation is significant with the Fab-scFv variant (v7091; P value = 0.023) but not the Fab-Fab format (v6093; P value = 0.053). The off-rate remained unchanged with varying antibody capture levels for the anti-HER2 monospecific control, v506.

[0489] Figure 47B shows the plot and linear regression analysis for the K_D (M) at different antibody capture levels with v6903 and v7091. Similar to the off-rate comparison, both v7091 and v6093 show a trend for increasing affinity (lower K_D value) with increasing surface capture levels. However, the correlation is significant with the Fab-scFv variant (v7091; P value = 0.04) but not the Fab-Fab format (v6093; P value = 0.51). The K_D remained unchanged with varying antibody capture levels for the anti-HER2 monospecific control, v506. The data in Figure 47 shows that the Fab-Fab and Fab-scFv anti-HER2 biparatopic antibody formats show trends of decreasing off -rates with increasing antibody surface capture levels; these trends are unique compared to a monospecific anti-Her2 antibody.

Example 44: Affinity and stability engineering of the Pertuzumab Fab

[0490] As indicated in Table 1, one variant (v10000) contains mutations in the Pertuzumab Fab. This Fab was derived from affinity and stability engineering in silico efforts, which were measured experimentally as monovalent or One-Armed Antibodies (OAAs).

[0491] Variant 9996: a monovalent anti-HER2 antibody, where the HER2 binding domain is a Fab derived from pertuzumab on chain A, with Y96A in VL region and T30A/A49G/L69F in VH region (Kabat numbering) and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A_Y407V (EU numbering) in Chain A, T350V_T366L_K392L_T394W (EU numbering) in Chain B, and the hinge region of Chain B having the mutation C226S; the antigen-binding domain binds to domain 4 of HER2.

[0492] Variant 10014: a monovalent anti-HER2 antibody, where the HER2 binding domain is a Fab derived from pertuzumab on chain A, with Y96A in VL region and T30A in VH region (Kabat numbering) and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A _Y407V (EU

numbering) in Chain A, T350V_T366L_K392L_T394W (EU numbering) in Chain B, and the hinge region of Chain B having the mutation C226S; the antigen-binding domain binds to domain 4 of HER2.

[0493] Variant 10013: a monovalent anti-HER2 antibody, where the HER2 binding domain is a Fab derived from wild type pertuzumab on chain A, and the Fc region is a heterodimer having the mutations T350V_L351Y_F405A_Y407V (EU numbering) in Chain A, T350V_T366L_K392L_T394W (EU numbering) in Chain B, and the hinge region of Chain B having the mutation C226S; the antigen-binding domain binds to domain 4 of HER2.

[0494] The following experiments were conducted to compare HER2 binding affinity and stability of the engineered Pertuzumab variants.

[0495] OAA variants were cloned and expressed as described in Example 1.

[0496] OAA were purified by protein A chromatography and Size Exclusion Chromatography, as described in Example 1.

[0497] Heterodimer purity (i.e. amount of OAA with a heterodimeric Fc) was assessed by non-reducing High Throughput Protein Express assay using Caliper LabChip GXII (Perkin Elmer #760499). Procedures were carried out according to HT Protein Express LabChip User Guide version2 LabChip GXII User Manual, with the following modifications. Heterodimer samples, at either 2 µl or 5 µl (concentration range 5-2000 ng/µl), were added to separate wells in 96 well plates (BioRad # HSP9601) along with 7 µl of HT Protein Express Sample Buffer (Perkin Elmer # 760328). The heterodimer samples were then denatured at 70°C for 15 mins. The LabChip instrument is operated using the HT Protein Express Chip (Perkin Elmer #760499) and the Ab-200 assay setting. After use, the chip was cleaned with MilliQ water and stored at 4°C.

[0498] The stability of the samples was assessed by measuring melting temperature or Tm, as determined by DSC with the protocol shown in example 24. The DSC was measured before and after SEC purification.

[0499] The affinity towards HER2 ECD of the samples was measured by SPR following the protocol from example 12. The SPR was measured before and after SEC purification. As summarized in Table 47A and 47B, the mutations in the variable domain have increased the HER2 affinity of the Fab compared to wild type pertuzumab, while maintaining WT stability. ([1] Purity determined by Caliper LabChip; [2] KD(WT)/KD(mut)

Table 47A:

OAA variant	Fab HC mutations	LC mut	Pr-A Yield (mg/L)	SPR pre-SEC				Het purity post - SEC[1]	SPR post-SEC			
				KD AVE (nM)	KD STDEV (nM)	n	Fold wrt WT[2]		KD AVE (nM)	KD STDEV (nM)	n	Fold wrt WT
v9996	T30A/A49G /L69F	Y96A	22	1.7E-09	1.7E-10	5	9.6	93%	1.8E-09	1.6E-11	2	8.4
v10014	T30A	Y96A	20	2.0E-09	3.1E-10	4	8.1	81%	2.1E-09	5.2E-10	3	7.0
v10013	WT	WT	18	1.6E-08	5.1E-09	16	1.0	91%	1.5E-08	3.5E-09	4	1.0

Table 47B:

OAA variant	DSC pre-SEC		DSC post-SEC	
	Tm (C)	ΔTm wrt WT (C)	Tm (C)	ΔTm wrt WT (C)
v9996	77.2	-0.2	77.2	-0.7
v10014	75.5	-1.9	75.5	-2.4
v10013	77.4	0.0	77.9	0.0

Example 45: Effect of v10000 on survival and tumor growth in a xenograft model of HER2-low, non-small cell lung cancer (NSCLC)

[0500] This experiment was performed to assess efficacy of v10000 compared to control IgG (v6908) in an A549 xenograft model of lung cancer. A549 cells are derived from non-squamous non-small cell lung cancer that is HER2-low, non-HER2 gene amplified, HER3+, EGFR-low and moderately sensitive to Cisplatin at the MTD (maximum tolerated dose). The study was carried out as described below.

[0501] Tumor cell suspensions were implanted subcutaneously into athymic nude mice. When tumors reached 158 mm^3 the animals were randomly assigned to groups as shown in Table A1, and treatment began in a blinded and controlled study. Animals were treated according to Regimen 1 on Day 1, followed by treatment according to Regimen 2 on subsequent days as indicated in Table A1.

Table A1. Study Design

Group (n)	Regimen 1				Regimen 2			
	Agent	Dosage (mg/kg)	Route	Schedule	Agent	Dosage (mg/kg)	Route	Schedule
1 (20)	v6908	15	iv	Day 1	v6908	10	iv	Days 4, 8, 11, 15, 18, 22 and 25
2 (20)	v10000	15	iv	Day 1	v10000	10	iv	Days 4, 8, 11, 15, 18, 22 and 25

[0502] Tumor volume was measured by calipers twice weekly. The study duration was 66 days with survival as the primary endpoint. Additional tumor response criteria were measured and are shown in Table A2. Mice were euthanized when tumor volume exceeded 800 mm^3, the surviving

percentage versus study day was plotted on a Kaplan-Meier and was statistically assessed using a log-rank test. Serum concentration of v10000 was determined by HER2 ELISA on study day 7.

[0503] The results are shown in Figure 48A (tumor volume) and Figure 48B (Kaplan-Meier survival). Variant 10000 reduced tumor growth compared to v6908 treated controls and significantly prolonged survival by log-rank test (Figure 48B and Table A3). Animals treated with v10000 had a median survival of greater than 66 days while those treated with v6908 had a median survival of 25.78 days (Fig 48B and Table A2). Tumor volume on study day 30 was 461 mm3 and 810 mm3 for v10000 and v6908 treated groups respectively (Fig 48A and Table A2). Serum exposure was 140.9 microg/mL on study day 7, indicating that the anticipated serum concentration was achieved.

[0504] These results show that treatment with v10000 was able to reduce tumor growth and prolong survival compared to treatment with a control hIgG in this HER2-low non-gene amplified NSCLC model.

Table A2: A549 Tumor Response Profile

	6908	**10000**
Tumor Response on Day 30		
Mean TV (mm^3)(%Δ from base line)	810(413%)	461(191%)
Treatment/Control Ratio	1.00	0.57
RECIST Scores		
CR(TV<20mm^3)	0/20	0/20
PR(>30% baseline regression)	0/20	1/20
PD(>20% baseline growth)	20/20	19/20
SD(neither PD or PR)	0/20	0/20
Median Time to Progression (days)	3.30	2.31
Survival Response		
Median Survival (days)	25.78	>66
CR-Complete Response PR-Partial Response PD-Progressive Disease SD-Stable Disease		

Table A3: Log Rank Summary

Group	**6908**
6908	-
10000	★★★
Legend: ns=not significant, ★=P<0.05. ★★=P<0.01, ★★★=P<0.001	

Example 46: Effect of v10000 on survival and tumor growth in a xenograft model of HER2-low, head and neck squamous cell carcinoma

[0505] This experiment was performed to assess efficacy of v10000 compared to Herceptin™ (v6336) and control human IgG (v6908) in the FaDu xenograft model of head and neck cancer. FaDu cells are derived from squamous cell cancer of the head and neck that is HER2 low, non-HER2 gene amplified, HER3+, EGFR + and highly sensitive to Cisplatin at the MTD. The study was carried out as described below.

[0506] Tumor cell suspensions were implanted subcutaneously into athymic nude mice. When tumors reached 121 mm^3 the animals were randomly assigned to groups as shown in Table A4, and treatment began in a blinded and controlled study. Cisplatin was purchased and provided for the study by Charles River Laboratories (Morrisville, NC). Animals were treated according to Regimen 1 at Day 1, followed by Regimen 2 on subsequent days as noted in Table A4.

Table A4. Study Design

Group (n)	**Regimen 1**				**Regimen 2**			
	Agent	Dosage (mg/kg)	Route	Schedule	Agent	Dosage (mg/kg)	Route	Schedule
1 (15)	v6908	15	iv	Day 1	v6908	10	iv	Days 4, 8, 11, 15, 18, 22 and 25
2 (15)	v6336	15	iv	D ay 1	v6336	10	iv	Days 4, 8, 11, 15, 18, 22 and 25
3 (15)	v10000	15	iv	Day 1	v10000	10	iv	Days 4, 8, 11, 15, 18, 22 and 25
4 (15)	Cisplatin	2	ip	Day 1, 3, 5, 7, 9, 11				

[0507] These results show that treatment with v10000 as a monotherapy was able to decrease tumor volume and prolong survival, compared to treatment with control IgG in this model of HER2-low non-gene amplified head and neck cancer. Overall, v10000 showed a trend towards

decreasing tumor volume compared to v6336 (Herceptin™).

[0508] Variant 10000 was also tested in combination with cisplatin. The combination of v10000 and cisplatin significantly prolonged survival compared to v6908, v6336, and single agent cisplatin (Table A5). The median survival of the v10000 and cisplatin combination was 53 days while the median survival of v6908, v6336, and single agent cisplatin was 25, 40, and 40 days, respectively.

[0509] These results demonstrate that treatment with v10000 in combination with cisplatin was able to decrease tumor growth and prolong survival compared to v6908 and v6336, in this model of head and neck cancer.

Table A5: FaDu Tumor Response Profile

	6908	6336	10000	cisplatin	10000+cisplatin
Tumor Response on Day 25					
Mean TV (mm^3)(%Δ from base line)	1979 (1532%)	1257 (929%)	1025 (782%)	1070 (782%)	816 (573%)
Treatment/Control Ratio	1.00	0.63	0.52	0.54	0.41
RECIST Scores					
CR(TV<20mm^3)	0/15	0/14	0/15	0/15	0/15
PR(>30% baseline regression)	0/15	0/14	0/15	0/15	0/15
PD(>20% baseline growth)	15/15	14/14	15/15	15/15	15/15
SD(neither PD or PR)	0/15	0/15	0/15	0/15	0/15
Median Time to Progression (days)	5.9	7.6	7.8	8.4	10.8
Survival Response					
Median Survival (days)	25	40	46	40	53
CR-Complete Response PR-Partial Response PD-Progressive Disease SD-Stable Disease					

Table A6: Log Rank Summary

Group	6908	6336	10000	Cisplatin
6908	-	-	-	-
6336	**	-	-	-
10000	★★★	n/s	-	-
Cisplatin	★★★	n/s	★	-
10000+Cisplatin	★★★	★	n/s	★★★
Legend: ns=not significant, ★=P<0.05. ★★=P<0.01, ★★★=P<0.001				

Example 47: Effect of v10000 on survival and tumor growth inhibition in a xenograft model of HER2 1+, ER+ breast cancer

[0510] This experiment was performed to assess efficacy of v10000 compared to a control IgG (v6908) or Herceptin™ (v6336) in the ST1337B xenograft model of breast cancer. ST1337B is a patient derived xenograft (PDX) established in nude mice from an ER+/PR- breast cancer with a luminal B molecular classification. ST1337 is HER2 1+ as measured by IHC. The study was carried out as described below.

[0511] Tumor fragments were implanted subcutaneously into athymic nude mice. When tumors reached 180 mm^3 the animals were randomly assigned to groups as shown in Table A7 and treatment began in a blinded and controlled study. Animals were treated according to Regimen 1 as shown in Table A7

Table A7. Study Design

Group (n)	Regimen 1			
	Agent	Dosage (mg/kg)	Route	Schedule
1 (15)	v6908	30	iv	Days 1, 4, 8, 11, 15, 18, 22, 25, 28, and 32
2 (15)	V6336	10	iv	Days 1, 4, 8, 11, 15, 18, 22, 25, 28, and 32
3 (15)	v10000	3	iv	Days 1, 4, 8, 11, 15, 18, 22, 25, 28, and 32
4 (15)	v10000	10	iv	Days 1, 4, 8, 11, 15, 18, 22, 25, 28, and 32
5 (15)	v10000	30	iv	Days 1, 4, 8, 11, 15, 18, 22, 25, 28, and 32

[0512] Tumor volume was measured by calipers twice weekly. The study duration was 63 days with survival as the primary endpoint. Additional tumor response criteria were measured and are shown in Table A8. Mice were euthanized when tumor volume exceeded 2000 mm^3, the surviving percentage versus study day was plotted on a Kaplan-Meier and was statistically assessed using a log-rank test. Serum concentration of v10000 and v6336 was determined by HER2 ELISA on study day 7 and on day 36, 4 days following the last dose on day 32.

[0513] The results are shown in Figure 50A (tumor volume) and Figure 50B (Kaplan-Meier survival). Treatment with variant 10000 at all doses tested reduced tumor growth compared to treatment with v6908 and significantly prolonged survival by log-rank test compared to v6908 (Figure 50B and Table A9). In addition, treatment with v10000 at 30 mg/kg significantly prolonged survival compared to treatment with v6336 at 10 mg/kg (Fig 50B and Table A8). Animals treated with v10000 had median survivals of 49, 59, and 59 days for the 3, 10 and 30 mg/kg doses respectively (Fig 50B and Table A8). Tumor volume on study day 29 for treatment with v10000 at 3, 10 and 30 mg/kg was 1010, 1016, and 931 mm3, respectively. Tumor volumes for v6908 and v6336 on study day 29 was 1898 and 1264 mm3 respectively (Fig 50A and Table A8). The serum exposure of v6336 and v10000 is shown in Table A10. These results confirm that increasing the dosage of v10000 results in an increase in serum concentration of v10000, and that similar doses of v10000 and v6336 result in similar serum concentrations of antibody.

[0514] These results indicate that treatment with v10000 is able to decrease tumor volume and prolong survival in this model of HER2-low ER+ breast cancer, when compared to the IgG control and to Herceptin™.

Table A8: ST1337b Tumor Response Profile

	6908, 30mg/kg	6336, 10mg/kg	10000, 3mg/kg	10000, 10mg/kg	10000, 30mg/kg
Tumor Response on Day 29					
Mean TV (mm^3)(%Δ from base line)	1898 (953%)	1264 (601%)	1010 (460%)	1016 (457%)	931 (411%)
Treatment/Control Ratio	1.00	0.66	0.53	0.53	0.49
RECIST Scores					
CR(TV<20mm^3)	0/15	0/15	0/15	0/15	0/15
PR(>30% baseline regression)	0/15	0/15	0/15	0/15	0/15
PD(>20% baseline growth)	15/15	15/15	15/15	15/15	15/15
SD(neither PD or PR)	0/15	0/15	0/15	0/15	0/15
Median Time to Progression (days)	11	10	14	26	13
Survival Response					
Median Survival (days)	29	43	49	59	59
CR-Complete Response PR-Partial Response PD-Progressive Disease SD-Stable Disease					

Table A9: Log Rank Summary

Group	6908, 30mg/kg	6336, 10mg/kg	10000, 3mg/kg	10000, 10mg/kg	10000, 30mg/kg
6908, 30mg/kg	-	-	-	-	
6336, 10mg/kg	★★	-	-	-	-
10000, 3mg/kg	★★	n/s	-	-	-
10000, 10mg/kg	★★★	n/s	n/s	-	-
10000, 30mg/kg	★★★	★	n/s	n/s	-
Legend: ns=not significant, ★=P<0.05. ★★=P<0.01, ★★★=P<0.001					

Table A10: Serum Exposure Summary

Sample Day	6336, 10mg/kg	10000, 3mg/kg	10000, 10mg/kg	10000, 30mg/kg
7	133.0	30.7	101.7	286.6
36	135.2	46.0	186.3	279.7

Example 48: Effect of v10000 on survival and tumor growth inhibition in a xenograft model of HER2 negative pancreatic cancer

[0515] This experiment was performed to assess efficacy of v10000 compared to a control IgG (v12470), Herceptin™ (v6336), and nab-paclitaxel as single agents and v10000 in combination with nab-paclitaxel (Abraxane™ Celgene) in the ST803 xenograft model of pancreatic cancer. ST803 is a patient-derived xenograft (PDX) of pancreatic cancer (South Texas Accelerated Research Therapeutics, San Antonio, TX 78229) that is HER2 negative as measured by IHC. The study was carried out as described below.

[0516] Tumor fragments were implanted subcutaneously into athymic nude mice. When tumors reached 170 mm^3 the animals were randomly assigned to groups as shown in Table A1 1 and treatment began in a blinded and controlled study. Animals were treated according to Regimen 1 and 2 as shown in Table A11. All treatments were administered intravenously.

Table A11. Study Design

Group (n)	Regimen 1			Regimen 2		
	Agent	Dosage (mg/kg)	Schedule	Agent	Dosage (mg/kg)	Schedule
1 (20)	v12470	30	Twice weekly for four weeks			

Table A12: ST803 Tumor Response Profile

	12470	6336	10000	12470+nab-pac*	12470+nab-pac*
Tumor Response on Day 54					
Mean TV (mm^3)(%Δ from base line)	1663 (+888%)	1494 (+806%)	1305 (+659%)	1365 (+693%)	1073 (+522%)
Treatment/Control Ratio	1.00	0.90	0.78	0.82	0.64
RECIST Scores					
CR(TV<20mm^3)	0/18	0/17	0/20	0/16	0/19
PR(>30% baseline regression)	0/18	0/17	0/20	0/16	0/19
PD(>20% baseline growth)	18/18	17/17	20/20	16/16	19/19
SD(neither PD or PR)	0/18	0/17	0/20	0/16	0/19
Median Time to Progression (days)	4.4	3.6	3.6	4.4	5.6
Survival Response					
Median Survival (days)	58.8	65.9	69.3	60.6	>71
CR-Complete Response PR-Partial Response PD-Progressive Disease SD-Stable Disease *nab-paclitaxel					

Table A13: Log Rank Summary

Group	12470	6336	10000	12470+nab-pac*	10000+nab-pac*
12470	-	-	-	-	
6336	ns	-	-	-	-
10000	ns	ns	-	-	-
12470+nab-pac	ns	-	Ns	-	-
10000, +nab-pac	★★	-	Ns	★★	-
Legend: ns=not significant, ★=P<0.05. ★★=P<0.01, ★★★=P<0.001 *nab-paclitaxel					

Table A14: Serum Exposure Summary

Sample Day	6336 (microg/mL)	10000 (microg/mL)	10000 (microg/mL) + nab-paclitaxel
14	426.7	279	391

Example 49: Effect of v10000 on tumor growth inhibition in a xenograft model of HER2 3+ gastric cancer

[0517] This experiment was performed to assess efficacy of v10000 compared to a control IgG (v12470) and Herceptin™ (v6336) as single agents in the GXA3054 xenograft model of gastric cancer. GXA3054 is a patient derived xenograft (PDX) of gastric cancer that is HER2 3+ (Oncotest GmbH, Am Flughafen 12-14, 79108 Freiburg, Germany). The study was carried out as described below.

[0518] Tumor fragments were implanted subcutaneously into athymic nude mice. When tumors reached 144 mm^3 the animals were randomly assigned to groups as shown in Table A15 and treatment began in a blinded and controlled study. Animals were treated according to Regimen 1 as shown in Table A15.

Table A15. Study Design

Group (n)	Regimen 1			
	Agent	Dosage (mg/kg)	Route	Schedule
1 (10)	v12470	30	IV	Twice weekly for five weeks
2 (10)	V6336	30	IV	Twice weekly for five weeks
3 (10)	v10000	30	IV	Twice weekly for five weeks

[0519] Tumor volume was measured by calipers twice weekly. The study duration was 59 days with tumor growth inhibition as the primary endpoint. Additional tumor response criteria were measured and are shown in Table A16. Mice were euthanized when tumor volume exceeded 2000 mm^3.

[0520] The results are shown in Figure 52 (tumor volume). Treatment with variant 10000 and v6336 reduced tumor growth compared to treatment with control IgG (v12470) (Figure 52 and Table A16). In addition, treatment with v10000 reduced tumor growth compared to treatment with v6336 (Fig 52 and Table A16). Mean tumor volume on study day 35 for treatment with control IgG, v10000 and v6336 was 1340, 236, and 7.8 mm3,

respectively. Tumor growth inhibition on day 35 for v10000 and v6336 was 111 and 92%, respectively (Table A16). On day 35 tumors treated with v10000 showed greater responses (7/10 complete and 3/10 partial responses) compared to tumors treated with v6336 (0/10 complete and 1/10 partial response) (Table A16). At the completion of the study, on day 59, 9/10 tumors treated with v10000 had complete responses with no evidence of recurrent tumor, while for v6336 treated tumors only 1/10 tumors had a complete response.

[0521] These results indicate that treatment with v10000 can regress tumors in this model of HER2 3+ gastric cancer. The tumor growth inhibition of v10000 was superior to IgG control and Herceptin™.

Table A16: GXA3054 Tumor Response Profile

	12470	6336	10000
Tumor Response on Day 35	Na	92	111
Tumor Growth Inhibition (%)			
RECIST Scores			
CR (≤ -95%)	0/10	0/10	7/10
PR (> -95% and < -66%)	0/10	1/10	3/10
SD (≥ -66% and ≤ +73%)	0/10	5/10	0/10
PD (> +73%)	10/10	4/10	0/10
CR-Complete Response PR-Partial Response PD-Progressive Disease SD-Stable Disease			

[0522] The reagents employed in the examples are generally commercially available or can be prepared using commercially available instrumentation, methods, or reagents known in the art. The foregoing examples illustrate various aspects described herein and practice of the methods described herein. The examples are not intended to provide an exhaustive description of the many different embodiments of the invention.

SEQUENCE TABLE

Variant	H1 clone name	H2 clone name	L1 clone name	L2 clone name
792	1011	1015	-2	-2
5019	3057	720	1811	NA
5020	719	3041	NA	1811
7091	3057	5244	1811	NA
10000	6586	5244	3382	NA
6903	5065	3468	5037	3904
6902	5065	3468	5034	3904
6717	3317	720	NA	NA
1040	4560	4553	NA	4561
630	719	716	NA	NA
4182	4560	3057	NA	1811
506	642	642	-2	-2
4184	3057	3041	1811	1811
9996	4372	6586	NA	3382

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
1	642	Full	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
2	642	Full	GAGGTGCAGCTGGTGGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCTCTGCGACTGAGTTGCGCCGCTTC AGGATTCAACATCAAGGACACCTACATTCACTGGGTGCGACAGGCTCCAGGAAAAGGACTGGAGTGGGTGGCTC GAATCTATCCCACTAATGGATACACCCGGTATGCCGACTCCGTGAAGGGGAGGTTTACTATAGCGCCGATACA TCCAAAAACACTGCTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACCGCTGTGTACTATTGCAGTCGATG GGGAGGAGACGGATTCTACGCTATGGATTATTGGGGACAGGGGACCCTGGTGACAGTGAGCTCCGCCTCTACCA
			AGGGCCCCAGTGTGTTCCCCTGGCTCCTTCTAGTAAATCCACCTCTGGAGGGACAGCCGCTCTGGGATGTCTG GTGAAGGACTATTCCCCGAGCCTGTGACCGTGAGTTGGAACTCAGGCGCCCTGACAAGCGGAGTGCACACTTT TCCTGCCGTGCTGCAGTCAAGCGGGCTGTACTCCCTGTCCTCTGTGGTGACAGTGCCAAGTCAAGCCTGGGCA CACAGACTTATATCTGCAACGTGAATCATAAGCCCTCAAATACAAAAGTGGACAAGAAAGTGGAGCCCAAGAGC TGTGATAAGACCCACACCTGCCCTCCCTGTCCAGCTCCAGAACTGCTGGGAGGACCTAGCGTGTTCCTGTTTCC CCCTAAGCCAAAAGACACTCTGATGATTTCCAGGACTCCCGAGGTGACCTGCGTGGTGGTGGACGTGTCTCACG AGGACCCCGAAGTGAAGTTCAACTGGTACGTGGATGGCGTGGAAGTGCATAATGCTAAGACAAAACCAAGAGAG

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			GAACAGTACAACTCCACTTATCGCGTCGTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGGAAGGA GTATAAGTGCAAAGTCAGTAATAAGGCCCTGCCTGCTCCAATCGAAAAAACCATCTCTAAGGCCAAAGGCCAGC CAAGGGAGCCCCAGGTGTACACACTGCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTGTCCCTGACATGT CTGGTGAAAGGCTTCTATCCTAGTGATATTGCTGTGGAGTGGGAATCAAATGGACAGCCAGAGAACAATTACAA GACCACACCTCCAGTGCTGGACAGCGATGGCAGCTTCTTCCTGTATTCCAAGCTGACAGTGGATAAATCTCGAT GGCAGCAGGGGAACGTGTTTAGTTGTTCAGTGATGCATGAAGCCCTGCACAATCATTACACTCAGAAGAGCCTG TCCCTGTCTCCCGGCAAA
3	642	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
4	642	VH	GAGGTGCAGCTGGTGGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCTCTGCGACTGAGTTGCGCCGCTTC AGGATTCAACATCAAGGACACCTACATTCACTGGGTGCGACAGGCTCCAGGAAAAGGACTGGAGTGGGTGGCTC GAATCTATCCCACTAATGGATACACCCGGTATGCCGACTCCGTGAAGGGGAGGTTTACTATTAGCGCCGATACA TCCAAAAACACTGCTTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACCGCTGTGTACTATTGCAGTCGATG GGGAGGAGACGGATTCTACGCTATGGATTATTGGGGACAGGGGACCCTGGTGACAGTGAGCTCC
5	642	H1	GFNIKDTY
6	642	H1	GGATTCAACATCAAGGACACCTAC
7	642	H3	SRWGGDGFYAMDY
8	642	H3	AGTCGATGGGGAGGAGACGGATTCTACGCTATGGATTAT
9	642	H2	IYPTNGYT
10	642	H2	ATCTATCCCACTAATGGATACACC
11	642	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKV
12	642	CH1	GCCTCTACCAAGGGCCCCAGTGTGTTTCCCCTGGCTCCTTCTAGTAAATCCACCTCTGGAGGGACAGCCGCTCT GGGATGTCTGGTGAAGGACTATTTCCCCGAGCCTGTGACCGTGAGTTGGAACTCAGGCGCCCTGACAAGCGGAG TGCACACTTTTCCTGCTGTGCTGCAGTCAAGCGGGCTGTACTCCCTGTCCTCTGTGGTGACAGTGCCAAGTTCA AGCCTGGGCACACAGACTTATATCTGCAACGTGAATCATAAGCCCTCAAATACAAAAGTGGACAAGAAAGTG
13	642	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
14	642	CH2	GCTCCAGAACTGCTGGGAGGACCTAGCGTGTTCCTGTTTCCCCCTAAGCCAAAAGACACTCTGATGATTTCCAG GACTCCCGAGGTGACCTGCGTGGTGGTGGACGTGTCTCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGG ATGGCGTGGAAGTGCATAATGCTAAGACAAAACCAAGAGAGGAACAGTACAACTCCACTTATCGCGTCGTGAGC GTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTCAGTAATAAGGCCCTGCC TGCTCCAATCGAAAAAACCATCTCTAAGGCCAAA
15	642	CH3	GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
16	642	CH3	GGCCAGCCAAGGGAGCCCCAGGTGTACACACTGCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTGTCCCT GACATGTCTGGTGAAAGGCTTCTATCCTAGTGATATTGCTGTGGAGTGGGAATCAAATGGACAGCCAGAGAACA ATTACAAGACCACACCTCCAGTGCTGGACAGCGATGGCAGCTTCTTCCTGTATTCCAAGCTGACAGTGGATAAA TCTCGATGGCAGCAGGGGAACGTGTTTAGTTGTTCAGTGATGCATGAAGCCCTGCACAATCATTACACTCAGAA GAGCCTGTCCCTGTCTCCCGGC
17	3468	Full	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDR SKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KGYFPEPVTVSWNSGALTSGVHTFPAVLKSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC
			DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSRDELTKNQVSLLCL VKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPG
18	3468	Full	GAAGTGCAGCTGGTCGAATCTGGAGGAGGACTGGTGCAGCCAGGAGGGTCCCTGCGCCTGTCTTGCGCCGCTAG TGGCTTCACTTTTACCGACTACACCATGGATTGGGTGCGACAGGCACCTGGAAAGGGCCTGGAGTGGGTCGCCG ATGTGAACCCAAATAGCGGAGGCTCCATCTACAACCAGCGGTTCAAGGGCCGGTTCACCCTGTCAGTGGACCGG AGCAAAAACACCCTGTATCTGCAGATGAATAGCCTGCGAGCCGAAGATACTGCTGTGTACTATTGCGCCCGGAA TCTGGGGCCCTCCTTCTACTTTGACTATTGGGGGCAGGGAACTCTGGTCACCGTGAGCTCCGCCTCCACCAAGG GACCTTCTGTGTTCCCACTGGCTCCCTCTAGTAAATCCACATCTGGGGGAACTGCAGCCCTGGGCTGTCTGGTG AAGGGCTACTTCCCAGAGCCCGTCACAGTGTCTTGGAACAGTGGCGCTCTGACTTCTGGGGTCCACACCTTTCC TGCAGTGCTGAAGTCAAGCGGGCTGTACAGCCTGTCCTCTGTGGTCACCGTGCCAAGTTCAAGCCTGGGAACAC AGACTTATATCTGCAACGTGAATCACAAGCCATCCAATACAAAAGTCGACAAGAAAGTGGAACCCAAGTCTTGT GATAAAACCCATACATGCCCCCCTTGTCCTGCACCAGAGCTGCTGGGAGGACCAAGCGTGTTCCTGTTTCCACC CAAGCCTAAAGATACACTGATGATTAGTAGGACCCCAGAAGTCACATGCGTGGTCGTGGACGTGAGCCACGAGG

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			ACCCCGAAGTCAAG.TTAACTGGTACG.GGACGGCGTCGAGGTGCATAATGCCAAGACTAAACCCAGGGAGGAA CAGTACAACAGCACCTACTCGCGTCGTGTCAGTCCTGACAGTGCTGCACCAGGATTGGCTGAACGGGAAAGAGTA TAAGTGCAAAGTGAGCAATAAGGCTCTGCCCGCACCTATCGAGAAAACAATTTCCAAGGCAAAAGGACAGCCCA GAGAACCACAGGTGTACGTGCTGCCTCCATCAAGGGATGAGCTGACAAAGAACCAGGTCAGCCTGCTGTCTG G.GAAAGGATTCTA.CCCTCTGACATTGCTGTGGAGTGGGAAAG.AATGGCCAGCCTGAGAACAATTACCTGAC CTGGCCCCCTGTGCTGGACTCAGATGGCAGCTTCTTTCTGTATAGCAAGCTGACCGTCGACAAATCCCGGTGGC AGCAGGGGAATGTGTTTAGTTGTTCAGTCATGCACGAGGCACTGCACAACCATTACACCCAGAAGTCACTGTCA C.TGTCACCAGGG
19	3468	VH	EVQLVESGGGLVQPGGSLRLSCAASGF.FTDY.MDWVRQAPGKGLEWVA.VNPNSGGS.YNQR.KGRFTLSVDR SKNTLYLQMNSLRAED.AVYYCARNLGPSFYFDYWGQGTLVTVSS
20	3468	VH	GAAGTGCAGCTGGTCGAATCTGGAGGAGGACTGGTGCAGCCAGGAGGGTCCCTGCGCCTGTCTTGCGCCGCTAG TGGC.TCACTT.TACCGACTACACCA.GGATTGGGTGCGACAGGCACCTGGAAAGGGCCTGGAGTGGGTCGCCG CATGTGAACCCAAATAGCGGAGGCTCCATCTACAACCAGCGGTTCAAGGGCCGGTTCACCCTGTCAGTGGACCGG AGCAAAAACACCCTGTACCTGCAGATGAATAGCCTGCGAGCCGAAGACACTGCTGTGTACTATTGCGCCCGGAA TCTGGGGCCCTCCTTCTACTTTGACTACTGGGGGCAGGGAACTCTGGTCACCGTGAGCTCC
21	3468	H1	GFTFTDYT
22	3468	H1	GGCTTCACTTTTACCGACTACACC
23	3468	H3	ARNLGPSFYFDY
24	3468	H3	GCCCGGAATCTGGGGCCCTCCTTCTACTTTGACTAT
25	3468	H2	VNPNSGGS
26	3468	H2	GTGAACCCAAATAGCGGAGGCTCC
27	3468	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKGYFPEPVTVSWNSGALTSGVHTFPAVLXSSGLYSLSSVVTVPSS SLG.QTYICNVNHKPSNTKVDAKV
28	3468	CH1	GCCTCCACCAAGGGACCTTCTGTGTTCCCACTGGCTCCC.CTAGTAAATCCACATCTGGGGGAACTGCAGCCC. GGGCTGTCTGGTGAAGGGCTACTTCCCAGAGCCCGTCACAGTGTCTTGGAACAGTGGCGCTCTGACTTCTGGGG TCCACACCTTTCCTGCAGTGCTGAAGTCAAGCGGGCTGTACAGCCTGTCCTCTGTGGTCACCGTGCCAAGTTCA AGCC.GGGAACACAGACTTATATCTGCAACGTGAATCACAAGCCATCCAATACAAAAGTCGACAAGAAAGTG
29	3468	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
30	3468	CH2	GCACCAGAGCTGCTGGGAGGACCAAGCGTGTTCCTGTTTCCACCCAAGCCTAAAGATACACTGATGATTAGTAG GACCCCAGAAGTCACATGCGTGGTCGTGGACGTGAGCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGG ACGGCGTCGAGGTGCATAATGCCAAGACTAAACCCAGGGAGGAACAGTACAACAGTACCTATCGCGTCGTGTCA GTCCTGACAGTGCTGCACCAGGATTGGCTGAACGGGAAAGAGTATAAGTGCAAAGTGAGCAATAAGGCTCTGCC CGCACCTA.CGAGAAAACAATTTCCAAGGCAAAA
31	3468	CH3	GQPREPQVYVLPPSREELTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
32	3468	CH3	GGACAGCC.AGAGAACCACAGGTGTACG.GCTGCCTCCA.CAAGGGATGAGCTGACAAAGAACCAGG.CAGCC. GCTG.GTC.GGTGAAAGGATTCTATCCCTCTGACATTGC.GTGGAGTGGGAAAGTAA.GGCCAGCC.GAGAACA ACTACCTGACCTGGCCCCCTGTGCTGGACTCAGATGGCAGCTTCTTTCTGTATAGCAAGCTGACCGTCGACAAA TCCCGGTGGCAGCAGGGGAATGTGTTTAGTTGTTCAGTCATGCACGAGGCACTGCACAACCATTACACCCAGAA GTCACTGTCACCTGTCACCAGGG
33	1811	Full	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
34	1811	Full	GACATTCAGATGACCCAGTCCCCAAGCTCCCTGAGTGCCTCAGTGGGCGACCGAGTCACCATCACATGCAAGGCC T.TCCCAGGATGTGTCTATTGGAGTCGCATGGTACCAGCAGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTA.A GCGCCTCC.ACCGG.ATACCGGCGTGCCCTCTAGATTC.CTGGCAGTGGGTCAGGAACAGACTTTACT.CTGACC ATCTCTAGTCTGCAGCCTGAGGATTTCGCTACC.ACTATTGCCAGCAGTACTATATCTACCCATATACCTTTGG CCAGGGGACAAAAG.GGAGATCAAGAGGACTG.GGCCGC.CCCTCCG.CTTCATTTT.CCCCCTTC.GACGAAC AGCTGAAAAGTGGCACAGCCAGCGTGG.CTGTC.GCTGAACAAT.TCCACCCTCGCGAAGCCAAAG.GCAGTGG AAGGTCGATAACGCCCTGCAGAGCGGCAACAGCCAGGAGTCTGTGACTGAACAGGACAGTAAAGATCCAACCTA TAGCCTGC.AGCACACCGACCTGAGCAAGGCAGACTACGAGAAGCACAAAGTGTATGCCTGCGAAGTCACAC A.TCAGGGGCTGTCC.CTCCTG.GACTAAGAGC.TCAACAGAGGAGAGTGT
35	1811	VL	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIK

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
36	1811	VL	GA.AT.CAGATGACCCAGTCCCCAAGCTCCCTGAGTGCC.CAGTGGGCGACCGAGTCACCATCACA.GCAAGGC TCCCAGGATGTGTCTATTGGAGTCGCATGGTACCAGCAGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTACA GCGCCTCCTACCGGTATACCGGCGTGCCCTCTAGATTCTCTGGCAGTGGGTCAGGAACAGACTTTACTCTGACC ATCTCTAGTCTGCAGCCTGAGGATTTCGCTACCTACTATTGCCAGCAGTACTATATCTACCCATATACCTTTGG CCAGGGGACAAAAGTGGAGATCAAG
37	1811	L1	QDVSIG
38	1811	L1	CAGGATGTGTCTATTGGA
39	1811	L3	QQYYIYPYT
40	1811	L3	CAGCAGTACTATATCTACCCATATACC
41	1811	L2	SAS
42	1811	L2	AGCGCCTCC
43	1811	CL	RTVAAPSVTIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
44	1811	CL	AGGACTGTGGCCGCTCCCTCCGTCTTCACTTTCCCCCTTCTGACGAACAGCTGAAAAGTGGCACAGCCAGCGT GGTCTGTCTGCTGAACAATTTCTACCCTCGCGAAGCCAAAGTGCAGTGGAAGGTCGATAACGCTCTGCAGAGCG GCAACAGCCAGGAG.CTGTGACTGAACGAGGACAGTAAAGA.TCAACCTA.AGCC.G.CAAGCACACTGACTCTG AGCAAGGCAGACTACGAGAAGCACAAAGTGTATGCCTGCGAAGTCACACATCAGGGGCTGTCCTCTCCCTGTGAC TAAGAGCTTTAACAGAGGAGAGTGT
45	5034	Full	.YKCDDDKDIQMTQSPSSLSASVGDRVT.TCRASQDVN.AVAWYQQKPG.APKLLIYSASFLYSGVPSRFSGSR SGTDFTL.ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVF.FPPSD.RLKSG.ASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
46	5034	Full	GAC.ACAAAGACGACGA.GACAAAGATATCCAGA.GACCCAGTCCCC.AGC.CCC.G.CCGC..CTG.GGGCGA TAGGGTCACTATTACCTGCCGCGCATCTCAGGACGTGAACACCGCAGTCGCCTGGTACCAGCAGAAGCCTGGGA AAGCTCCAAAGCTGCTGATCTACAGTGCATCATCCTGTATTCAGGAGTGCCCAGCCGGTTTAGCGGCAGCAGA TCTGGCACCGATTTCACAC.GAC.ATTTC.AGTCTGCAGCCTGAGGACTT.GCCACATAC.A.TGCCAGCAGCA CTATACCACACCCCCTACTTTCGGCCAGGGGACCAAAGTGGAGATCAAGCGAACTGTGGCGGCTCCAAGTGTCT TCATTTTTCCACCCAGCGATGAAAGACTGAAGTCCGGCACAGCTTCTGTGGTCTGTCTGCTGAACAATTTTTAC CCCAGAGAGGCCAAAG.GCAGTGGAAGG.CGACAACGCTCTGCAGAGTGGCAACAGCCAGGAGAGCG.GACAGA
			ACAGGAT.CCAAAGACTC.ACTTATAGTCTG.CAAGCACCCTGACACTGAGCAAGGCAGACTACGAAAAGCA.A AAG.GTA.GCCTGTGAGG.CACACA.CAGGGGCTGTCATCACCAGTCACCAAA.CA.TCAATCGGGGGGAGTGC
47	5034	VL	DIQMTQSPSSLSASVGDRVT.TCRASQDVNTAVAWYQQKPGKAPKLL.YSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVE.K
48	5034	VL	GATATCCAGATGACCCAGTCCCCTAGCTCCCTGTCCGCTCCTGTGGGCGATAGGGTCACTATTACCTGCCGCGC ATCTCAGGACGTGAACACCGCAGTCGCCTGGTACCAGCAGAAGCCTGGGAAAGCTCCAAAGCTGCTGATCTACA GTGCATCATTCCTGTATTCAGGAGTGCCCAGCCGGTTTAGCGGCAGCAGATCTGGCACCGATTTCACACTGACT ATTTCTAGTCTGCAGCCTGAGGACTTTGCCACATACTATTGCCAGCAGCACTATACCACACCCCCTACTTTCGG CCAGGGGACCAAAGTGGAGATCAAG
49	5034	L1	QDVNTA
50	5034	L1	CAGGACGT GAACACC GCA
51	5034	L3	QQHYTTPPT
52	5034	L3	CAGCAGCACTATACCACACCCCCTACT
53	5034	L2	SAS
54	5034	L2	AGTGCATCA
55	5034	CL	RTVAAPSVFIFPPSDEALKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
56	5034	CL	CGAACTGTGGCCGCTCCAAGTGTCTTCATTTTCCCACCCAGCGA.GAAAGACTGAAGTCCGGCACAGCTTCTG.G GGTCTGTCTGCTGAACAATTTCTACCCAGAGAGGCCAAAG.GCAGTGGAAGG.CGACAACGCTCTGCAGAG.G GCAACAGCCAGGAGAGCGTGACAGAACAGGA.TCCAAAGAC.CTCACTTA.AGTC.G.CAAGCACCC.GACAC.G AGCAAGGCAGAC.ACGAAAAGCAC.AAAG.GTATGCCTGTGAGG.CACACA.CAGGGGCTG.CATCACCAGTCAC CAAA.CA.TCAA.CGGGGGGAGTGC
57	5037	Full	DYKDDDDKDIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSR SGTDFTLTISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDERLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSKESVTEQDSKDSTYSLSSRLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
58	5037	Full	GACTACGAAAGACGACGATGACAAAGA.ATCCAGATGACCCAGTCCCCTAGCTCCCTGTCCGCTTCTG.GGGCGA TAGGGTCACTATTACCTGCCGCGCATC.CAGGACGTGAACACCGCAG.CGCCTGGTACCAGCAGAAGCCTGGGA AAGCTCCAAAGCTGCTGATCTACAGTGCATCATTCCTGTATTCAGGAGTGCCCAGCCGGTTTAGCGGCAGCAGA TCTGGCACCGATTTCACACTGACTATTCTAGTCTGCAGCCTGAGGACTTTGCCACATACTATTGCCAGCAGCA C.ATACCACACCCCTACTTTCGGCCAGGGGACCAAAG.GGAGA.CAAGCGAACTGTGGCCGCTCCAAGTGTC. TCATTTTCCACCCAGCGATGAAAGACTGAAGTCCGGCACAGCTTCTGTGGTCTGTCTGCTGAACAATTTTAC CCCAGAGAGGCCAAAGTGCAGTGGAAGGTCGACAACGCCTGCAGAGTGGCAACAGCAAGGAGAGCGTGACAGA ACAGGAT.CCAAAGACTCTAC.TATAG.CTGTCAAGCAGACTGACAC.GAGCAAGGCAGACTACGAAAAGCA.A AAGTGTA.GCCCGTGAGGTCACACATCAGGGGCCTGTCA.CACCAGTCACCAAATCATCAATCGGGGGGAGTGC
59	5037	VL	DIQMTQSPSSLSASVGDRVT.TCRASQDVNTAVAWYQQKPGKAPKLL.YSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFA.YYCQQHYTTPPT.FGQGTKVEIK
60	5037	VL	GA.ATCCAGATGACCCAGTCCCCTAGCTCCCTGTCCGCTTCTGTGGGCGATAGGGTCACTATTACCTGCCGCGC A.CTCAGGACGTGAACACCGCAGTCGCCTGGTACCAGCAGAAGCCTGGGAAAGCTCCAAAGCTGCTGATCTACA G.GCATCA.TCCTG.AT.CAGGAGTGCCCAGCCGGTTTAGCGGCAGCAGATCTGGCACCGATTTCACACTGAC. A.TTCTAG.CTGCAGCC.GAGGACTT.GCCACA.ACTA..GCCAGCAGCACTATACCACACCCCTACTTTCGG CCAGGGGACCAAAGTGGAGATCAAG
61	5037	L1	QDVNTA
62	5037	L1	CAGGACGT GAACACC GCA
63	5037	L3	QQHYTTPPT
64	5037	L3	CAGCAGCACTATACCACACCCCCTACT
65	5037	L2	SAS
66	5037	L2	AGTGCATCA
67	5037	CL	RTVAAPSVTIFPPSDERLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSKESVTEQDSKDSTYSLSSRLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
68	5037	CL	CGAACTGTGGCCGCTCCAAGTGTCTTCATTTTCCACCCAGCGATGAAAGACTGAAGTCCGGCACAGCTCTGT GGTCTGTCTGCTGAACAATTTTACCCCAGAGAGGCCAAAGTGCAGTGGAAGGTCGACAACGCTCTGCAGAGTG GCAACAGCAAGGAGAGCGTGACAGAACAGGATTCCAAAGACTCTACTTATAGTCTGTCAAGCAGACTGACACTG AGCAAGGCAGACTACGAAAAGCACTAAAGTGTATGCCTGTGAGGTCACACATCAGGGGCTGTCATCACCAGTCAC CAAA.CA.TCAATCGGGGGGAGTGC
69	3382	Full	DIQMTQSPSSLSASVGDRVT.TCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRY.GVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQYYIYPATFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
70	3382	Full	GA.AT.CAGATGACCCAGTCCCCAAGCTCCCTGAGTGCC.CAGTGGGCGACCGAGTCACCATCACA.GCAAGGC T.CCCAGGATGTGTCTATTGGAGTCGCATGGTACCAGCAGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTA.A GCGCCTCCTACCGGTATACCGGCGTGCCCTCTAGATTCTCTGGCAGTGGGTCAGGAACAGACTTTACTCTGACC A.TCTCTAG.CTGCAGCC.GAGGATTTCGCTACC.ACTA..GCCAGCAGTACTATATC.ACCCAGCCACCTTTGG CCAGGGGACAAAAG.GGAGATCAAGAGGACTG.GGCCGC.CCCTCCG.CTTCATTTT.CCCCCTTC.GACGAAC AGCTGAAAAGTGGCACAGCCAGCGTGGTCTGTCTGCTGAACAATTTCTACCCTCGCGAAGCCAAAGTGCAGTGG AAGGTCGATAACGCCCTGCAGAGCGGCAACAGCCAGGAGTCTGTGACTGAACAGGACAGTAAAGATTCAACCTA TAGCCTG.CAAGCACAC.GAC.CTGAGCAAGGCAGACTACGAGAAGCACAAAGTGTA.GCCTGCGAAGTCACAC ATCAGGGGCTGTCCCTCTCCTGTGACTAAGAGCTTCAACAGAGGAGAGTGT
71	3382	VL	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQYYIYPATFGQGTKVEIK
72	3382	VL	GACATTCAGATGACCCAGTCCCCAAGCTCCCTGAGTGCCTCAGTGGGCGACCGAGTCACCATCACATGCAAGGC TCCCAGGATGTGTCTACTGGAGTCGCATGGTACCAGCAGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTACA GCGCCTCCTACCGGTATACCGGCGTGCCCTCTAGATTCTCTGGCAGTGGGTCAGGAACAGACTTTACTCTGACC A.TCTCTAG.CTGCAGCC.GAGGATTTCGCTACC.ACTA..GCCAGCAGTACTATATC.ACCCAGCCACCTTTGG CCAGGGGACAAAAG.GGAGATCAAG
73	3382	L1	QDVSIG
74	3382	L1	CAGGATGTGTCTATTGGA
75	3382	L3	QQYYIYPAT
76	3382	L3	CAGCAGTACTATATCTACCCAGCCACC
77	3382	L2	SAS
78	3382	L2	AGCGCCTCC
79	3382	CL	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
80	3382	CL	AGGACTG.GGCCGC.CCCTCCG.CT.CATTTT.CCCCCT.CTGACGAACAGCTGAAAAG.GGCACAGCCAGCG. GGTCTGTCTGCTGAACAACTTCTACCCTCGCGAAGCCAAAGTGCAGTGGAAGGTCGATAACGCTCTGCAGAGCG GCAACAGCCAGGAGTCTGTGACTGAACAGGACAGTAAAGACTCAACCTATAGCCTGTCAAGCACACTGACTCTG AGCAAGGCAGACTACGAGAAGCACAAAGTGTATGCCTGCGAAGTCACACATCAGGGGCTGTCCTCTCCTGTGAC TAAGAGCTTTAACAGAGGAGAGTGT
81	5065	Full	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCE VTTY.PEPV.VSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY.CNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPG
82	5065	Full	GAGGTGCAGCTGGTGGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGGTCACTGCGACTGAGCTGCGCAGCTTC CGGCTTCAACATCAAGGACACCTACATCACTGGGTCCGCCAGGCTCCTGGAAAAGGCCTGGAGTGGGTGGCAC GAATCTATCCAACTAATGGATACACCCGGTATGCCGACTCCGTGAAGGGCCGGTTCACCATTTCTGCAGATACA AGTAAAAACACTGCCTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACAGCCGTGTACTATTGCAGCCGATG GGGAGGCGACGGCTTCTACGCTATGGATTATTGGGGGCAGGGAACCCTGGTCACAGTGAGCTCCGCATCAACAA AGGGGCCAAGCGTGTTCCACCTGGCCCCCTCTAGTAAACCCACCCTGGGGGAACAGCAGCCCTGGGATGTGAG
			GAGACCGACTACTTCCCAGAGCCCGTCACTGTGAGCTGGAACTCCGGCGCCCTGACATCTGGGGTCCATACTTC TCCTGCCGTGCTGCAGTCAAGGGGCCTGTACAGCCTGTCCTCTGTGGTCACTGTGCCAAGTCAAGCCTGGGGA CTCAGACCTATATCTGCAACGTGAATCACAAGCCATCCAATACCAAAGTCGACAAGAAAGTGGAACCCAAGTCT TGTGATAAAACACATGCCCCCCTTGTCCTGCACCAGAGCTGCTGGGAGGACCAAGCGTGTTCCTGTTTCC ACCCAAGCCTAAAGACACCCTGATGATTAGTAGGACTCCAGAAGTCACCTGCGTGGTCGTGGACGTGAGCCACG AGGACCCCGAAGTCAAGTTCAACTGGTACGTGGATGGCGTCGAGGTGCATAATGCCAAGACAAAACCCAGGGAG GAACAGTACAACTCCACCTATCGCGTCGTGTCTGTCCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGA GTATAAGTGCAAAGTGAGCAATAAGGCTCTGCCCGCACCTATCGAGAAAACAATTTCCAAGGCTAAAGGGCAGC CTAGAGAACCACAGGTGTACGTACCCTCCATCTAGGGACGAGCTGACCAAGAACCAGGTCAGTCTGACATGT CTGGTGAAAGGGTTCTATCCCAGCGATATCGCAGTGGAGTGGGAATCCAATGGACAGCCTGAGAACAATTACAA GACCACACCCCCTGTGCCTGGACTCTGATGGAAGTTTCGCCCTGGTGAGTAAGCTGACCGTGGATAAATCACGGT GGCAGCAGGGCAACGTGTTCAGCTGTTCAGTGATGCACGAAGCACTGCACAACCACTACACCCAGAAAAGCCTG TCCCTGTCCCCGGG
83	5065	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
84	5065	VH	GAGGTGCAGCTGGTGGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGGTCACTGCGACTGAGCTGCGCAGCTTC CGGCTTCAACATCAAGGACACCTACATCACTGGGTCCGCCAGGCTCCTGGAAAAGGCCTGGAGTGGGTGGCAC GAATCTATCCAACTAATGGATACACCCGGTATGCCGACTCCGTGAAGGGCCGGTTCACCATTTCTGCAGATACA AGTAAAAACACTGCCTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACAGCCGTGTACTATTGCAGCCGATG GGGAGGCGACGGCTTCTACGCTATGGATTATTGGGGGCAGGGAACCCTGGTCACAGTGAGCTCC
85	5065	H1	GFNIKDTY
86	5065	H1	GGCTTCAACATCAAGGACACCTAC
87	5065	H3	SRWGGDGFYAMDY
88	5065	H3	AGCCGATGGGGAGGCGACGGCTTCTACGCTATGGATTAT
89	5065	H2	IYPTNGYT
90	5065	H2	ATCTATCCAACTAATGGATACACC
91	5065	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCEVTDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKV
92	5065	CH1	GCATCAACAAAGGGGCCTAGCGTGTTTCCACTGGCCCCCTCTAGTAAATCCACCTCTGGGGGAACAGCAGCCCT GGGATGTGAGGTGACCGACTACTTCCCAGAGCCCGTCACTGTGAGCTGGAACTCCGGCGCCCTGACATCTGGGG TCCATACTTTCCTGCTGCTGCAGTCAAGCGGCCTGTACAGCCTGTCCTCTGTGGTCACTGTGCCAAGTTCA AGCCTGGGGACTCAGACCTATATCTGCAACGTGAATCACAAGCCATCCAATACCAAAGTCGACAAGAAAGTG
93	5065	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
94	5065	CH2	GCACCAGAGCTGCTGGGAGGACCAAGCGTGTTCCTGTTCCCACCCAAGCCTAAAGACACCCTGATGATTAGTAG GACTCCAGAAGTCACCTGCGTGGTCGTGGACGTGAGCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGG ATGGCGTCGAGGTGCATAATGCCAAGACAAAACCCAGGGAGGAACAGTACAACTCCACTTACCGCGTCGTGTCT GTCCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTATAAGTGCAAAGTGAGCAATAAGGCTCTGCC CGCACCTATCGAGAAAACAATTTCCAAGGCTAAA
95	5065	CH3	GQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
96	5065	CH3	GGGCAGCCXAGAGAACCACAGGTGTACGXGTACCCTCCA.CXAGGGACGAGCTGACCAAGAACCAGGXCAGTC. GACA.GXC.GG.GAAAGGGTTCTATCCCAGCGA.ATCGCAGTGGAGTGGGAATCCAA.GGACAGCC.GAGAACA ATACAAGACCACACCCCCTGXGCTGGACTCTGATGGAAGTTTCGCCCTGGTGAGTAAGCXGACCGXCGATAAA TCACGG.GGCAGCAGGGCAACGTGTTCAGCTG.XCAGTGATGCACGAAGCACTGCACAACCACTACACCCAGAA AAGCCTG.CCC.GTCCCCGGC
97	6586	Full	EVQLVESGGGLVQPGGSLRLSCAASGFTFADYTMDWVRQAPGKGLEWVGDVNPNSGGSIYNQRFKGRFTFSVDR SKNTLYLQMNSLKAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKS.SGG.AALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSN.KVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
			QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP.EKT.SKAKGQPREPQVYVYPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPG
98	6586	Full	GAGGTGCAGCTGGTGGAATCAGGAGGGGGCCTGGTGCAGCCCGGAGGGTCTCTGCGACTG.CATGTGCCGCTTC TGGGTTCACTTTCGCAGACTACACAACTGGATTGGGTGCGACAGGCCCCCGGAAAGGGACTGGAGTGGGTGGGCG A.GTCAACCCTAAT.CTGGCGGGAGTA.CTACAACCAGCGGTTCAAGGGGAGATTCACTT.TTCAG.GGACAGA AGCAAAAACACCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAAGACTACCGCTGTCTACTATTGCGCTCGCAA TCTGGGCCCCAGTT.CTACTT.GACTA.TGGGGGCAGGGAACCC.GGTGACAGTCAGCTCCGCTAGCACTAAGG GGCCTTCCGTGTTTCCACTGGCTCCCTCTAGTAAATCCACCTCTGGAGGCACAGCTGCACCGGGATGCTGGCG AAGGATTACTTCCCCGAACCAGTCACAGTGAGTTGGAACTCAGGGGCCTGACAAGTGGAGTCCATACTTTCC CGCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGGTCACCGTGCCTAGTTCAAGCCTGGGCACCC AGACATA.ATC.GCAACGTGAATCACAAGCCA.CAAATACAAAAGTCGACAAGAAAG.GGAGCCCAAGAGC.G. GATAAAAC.CA.ACCTGCCCACCTTG.CCGGCGCCAGAACTGCTGGGAGGACCAAGCGTG.TCCTG.T.TCCACC CAAGCC.AAAGACACCC.GATGATTTCCCGGAC.CCTGAGGTCACCTGCGTGGTCGTGGACGTGTC.CACGAGG ACCCCGAAGTCAAGTTCAACTGGTACGTGGATGGCGTGGAAGTGCATAATGCCAAGACCAAACCCCGGGAGGAA CAGTACAACTCTACCTATAGAGTCGTGAGTGTCCTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTA TAAGTGTAAAGTGAGCAACAAAGCCCTGCCCGCCCCAA.CGAAAAACAATCTCTAAAGCAAAAGGACAGCCCC GCGAACCACAGGTC.ACGTCTACCCCCCATCAAGAGATGAACTGACAAAAAATCAGGTCTCTCTGACATGCC.G GTCAAAGGATTCTACCCCTCCGACATCGCCGTGGAGTGGGAAAGTAACGGCCAGCCCGAGAACAATTACAAGAC CACACCCCCTGTCC.GGACTC.GATGGGAGTT.CGCTC.GGTGTCAAAGCTGACGTCGA.AAAAGCCGGTGGC AGCAGGGCAATGTGTTAGCTGCTCCGTCATGCACGAAGCCCTGCACAATCACTACACACAGAAGTCCCTGAGC CTGAGCCCTGGC
99	6586	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFADYTMDWVRQAPGKGLEWVGDVNPNSGGSIYNQRFKGRFTFSVDR SKNTLYLQMNSLKAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSS
100	6586	VH	GAGGTGCAGCTGGTGGAATCAGGAGGGGGCCTGGTGCAGCCCGGAGGGTCTCTGCGACTGTCATGTGCCGCTTC TGGG.TCACTTTCGCAGACTACACAA.TGGATTGGGTGCGACAGGCCCCCGGAAAGGGACTGGAGTGGGTGGGCG A.GTCAACCCTAAT.CTGGCGGGAGTA.CTACAACCAGCGGTTCAAGGGGAGATTCACTT.TTCAG.GGACAGA AGCAAAAACACCCTGTACCTGCAGATGAACAGCCTGAGGGCCGAAGACTACCGCTGTCTACTATTGCGCTCGCAA TCTGGGCCCCAGTT.CTACTT.GACTA.TGGGGGCAGGGAACCC.GGTGACAGTCAGCTCC
101	6586	H1	GFTFADYT
102	6586	H1	GGGTTCACTTTCGCAGACTACACA
103	6586	H3	ARNLGPSFYFDY
104	6586	H3	GCTCGCAATCTGGGCCCCAGTTTCTACTTTGACTAT
105	6586	H2	VNPNSGGS
106	6586	H2	GTCAACCCTAATCTGGCGGGAGT
107	6586	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKV
108	6586	CH1	GC.AGCAC.AAGGGGCCTTCCGTGTT.CCACTGGCTCCC.CT.AGTAAATCCACCTCTGGAGGCACAGCTGCAC. GGGATGTCTGGTGAAGGATTACTTCCCCGAACCAGTCACAGTGAGTTGGAACTCAGGGGCCTGACAAGTGGAG TCCATACTTTTCCCGCAGTGCTGCAGTCAAGCGGACTGTACTCCCTGTCCTCTGTGGTCACCGTGCCTAGTTCA AGCC.GGGCACCCAGACATATATCTGCAACGTGAATCACAAGCCATCAAATACAAAAGTCGACAAGAAAGTG
109	6586	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAP.EKT.SKAK
110	6586	CH2	GCGCCAGAACTGCTGGGAGGACCAAGCGTGTTCCTGTTTCCACCCAAGCCTAAAGACACCCTGATGATTTCCCG GACTCCTGAGGTCACCTGCGTGGTCGTGGACGTGTCTCACGAGGACCCCGAAG.CAAGTTCAACTGG.ACGTGG A.GGCGTCGAAGTGCATAATGCCAAGACCAAACCCCGGGAGGAACAGTACAAC.CTACCTA.AGAG.CGTGAG. GTCCTGACAGTGCTGCACCAGGACTGGCTGAATGGGAAGGAGTATAAGTGTAAAGTGAGCAACAAAGCCCTGCC CGCCCCAA.CGAAAAAACAATCTC.AAAGCAAAA

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
111	6586	CH3	GQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
112	6586	CH3	GGACAGCCTCGCGAACCACAGGTCTACGTCTACCCCCCATCAAGAGATGAACTGACAAAAAATCAGGTCTCTCT GACATGCCTGGTCAAAGGATTCTACCCTTCCGACATCGCCGTGGAGTGGGAAAGTAACGGCCAGCCCGAGAACA ATTACAAGACCACACCCCCTGTCCTGGACTCTGATGGGAGTTTCGCTCTGGTGTCAAAGCTGACCGTCGATAAA AGCCGGTGGCAGCAGGGCAATGTGTTTAGCTGCTCCGTCATGCACGAAGCCCTGCACAATCACTACACACAGAA GTCCCTGAGCCTGAGCCCTGGC
113	3904	Full	YPYDVPDYATGSDIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKRTVAAPSVFIFPPSDEELKSGTASVVCLL NNFYPREAKVQWKVDNALQSGNSEESVTEQDSKDSTYSLSSTLELSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC
114	3904	Full	TATCCCTACGATGTGCCTGACTACGCTACTGGCTCCGATATCCAGATGACCCAGTCTCCAAGCTCCCTGAGTGC ATCAGTGGGGGACCGAGTCACCATCACATGCAAGGCTTCCCAGGATGTGTCTATTGGAGTCGCATGGTACCAGC AGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTACAGCGCCTCCTACCGGTATACTGGGGTGCCTTCCAGATTC TCTGGCAGTGGGTCAGGAACCGACTTTACTCTGACCATCTCTAGTCTGCAGCCCGAGGATTTCGCCACCTACTA TTGCCAGCAGTACTATATCTACCCTTATACCTTTGGCCAGGGGACAAAAGTGGAGATCAAGAGGACAGTGGCCG CTCCAAGTGTCTTCATTTTTCCCCCTTCCGACGAAGAGCTGAAAAGTGGAACTGCTTCAGTGGTCTGTCTGCTG AACAATTTCTACCCCCGCGAAGCCAAAGTGCAGTGGAAGGTCGATAACGCTCTGCAGAGCGGCAATTCCGAGGA GTCTGTGACAGAACAGGACAGTAAAGATTCAACTTATAGCCTGTCAAGCACACTGGAGCTGTCTAAGGCAGACT ACGAGAAGCACAAAGTGTATGCCTGCGAAGTCACCCATCAGGGGCTGTCCTCTCCCGTGACAAAGAGCTTTAAC AGAGGAGAGTGT
115	3904	VL	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIK
116	3904	VL	GATATCCAGATGACCCAGTCTCCAAGCTCCCTGAGTGCATCAGTGGGGGACCGAGTCACCATCACATGCAAGGC TTCCCAGGATGTGTCTATTGGAGTCGCATGGTACCAGCAGAAGCCAGGCAAAGCACCCAAGCTGCTGATCTACA GCGCCTCCTACCGGTATACTGGGGTGCCTTCCAGATTCTCTGGCAGTGGGTCAGGAACCGACTTTACTCTGACC ATCTCTAGTCTGCAGCCCGAGGATTTCGCCACCTACTATTGCCAGCAGTACTATATCTACCCTTATACCTTTGG CCAGGGGACAAAAGTGGAGATCAAG
117	3904	L1	QDVSIG
118	3904	L1	CAGGATGTGTCTATTGGA
119	3904	L3	QQYYIYPYT
120	3904	L3	CAGCAGTACTATATCTACCCTTATACC
121	3904	L2	SAS
122	3904	L2	AGCGCCTCC
123	3904	CL	RTVAAPSVFIFPPSDEELKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSEESVTEQDSKDSTYSLSSTLEL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
124	3904	CL	AGGACAGTGGCCGCTCCAAGTGTCTTCATTTTTCCCCCTTCCGACGAAGAGCTGAAAAGTGGAACTGCTTCAGT GGTCTGTCTGCTGAACAATTTCTACCCCCGCGAAGCCAAAGTGCAGTGGAAGGTCGATAACGCTCTGCAGAGCG GCAATTCCGAGGAGTCTGTGACAGAACAGGACAGTAAAGATTCAACTTATAGCCTGTCAAGCACACTGGAGCTG TCTAAGGCAGACTACGAGAAGCACAAAGTGTATGCCTGCGAAGTCACCCATCAGGGGCTGTCCTCTCCCGTGAC AAAGAGCTTTAACAGAGGAGAGTGT
125	4553	Full	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
126	4553	Full	GAAGTCCAGCTGGTCGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGGTCTCTGCGACTGAGTTGCGCCGCTTC AGGCTTCAACATCAAGGACACCTACATTCACTGGGTGCGCCAGGCTCCTGGAAAAGGCCTGGAGTGGGTGGCAC GAATCTATCCAACTAATGGATACACCCGGTATGCAGACAGCGTGAAGGGCCGGTTCACCATTAGCGCAGATACA TCCAAAAACACTGCCTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACTGCTGTGTACTATTGCAGTCGGTG
			GGGAGGCGACGGCTTCTACGCTATGGATTATTGGGGGCAGGGAACCCTGGTCACAGTGAGCTCCGCATCTACAA AGGGGCCTAGTGTGTTTCCACTGGCCCCCTCTAGTAAATCCACCTCTGGGGGAACAGCAGCCCTGGGATGTCTG GTGAAGGACTATTTCCCAGAGCCCGTCACTGTGAGTTGGAACTCAGGCGCCCTGACATCCGGGGTCCATACTTT TCCTGCTGTGCTGCAGTCAAGCGGCCTGTACTCTCTGTCCTCTGTGGTCACCGTGCCAAGTTCAAGCCTGGGGA CTCAGACCTATATCTGCAACGTGAATCACAAGCCAAGCAATACAAAAGTCGACAAGAAAGTGGAACCCAAGAGC [illegible]

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			[illegible] ACCCAAGCCTAAAGACACCCTGATGATTTCCAGGAGTCCAGAAGTCACCTGCGTGGTCGTGGACGTGTCTCACG AGGACCCCGAAGTCAAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCATAATGCCAAGACAAAACCCAGGGAG GAACAGTACAACTCAACTATCGCGTCGTGAGCGTCCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGA GTATAAGTGCAAGGTGAGCAATAAGGCCCTGCCCGCACCTATCGAGAAAACCATTAGCAAGGCCAAAGGGCAGC CCAGAGAACCACAGGTCTACGTGTATCCTCCAAGCAGGGACGAGCTGACCAAGAACCAGGTCTCCCTGACATGT CTGGTGAAAGGGTTTTACCCCAGTGATATCGCCGTGGAGTGGGAATCAAATGGACAGCCTGAAAACAATTATAA GACCACACCCCCTGTGCTGGACAGCGATGGCAGCTTCGCCCTGGTCTCCAAGCTGACCGTGGATAAATCTCGGT GGCAGCAGGGCAACGTCTTTAGTTGTTCAGTGATGCATGAGGCACTGCACAATCATTACACCCAGAAGAGCCTG TCCCTGTCTCCCGGCAAA
127	4553	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
128	4553	VH	GAAGTCCAGCTGGTCGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGGTCTCTGCGACTGAGTTGCGCCGCTTC AGGCTTCAACATCAAGGACACCTACATCCACTGGGTGCGCCAGGCTCCTGGAAAAGGCCTGGAGTGGGTGGCAC GAATCTATCCAACTAATGGATACACCCGGTATGCAGACAGCGTGAAGGGCCGGTTCACCATAGCGCAGATACA TCCAAAAACACTGCCTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACTGCTGTGTACTATTGCAGTCGGTG GGGAGGCGACGGCTTCTACGCTATGGATTATTGGGGGCAGGGAACCCTGGTCACAGTGAGCTCC
129	4553	H1	GFNIKDTY
130	4553	H1	GGCTTCAACATCAAGGACACCTAC
131	4553	H3	SRWGGDGFYAMDY
132	4553	H3	AGTCGGTGGGGAGGCGACGGCTTCTACGCTATGGATTAT
133	4553	H2	IYPTNGYT
134	4553	H2	ATCTATCCAACTAATGGATACACC
135	4553	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKV
136	4553	CH1	GCATCTACAAAGGGGCCTAGTGTGTTTCCACTGGCCCCCTCTAGTAAATCCACCTCTGGGGGAACAGCAGCCCT GGGCATGTCTGGTAAGGACTATTTCCCAGAGCCCGTCACTGTGAGTTGGAACTCAGGCGCCCTGACATCCGGGG TCCATACATTTCCTGCTGTGCAGTCAAGTCGGCCTGTACTCTCTGTCCTCTGTGGTCACCGTGCCAAGTTCA AGCCTGGGGACCTCAGACCTATATCTGCAACGTGAATCACAAGCCAAGCAATACAAAAGTCGACAAGAAAGTG
137	4553	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
138	4553	CH2	GCACCAGAGCTGCTGGGAGGACCATCCGTGTTCCTGTTTCCACCGAAGCCTAAAGACACCCTGATGATTTCCAG GACTCCAGAAGTCACCTGCGTGGTCGTGGACGTGTCTCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGG ATGGCGTGGAGGTGCATAATGCCAAGACAAAACCCAGGGAGGAACAGTACAACTCAACTATCGCGTCGTGAGC GTCCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAGGAGTATAAGTGCAAAGTGAGCAATAAGGCCCTGCC CGCACCTATCGAGAAAACCATTAGCAAGGCCAAA
139	4553	CH3	GQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
140	4553	CH3	GGGCAGCCTAGAGAACCACAGGTCTACGTGTATCCTCCAAGCAGGGACGAGCTGACCAAGAACCAGGTCTCCCT GACATGTCTGGTAAAGGGTTTTACCCCAGTGATATCGCCGTGGAGTGGGAATCAAATGGACAGCCTGAAAACA ATTATAAGACCACACCCCCTGTGCTGGACAGCGATGGCAGCTTCGCCCTGGTCTCCAAGCTGACCGTGGATAAA TCTCGGTGGCAGCAGGGCAACGTCTTTAGTTGTTCAGTGATGCATGAGGCACTGCACAATCATTACACCCAGAA GAGCCTGTCCCTGTCTCCCGGC
141	716	Full	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
			SLICLVKGFYPSDIAVEWESNGQPENRYMTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK
142	716	Full	GAGCCCAAGAGCAGCGATAAGACCCACACCTGCCCTCCCTGTCCAGCTCCAGAACTGCTGGGAGGACCTAGCGT GTTCCTGTTTCCCCCTAAGCCAAAAGACACTCTGATGATTTCCAGGACTCCCGAGGTGACCTGCGTGGTGGTGG ACGTGTCTCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGGATGGCGTGGAAGTGCATAATGCCAAGACA AAACCAAGAGAGGAACAGTACAACTCCACTTATCGCGTCGTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCT GAACGGGAAGGAGTATAAGTGCAAAGTCAGTAATAAGGCCCTGCCTGCTCCAATCGAAAAAACCATCTCTAAGG CCAAAGGCCAGCCAAGGGAGCCCCAGGTGTACACACTGCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTG TCCCTGACTCTGCTGGTGAAAGGCTTCTATCCTAGTGATATTGCTGTGGAGTGGGAATCAAATGGACAGCCAGA GAACAGATACAAGACCTGGCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCCTGTATTCCAAGCTGACAGTGG ATAAATCTCGATGGCAGCAGGGGAACGTTTAGTTGTTCAGTGATGCATGAAGCCCTGCACAATCATTACACT CAGAAGAGCCTGTCCCTGTCTCCCGGCAAA

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143	716	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
144	716	CH2	GCTCCAGAATTGCTGGGAGGACCTAGCGTGTTCCTGTTCCCCCCAAGCCAAAAGACACTCTGATGATCTCCAG GACTCCCGAGGTGACCTGCGTGGTGGACGTGTCTCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGG ACGGCGTGGAAGTGCATAATGCTAAGACAAAACCAAGAGAGGAACAGTACAACTCCACTTACCGCGTCGTGAGC GTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTCAGTAATAAGGCCCTGCC TGCTCCAATCGAAAAAACCATCTCTAAGGCCAAA
145	716	CH3	GQPREPQVYTLPPSRDELTKNQVSLICLVKGFYPSDIAVEWESNGQPENRYMTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
146	716	CH3	GGCCAGCCAAGGGAGCCCCAGGTGTACACACTGCCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTGTCCCT GATCTGTCTGGTGAAAGGCTTCTATCCCAGTGATATTGCTGTGGAGTGGGAATCAAATGGACAGCCAGAGAACA GATACAAGACCTGGCCTCCAGTGCTGGACAGCGATGGCAGCTTCTTCCTGTATTCCAAGCTGACAGTGGATAAA TCTCGATGGCAGCAGGGGAACGTGTTTAGTTGTTCAGTGATGCATGAAGCCCTGCACAATCATTACACTCAGAA GAGCCTGTCCCTGTCTCCCGGC
147	719	Full	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGSGGGSGGGSGGGSGGGSEVQLVESGGGLVQPGGSLRLS CAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRWGGDGFYAMDYWGQGTLVTVSSAAEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLICLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
148	719	Full	GACATCCAGATGACCCAGTCTCCATCCTCCCTGCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC AAGTCAGGACGTTAACACCGCTGTAGCTTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTACT CTGCATCCTTTTTGTACAGTGGGGTCCCATCAAGGTTCAGTGGCAGTCGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGCATTACACTACCCCACCCACTTTCGG CCAAGGGACCAAAGTGGAGATCAAAGGTGGTTCTGGTGGTGGTTCTGGTGGTGGTTCTGGTGGTGGTTCTGGTG GTGGTTCTGGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCGGGTCCCTGAGACTCTCC TGTGCAGCCTCCGGATTCAACATTAAAGATACTTATATCCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGA GTGGGTCGCACGTATTTATCCCACAAATGGTTACACACGGTATGCGGACTCTGTGAAGGGCCGATTCACCATCT CCGCAGACACTTCCAAGAACACCGCGGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCGTTTATTAC TGTTCAAGATGGGGCGGAGACGGTTTCTACGCTATGGACTACTGGGGCCAAGGGACCCTGGTCACCGTCTCCTC AGCCGCCGAGCCCAAGAGCAGCGGATAAGACCCACACCTGCCCTCCCTGTCCAGCTCCAGAACTGCTGGGAGGAC CTAGCGTGTTCCTGTTTCCCCCTAAGCCAAAAGACACTCTGATGATTTCCAGGACTCCCGAGGTGACCTGCGTG GTGGTGGACGTGTCTCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGGATGGCGTGGAAGTGCATAATGC TAAGACAAAACCAAGAGAGGAACAGTACAACTCCACTTACCGCGTCGTGAGCGTGCTGACCGTGCTGCACCAGG ACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTCAGTAATAAGGCCCTGCCTGCTCCAATCGAAAAAACCATC TCTAAGGCCAAAGGCCAGCCAAGGGAGCCCCAGGTGTACACATACCCACCCAGCAGAGACGAACTGACCAAGAA CCAGGTGTCCCTGACATGTCTGGTGAAAGGCTTCTATCCCAGTGATATTGCTGTGGAGTGGGAATCAAATGGAC AGCCAGAGAACAATTACAAGACCACACCTCCAGTGCTGGACGAGGATGGCAGCTTCGCCCTGGTGTCCAAGCTG
			ACAGTGGATAAATCTCGATGGCAGCAGGGGAACGTGTTTAGTTGTTCAGTGATGCATGAAGCCCTGCACAATCA TTACACTCAGAAGAGCCTGTCCCTGTCTCCCGGCAAA
149	719	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK
150	719	VL	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC AAGTCAGGACGTTAACACCGCTGTAGCTTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTACT CTGCATCCTTTTTGTACAGTGGGGTCCCATCAAGGTTCAGTGGCAGTCGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGCATTACACTACCCCACCCACTTTCGG CCAAGGGACCAAAGTGGAGATCAAA
151	719	L1	QDVNTA
152	719	L1	CAGGACGTTAACACCGCT
153	719	L3	QQHYTTPPT
154	719	L3	CAACAGCATTACACTACCCCACCCACT
155	719	L2	SAS
156	719	L2	TCTGCATCC
157	719	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
158	719	VH	GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCGGGTCCCTGAGACTCTCCTGTGCAGCCTC

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			TGGATTCAACATTAAAGATACTTATATACCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCGCAC GTATTTATCCCACAAATGGTTACACAGGTATGCGGACTCTGTGAAGGGCCGATTCACCATCTCCGCAGACACT TCCAAGAACACCGCGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCCGTTTATTACTGTCAAGATG GGGCGGAGACGGTTTCTACGCTATGGACTACTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA
159	719	H1	GFNIKDTY
160	719	H1	GGATTCAACATTAAAGATACTTAT
161	719	H3	SRWGGDGFYAMDY
162	719	H3	TCAAGATGGGGCGGAGACGGTTTCTACGCTATGGACTAC
163	719	H2	IYPTNGYT
164	719	H2	ATTTATCCCACAAATGGTTACACA
165	719	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
166	719	CH2	GCTCCAGAACTGCTGGGAGGACCTAGCGTGTTCCTGTTCCCCCAAGCCAAAAGACACTCTGATGATTTCCAG GACTCCCGAGGTGACCTGCGTGGTGGACGTGTCTCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGG ATGGCGTGGAAGTGCATAATGCTAAGACAAAACCAAGAGAGGAACAGTACAACTCCACTTATCGCGTGGTGAGC GTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTCAGTAATAAGGCCCTGCC TGCTCCAATCGAAAAAACCATCTCTAAGGCCAAA
167	719	CH3	GQPREPQVYTYPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
168	719	CH3	GGCCAGCCAAGGGAGCCCCAGGTGTACACATACCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTGTCCCT GACATGTCTGGCGAAAGGCTTCTATCCCAGTGATATTGCTGTGGAGTGGGAATCAAACTGGACAGCCAGAGAACA ATACAAGACCACACCTCCAGTGCTGGACGAGGATGGCAGCTTCGCCCTGGTGTCCAAGCTGACAGTGGATAAA TCTCGATGGCAGCAGGGGAACGTGTTTAGTTGCTCAGTGATGCATGAAGCCCTGCACAATCATTACACTCAGAA GAGCCTGTCCCTGTCTCCCGGC
169	720	Full	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGGSGGGSGGGSGGGSGGGSEVQLVESGGGLVQPGGSLRLS CAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRWGGDGFYAMDYWGQGTLVTVSSAAEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENRYMTWPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
170	720	Full	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC AAGTCAGGACGTTAACACCGCTGTAGCTTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATT CTGCATCCTTTTTGTACAGTGGGGTCCCATCAAGGTTCAGTGGCAGTCGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGCATTACACTACCCCACCCACTTTCGG CCAAGGGACCAAAGGGAGATCAAAGGTGGTTCTGGTGGTGGTTCTGGTGGTGGTTCTGGTGGTGGTTCTGGTG GTGGTCTGGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCGGGTCCCTGAGACTCTCC TGTGCAGCCTCTGGATTCAACATTAAAGATACTTATATCCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGA GTGGGTCGCACGTATTTATCCCACAAATGGTTACACACGGTATGCGGACTCTGTGAAGGGCCGATTCACCATCT CCGCAGACACTTCCAAGAACACCGCGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCGTTTATTAC TGTTCAAGATGGGGCGGAGACGGTTTCTACGCTATGGACTACTGGGGCCAAGGGACCCTGGTCACCGTCTCCTC AGCCGCCGAGCCCAAGAGCAGCGATAAGACCCACACCTGCCCTCCCTGTCCAGCTCCAGAACTGCTGGGAGGAC CTAGCGTGTTCCTGTTCCCCCTAAGCCAAAAGACACTCTGATGATTTCCAGGACTCCCGAGGTGACCTGCGTG GTGGTGGACGTGTCTCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGGATGGCGTGGAAGTGCATAATGC TAAGACAAAACCAAGAGAGGAACAGTACAACTCCACTTATCGCGTGGTGAGCGTGCTGACCGTGCTGCACCAGG ACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTCAGTAATAAGGCCCTGCCTGCTCCAATCGAAAAAACCATC TCTAAGGCCAAAGGCCAGCCAAGGGAGCCCCAGGTGTACACACTGCCACCCAGCAGAGACGAACTGACCAAGAA CCAGGTGTCCCTGACTGTCTGGTGAAAGGCTTCTATCCCAGTGATATTGCTGTGGAGTGGGAATCAAATGGAC AGCCAGAGAACAGATACAAGACCACACCTCCAGTGCTGGACGAGGATGGCAGCTTCTTCCTGTATTCCAAGCTG ACAGTGGATAAATCTCGATGGCAGCAGGGGAACGTGTTTAGTTGCTCAGTGATGCATGAAGCCCTGCACAATCA TTACACTCAGAAGAGCCTGTCCCTGTCTCCCGGCAAA
171	720	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK
172	720	VL	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC AAGTCAGGACGTTAACACCGCTGTAGCTTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATT CTGCATCCTTTTTGTACAGTGGGGTCCCATCAAGGTTCAGTGGCAGTCGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGCATTACACTACCCCACCCACTTTCGG CCAAGGGACCAAAGTGGAGATCAAA
173	720	L1	QDVNTA
174	720	L1	CAGGACGTTAACACCGCT
173	720	L3	QQHYTTPPT

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
176	720	L3	CAACAGCATTACACTACCCCACCCACT
177	720	L2	SAS
178	720	L2	TCTGCATCC
179	720	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
180	720	VH	GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGCGGGTCCCTGAGACTCTCCTGTGCAGCCTC TGGATTCAACATTAAAGATACTTATATCCACTGGGTCCGGCAAGCTCCAGGGAAGGGCCTGGAGTGGGTCGCAC GTATTTATCCCACAAATGGTTACACACGGTATGCGGACTCTGTGAAGGGCCGATTCACCATCTCCGCAGACACT TCCAAGAACACCGCGTATCTGCAAATGAACAGTCTGAGAGCTGAGGACACGGCCGTTTATTACTGTTCAAGATG GGGCGGAGACGGTTTCTACGCTATGGACTACTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA
181	720	H1	GFNIKDTY
182	720	H1	GGATTCAACATTAAAGATACTTAT
183	720	H3	SRWGGDGFYAMDY
184	720	H3	TCAAGATGGGGCGGAGACGGTTTCTACGCTATGGACTAC
185	720	H2	IYPTNGYT
186	720	H2	ATTTATCCCACAAATGGTTACACA
187	720	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVWDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRWS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
188	720	CH2	GCTCCAGAACTGCTGGGAGGACCTAGCGTGTTCCTGTTTCCCCCTAAGCCAAAAGACACTCTGATGATTTCCAG GACTCCCGAGGTGACCTGCGTGGTGGTGGACGTGTCTCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGG ATGGCGTGGAAGTGCATAATGCTAAGACAAAACCAAGAGAGGAACAGTACAACTCCACTTATCGCGTCGTGAGC GTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTCAGTAATAAGGCCCTGCC TGCTCCAATCGAAAAAACCATCTCTAAGGCCAAA
189	720	CH3	GQPREPQVYTLPPSRDELTKNQVSLICLVKGFYPSDIAVEWESNGQPENRYMTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
190	720	CH3	GGCCAGCCAAGGGAGCCCCAGGTGTACACACTGCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTGTCCCT GATCTGTCTGGTGAAAGGCTTCTATCCTAGTGATATTGCTGTGGAGTGGGAATCAAATGGACAGCCAGAGAACA GATACATGACCTGGCCTCCAGTGCTGGACAGCGATGGCAGCTTCTTCCTGTATTCCAAGCTGACAGTGGATAAA TCTCGATGGCAGCAGGGGAACGTGTTTAGTTGTTCAGTGATGCATGAAGCCCTGCACAATCATTACACTCAGAA GAGCCTGTCCCTGTCTCCCGGC
191	4561	Full	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
192	4561	Full	GATATTCAGATGACCCAGTCCCCTAGCTCCCTGTCCGCTTCTGTGGGCGACAGGGTCACTATCACCTGCCGCGC ATCTCAGGATGTGAACACCGCAGTCGCCTGGTACCAGCAGAAGCCTGGGAAAGCTCCAAAGCTGCTGATCTACA GTGCATCATTCCTGTATTCAGGAGTGCCCAGCCGGTTTAGCGGCAGCAGATCTGGCACCGACTTCACACTGACT ATCTCTAGTCTGCAGCCTGAGGATTTTGCCACATACTATTGCCAGCAGCACTATACCACACCCCCTACTTTCGG CCAGGGGACCAAAGTGGAGATCAAGCGAACTGTGGCCGCTCCAAGTGTCTTCATTTTTCCACCCAGCGACGAAC AGCTGAAATCCGGCACAGCTTCTGTGGTCTGTCTGCTGAACAACTTCTACCCCAGAGAGGCCAAAGTGCAGTGG AAGGTCGATAACGCTCTGCAGAGTGGCAACAGCCAGGAGAGCGTGACAGAACAGGACTCCAAAGATTCTACTTA TAGTCTGTCAAGCACCCTGACACTGAGCAAGGCAGACTACGAAAAGCATAAAGTGTATGCCTGTGAGGTGACCC ATCAGGGGCTGTCTTCTCCCGTGACCAAGTCTTTCAACCGAGGCGAATGT
193	4561	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK
194	4561	VL	GATATTCAGATGACCCAGTCCCCTAGCTCCCTGTCCGCTTCTGTGGGCGACAGGGTCACTATCACCTGCCGCGC ATCTCAGGATGTGAACACCGCAGTCGCCTGGTACCAGCAGAAGCCTGGGAAAGCTCCAAAGCTGCTGATCTACA GTGCATCATTCCTGTATTCAGGAGTGCCCAGCCGGTTTAGCGGCAGCAGATCTGGCACCGACTTCACACTGACT ATCTCTAGTCTGCAGCCTGAGGATTTTGCCACATACTATTGCCAGCAGCACTATACCACACCCCCTACTTTCGG CCAGGGGACCAAAGTGGAGATCAAG
195	4561	L1	QDVNTA
196	4561	L1	CAGGATGT GAACACC GCA
197	4561	L3	QQHYTTPPT
198	4561	L3	CAGCAGCACTATACCACACCCCCTACT
199	4561	L2	SAS
200	4561	L2	AGTGCATCA

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
201	4561	CL	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
202	4561	CL	CGAACTGTGGCCGCTGCAAGTGTTTCATTTTCCACCCAGCGACGAACAGCTGAAATCCGGCACAGCTTCTGT GGTCTGTCTGCTGAACAACTTCTATCCCAGAGAGGCCAAAGTGCAGTGGAAGGTCGATAACGCTCTGCAGAGTG GCAACAGCCAGGAGAGCGTGACAGAACAGGACTCCAAAGATTCTACTTATAGTCTGTCAAGCACCCTGACACTG AGCAAGGCAGACTACGAAAAGCACAAAGTGTATGCCTGTGAGGTGACCCATCAGGGGCTGTCTTCTCCCGTGAC CAAGTCTTTCAACCGAGGCGAATGT
203	3041	Full	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDR SKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSRDELTKNQVSLTCL
			VKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPG
204	3041	Full	GAAGTGCAGCTGGTCGAATCTGGAGGAGGACTGGTGCAGCCAGGAGGGTCCCTGCGCCTGTCTTGCGCCGCTAG TGGCTTCACTTTTACCGACTACACCATGGATTGGGTGCGACAGGCACCTGGAAAGGGCCTGGAGTGGGTCGCCG ATGTGAACCCAAATAGCGGAGGCTCCATCTACAACCAGCGGTTCAAGGGCCGGTTCACCCTGTCAGTGGACCGG AGCAAAAACACCCTGTACCTGCAGATGAATAGCCTGCGAGCCGAAGACACTGCTGTGTACTATTGCGCCCGGAA TCTGGGGCCCTCCTTCTACTTTGACTATTGGGGGCAGGGAACTCTGGTCACCGTGAGCTCCGCCTCCACCAAGG GACCTTCTGTGTTCCCACTGGCTCCCCTAGTAAATCCACATCTGGGGGAACTGCAGCCCTGGGCTGCTGGTG AAGGACTACTTCCCAGAGCCCGTCACAGTGTCTTGGAACAGTGGCGCTCTGACTTCTGGGGTCCACACCTTCC TGCAGTGCTGCAGTCAAGCGGGGCTGTACAGCCTGTCCTCTGTGGTCACCGTGCCAAGTTCAAGCCTGGGAACAC AGACTTACATCTGCAACGTGAATCACAAGCCATCGAATACAAAAGTCGACAAGAAAGTGGAACCCAAGTCTTGT GATAAAACCCACACATGCCCCCCTTGTCCTGCACCAGAGCTGCTGGGAGGACCAAGCGTGTTCCTGTTTCCACC CAAGCCTAAAGATACACTGATGATTAGTAGGACCCCAGAAGTCACATGCGTGGTCGTGGACGTGAGCCACGAGG ACCCCGAAGTCAAGTTAACTGGTACGTGGACGGCGTCGAGGTGCATAATGCCAAGACTAAACCCAGGGAGGAA CAGTACAACAGCACCTACCGCGTCGTGTCAGTCCTGACAGTGCTGCATCAGGATTGGCTGAACGGGAAAGAGTA TAAGTGCAAAGTGAGCAATAAGGCTCTGCCCGCACCTATCGAGAAAACAATTTCCAAGGCAAAAGGACAGCCTA GAGAACCACAGGTGTACGTGCCTCCATCAAGGATGAGCTGACAAAGAACCAGGTCAGCCTGCCTGTGTCCTG GTGAAAGGATTCTATCCCTCTGACATTGCTGTGGAGTGGGAAAGTAATGGCCAGCCTGAGAACAATTACCTGAC CTGGCCCCCTGTGCTGGACTCAGATGGCAGCTTCTTTCTGTATAGCAAGCTGACCGTCGACAAATCCCGGTGGC AGCAGGGGAATGTGTTTAGTTGTTCAGTCATGCACGAGGCACTGCACAACCATTACACCCAGAAGTCACTGTCA CTGTCACCAGGG
205	3041	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDR SKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSS
206	3041	VH	GAAGTGCAGCTGGTCGAATCTGGAGGAGGACTGGTGCAGCCAGGAGGGTCCCTGCGCCTGTCTTGCGCCGCTAG TGGCTTCACTTTTACCGACTACACCATGGATTGGGTGCGACAGGCACCTGGAAAGGGCCTGGAGTGGGTCGCCG ATGTGAACCCAAATAGCGGAGGCTCCATCTACAACCAGCGGTTCAAGGGCCGGTTCACCCTGTCAGTGGACCGG AGCAAAAACACCCTGTACCTGCAGATGAATAGCCTGCGAGCCGAAGACACTGCTGTGTACTATTGCGCCCGGAA TCTGGGGCCCTCCTTCTACTTTGACTATTGGGGGCAGGGAACTCTGGTCACCGTGAGCTCC
207	3041	H1	GFTFTDYT
208	3041	H1	GGCTTCACTTTTACCGACTACACC
209	3041	H3	ARNLGPSFYFDY
210	3041	H3	GCCCGGAATCTGGGGCCCTCCTTCTACTTTGACTAT
211	3041	H2	VNPNSGGS
212	3041	H2	GTGAACCCAAATAGCGGAGGCTCC
213	3041	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKV
214	3041	CH1	GCCTCCACCAAGGGACCTTCTGTGTTCCCACTGGCTCCCTCTAGTAAATCCACATCTGGGGGAACTGCAGCCCT GGGCTGCTGGTGAAGGACTACTTCCCAGAGCCCGTCACAGTGTCTTGGAACAGTGGCGCTCTGACTTCTGGGG TCCACACCTTCCTGCAGTGCTGCAGTCAAGCGGGGCTGTACAGCCTGTCCTCTGTGGTCACCGTGCCAAGTTCA AGCCTGGGAACACAGACTTATATCTGCAACGTGAATCACAAGCCATCCAATACAAAAGTCGACAAGAAAGTG
215	3041	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
216	3041	CH2	GCACCAGAGCTGCTGGGAGGACCAAGCGTGTTCCTGTTTCCCCACCCAAGCCTAAAGATACACTGATGATTAGTAG GACCCCAGAAGTCACATGCGTGGTCGTGGACGTGAGCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGG ACGGCGTCGAGGTGCATAATGCCAAGACTAAACCCAGGGAGGAACAGTACAACAGTACCTATCGCGTCGTGTCA GTCCTGACAGTGCTGCATCAGGATTGGCTGAACGGGAAAGAGTATAAGTGCAAAGTGAGCAATAAGGCTCTGCC

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			CGCACCTATCGAGAAAACAATTTCCAAGGCAAAA
217	3041	CH3	GQPREPQVYVLPPSRDELTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
218	3041	CH3	GGACAGCCTAGAGAACCACAGGTGTACGTGCTGCCTCCATCAAGGGATGAGCTGACAAAGAACCAGGTCAGCCT GCTGTGCCTGGTCAAAGGATTCTATCCCAGCGACATTGCCGTGGAGTGGGAAAGTAATGGCCAGCCTGAGAACA ACTACCTGACCTGGCCCCCTGTGCTGGACTCAGATGGCAGCTTCTTTCTGTATAGCAAGCTCACCGTGGACAAA TCCCGGTGGCAGCAGGGGAATGTGTTTAGTTGTCAGTCATGCACGAGGCACTGCACAACCATTACACCCAGAAA GTCACTGTCACCAGGG
219	3057	Full	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDR SKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSC DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPG
220	3057	Full	GAAGTGCAGCTGGTCGAATCTGGAGGAGGACTGGTGCAGCCAGGAGGGTCCCTGCGCCTGTCTTGCGCCGCTAG TGGCTTCACTTTTACCGACTACACCATGGATTGGGTGCGACAGGCACCTGGAAAGGGCCTGGAGTGGGTCGCCG ATGTGAACCCAAATAGCGGAGGCTCCATCTACAACCAGCGGTTCAAGGGCCGGTTCACCCTGTCAGTGGACCGG AGCAAAAACACCCTGTATCTGCAGATGAATAGCCTGCGAGCCGAAGATACTGCTGTGTACTATTGCGCCCGGAA TCTGGGGCCCTCCTTCTACTTTGACTATTGGGGCCAGGGAACTCTGGTCACCGTGAGCTCCGCCTCCACCAAGG GACCTTCTGTGTTCCCACTGGCTCCCTCTAGTAAATCCACATCTGGGGGAACTGCAGCCCTGGGCTGTCTGGTG AAGGACTACTTCCCAGAGCCCGTCACAGTGTCCTGGAACAGTGGCGCTCTGACTTCTGGGTCCACACCTTTCC TGCAGTGCTGCAGTCAAGCGGGCTGTACAGCCTGTCCTCTGTGGTCACCGTGCCAAGTTCAAGCCTGGGAACAC AGACTTATATCTGCAACGTGAATCACAAGCCATCCAATACAAAAGTCGACAAGAAAGTCGGAACCCAAGTCTTGT GATAAAACCCATACATGCCCCCCTTGTCCTGCACCAGAGCTGCTGGGAGGACCAAGCGTGTTCCTGTTCCACC CAAGCCTAAAGATACACTGATGATTAGTAGGACCCCAGAAGTCACATGCGTGGTCGTGGACGTGAGCCACGAGG ACCCCGAAGTCAAGTTTAACTGGTACGTGGACGGCGTCGAGGTGCATAATGCCAAGACTAAACCCAGGGAGGAA CAGTACAACAGCACCTACCGCGTCGTGTCAGTCCTGACAGTGCTGCATCAGGATTGGCTGAACGGGAAAGAGTA TAAGTGCAAAGTGAGCAATAAGGCTCTGCCCGCACCTATCGAGAAAACAATTTCCAAGGCAAAAGGACAGCCCA GAGAACCACAGGTGTACGTGTATCCTCCATCAAGGGATGAGCTGACAAAGAACCAGGTCAGCCTGACTTGTCTG GTGAAAGGATTCTATCCCTCTGACATTGCTGTGGAGTGGGAAAGTAATGGCCAGCCTGAGAACAATTACAAGAC CACACCCCTGTGCTGGACTCAGATGGCAGCTTCGCGCTGGTGAGCAAGCTGACCGTGGACAAATCCCGGTGGC AGCAGGGGAATGTGTTTAGTTGTTCAGTCATGCACGAGGCACTGCACAACCATTACACCCAGAAGTCACTGTCA CTGTCACCAGGG
221	3057	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDR SKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSS
222	3057	VH	GAAGTGCAGCTGGTCGAATCTGGAGGAGGACTGGTGCAGCCAGGAGGGTCCCTGCGCCTGTCTTGCGCCGCTAG TGGCTTCACTTTTACCGACTACACCATGGATTGGGTGCGACAGGCACCTGGAAAGGGCCTGGAGTGGGTCGCCG ATGTGAACCCAAATAGCGGAGGCTCCATCTACAACCAGCGGTTCAAGGGCCGGTTCACCCTGTCAGTGGACCGG AGCAAAAACACCCTGTATCTGCAGATGAATAGCCTGCGAGCCGAAGATACTGCTGTGTACTATTGCGCCCGGAA TCTGGGGCCCTCCTTCTACTTTGACTATTGGGGCCAGGGAACTCTGGTCACCGTGAGCTCC
223	3057	H1	GFTFTDYT
224	3057	H1	GGCTTCACTTTTACCGACTACACC
225	3057	H3	ARNLGPSFYFDY
226	3057	H3	GCCCGGAATCTGGGGCCCTCCTTCTACTTTGACTAT
227	3057	H2	VNPNSGGS
228	3057	H2	GTGAACCCAAATAGCGGAGGCTCC
229	3057	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKV
230	3057	CH1	GCCTCCACCAAGGGACCTTCTGTGTTCCCACTGGCTCCCTCTAGTAAATCCACATCTGGGGGAACTGCAGCCCT GGGCTGTCTGGTGAAGGACTACTTCCCAGAGCCCGTCACAGTGTCCTGGAACAGTGGCGCTCTGACTTCTGGGG TCCACACCTTTCCTGCAGTGCTGCAGTCAAGCGGGCTGTACAGCCTGTCCTCTGTGGTCACCGTGCCAAGTTCA AGCCTGGGAACACAGACTTATATCTGCAACGTGAATCACAAGCCATCCAATACAAAAGTCGACAAGAAAGTG
231	3057	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
232	3057	CH2	GCACCAGAGCTGCTGGGAGGACCAAGCGTGTTCCTGTTCCCACCCAAGCCTAAAGATACACTGATGATTAGTAG GACCCCAGAAGTCACATGCGTGGTCGTGGACGTGAGCCACGAGGACCCCGAAGTCAAGTTTAACTGGTACGTGG ACGGCGTCGAGGTGCATAATGCCAAGACTAAACCCAGGGAGGAACAGTACAACAGTACCTATCGCGTCGTGTCA GTCCTGACAGTGCTGCATCAGGATTGGCTGAACGGGAAAGAGTATAAGTGCAAAGTGAGCAATAAGGCTCTGCC CGCACCTATCGAGAAAACAATTTCCAAGGCAAAA

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
233	3057	CH3	GQPREPQVYVYPPSREELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
234	3057	CH3	GGACAGCCTAGAGAACCACAGGTGTACGTGTACCCTCCATCCAAGGGATGAGCTGACAAAGAACCAGGTCAGCCT GACTTGTCTGGTGAAAGGATTCTATCCCTCTGACATTGCCGTGGAGTGGGAAAGTAATGGCCAGCCTGAGAACA ATTACAAGACCACACCCCCTGTGCTGGACTCAGATGGCAGCTTCGGGCTGGTGAGCAAGCTGACCGTCGACAAA TCCCGGTGGCAGCAGGGGAATGTGTTTAGTTGTCAGTCATGCACGAGGCACTGCACAACCATTACACCCAGAA GTCACTGTCACCGTCACCAGGG
235	1011	Full	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVYPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
236	1011	Full	GAGGTGCAGCTGGTGGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCTCTGCGACTGAGTTGCGCCGCTTC AGGATTCAACATCAAGGACACCTACATTCACTGGGTGCGACAGGCTCCAGGAAAAGGACTGGAGTGGGTGGCTC GAATCTACCCCACTAATGGATACACCCGGTATGCCGACTCCGTGAAGGGGAGGTTTACTATAGCGCCGATACA TCCAAAAACACTGCTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACCGCTGTGTACTATTGCAGTCGATG GGGAGGAGACGGATTCTACGCTATGGATTATTGGGGACAGGGGACCCTGGTGACAGTGAGCTCCGCCTCTACCA AGGGCCCCAGTGTGTTTCCCCTGGCTCCTTCTAGTAAATCCACCTCTGGAGGGACAGCCGCTCTGGGATGTCTG GTGAAGGACTATTTCCCCGAGCCTGTGACCGTGAGTTGGAACTCAGGCGCCCTGACAAGCGGAGTGCACACTT TCCTGCTGTGCAGTCAAGCGGGCTGTACTCCCTGTCCTCTGTGGTGACAGTGCCAAGTCAAGCCTGGGCA CACAGACCTATATCTGCAACGTGAATCATAAGCCCTCAAATACAAAAGTGGACAAGAAAGTGGAGCCCAAGAGC TGTGATAAGACCCACACCTGCCCTCCCTGTCCAGCTCCAGAACTGCTGGGAGGACCTAGCGTGTTCCTGTTCC CCCTAAGCCAAAAGACACTCTGATGATCTCCAGGACTCCCGAGGTGACCTGCGTGGTGGTGGACGTGTCTCACG AGGACCCCGAAGTGAAGTTCAACTGGTACGTGGATGGCGTGGAAGTGCATAATGCTAAGACAAAACCAAGAGAG GAACAGTACAACTCCACTTATCGCGTCGTGAGCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGGAAGGA GTATAAGTGCAAAGTCAGTAACAAGGCCCTGCCTGCTCCAATCGAAAAAACCATCTCTAAGGCCAAAGGCCAGC CAAGGGAGCCCCAGGTGTACGTGTACCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTGTCCCTGACATGT CTGGTGAAAGGCTTCTATCCTAGTGATATTGCCGTGGAGTGGGAATCAAATGGACAGCCAGAGAACAATTACAA GACCACACCTCCAGTGCTGGACAGCGATGGCAGCTTCGCCCTGGTGTCCAAGCTGACAGTGGATAAATCTCGAT GGCAGCAGGGGAACGTGTTTAGTTGTCAGTGATGCATGAAGCCCTGCACAATCATTACACTCAGAAGAGCCTG TCCCTGTCTCCCGGCAAA
237	1011	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
238	1011	VH	GAGGTGCAGCTGGTGGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCTCTGCGACTGAGTTGCGCCGCTTC AGGATTCAACATCAAGGACACCTACATTCACTGGGTGCGACAGGCTCCAGGAAAAGGACTGGAGTGGGTGGCTC GAATCTACCCCACTAATGGATACACCCGGTATGCCGACTCCGTGAAGGGGAGGTTTACTATAGCGCCGATACA TCCAAAAACACTGCTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACCGCTGTGTACTATTGCAGTCGATG GGGAGGAGACGGATTCTACGCTATGGATTATTGGGGACAGGGGACCCTGGTGACAGTGAGCTCC
239	1011	H1	GFNIKDTY
240	1011	H1	GGATTCAACATCAAGGACACCTAC
241	1011	H3	SRWGGDGFYAMDY
242	1011	H3	AGTCGATGGGGAGGAGACGGATTCTACGCTATGGATTAT
243	1011	H2	IYPTNGYT
244	1011	H2	ATCTATCCCACTAATGGATACACC
245	1011	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKV
246	1011	CH1	GCCTCTACCAAGGGCCCCAGTGTGTTTCCCCTGGCTCCTTCTAGTAAATCCACCTCTGGAGGGACAGCCGCTCT GGGATGTCTGGTGAAGGACTATTTCCCCGAGCCTGTGACCGTGAGTTGGAACTCAGGCGCCCTGACAAGCGGAG TGCACACTTTCCTGCTGTGCAGTCAAGCGGGCTGTACTCCCTGTCCTCTGTGGTGACAGTGCCAAGTTCA AGCCTGGGCACACAGACCTATATCTGCAACGTGAATCATAAGCCCTCAAATACAAAAGTGGACAAGAAAGTG
247	1011	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
248	1011	CH2	GCTCCAGAACTGCTGGGAGGACCTAGCGTGTTCCTGTTTCCCCCTAAGCCAAAAGACACTCTGATGATCTCCAG GACTCCCGAGGTGACCTGCGTGGTGGTGGACGTGTCTCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGG ATGGCGTGGAAGTGCATAATGCTAAGACAAAACCAAGAGAGGAACAGTACAACTCCACTTATCGCGTCGTGAGC GTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTCAGTAACAAGGCCCTGCC TGCTCCAATCGAAAAAACCATCTCTAAGGCCAAA

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
249	1011	CH3	GQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
250	1011	CH3	GGCCAGCCAAGGGAGCCCCAGGTGTACGTGTACCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTGTCCC TGACATGCCTGGTGAAAGGCTTCTATCCTAGTGATATTGCTGTGGAGTGGGAATCAAATGGACAGCCAGAGAACA ACTACAAGACCACACCTCCAGTGCTGGACAGCGACGGCAGCTTCGCCCTGGTGTCCAAGCTGACAGTGGATAAA TCTCGATGGCAGCAGGGGAACGTGTTTAGTTGCTCAGTGATGCATGAAGCCCTGCACAATCATTACACTCAGAA GAGCCTGTCCCTGTCTCCCGGC
251	4560	Full	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSRDELTKNQV SLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
252	4560	Full	GAACCTAAAAGCAGCGACAAGACCCACACATGCCCCCCCTTGTCCAGCTCCAGAACTGCTGGGAGGACCAAGCGT GTTCCTGTTTCCACCCAAGCCCAAAGATACACCGATGATCAGCCGAACTCCCGAGGTCACCTGCGTGGTCGTGG ACGTGTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGGACGGCGTCGAAGTGCATAATGCAAAGACT AAACCACGGGAGGAACAGTACAACTCTACATATAGAGTGGTGAGTGTCCTGACTGTGCTGCATCAGGATTGGCT GAACGGCAAAGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCCTGCTCCAATCGAGAAAACTATCAGTAAGG CAAAAGGGCAGCCCAGGGAACCTCAGGTCTACGTGCTGCCTCCAAGTCGCGACGAGCTGACCAAGAACCAGGTC TCACTGCCTGTGTCTGGTGAAAGGATTCTATCCTTCGATATTGCGTGGAGTGGGAACCTAATGGCCAGCCAGA GAACAATTACCTGACCTGGCCCCCTGTGCTGGACAGCGATGGGTCCTTCTTTCTGTATTCAAAGCTGACAGTGG ACAAAAGCAGATGGCAGCAGGGAAACGTCTTTAGCTGTTCCGTGATGCACGAAGCCCTGCACAATCATTACACC CAGAAGTCCCTGAGTCTGTCACCTGGCAAA
253	4560	CH2	APELLGGPSVTLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
254	4560	CH2	GCTCCAGAACTGCTGGGAGGACCAAGCGTGTTCCTGTTTCCACCCAAGCCCAAAGATACACCGATGATCAGCCG AACTCCCGAGGTCACCTGCGTGGTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGG ACGGCGTCGAAGTGCATAATGCAAAGACTAAACCACGGGAGGAACAGTACAACTCTACATATAGAGTGGTGAGT GTCCTGACTGTGCTGCATCAGGATTGGCTGAACGGCAAAGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCC TGCTCCAATCGAGAAAACTATCAGTAAGGCAAAA
255	4560	CH3	GQPREPQVYVLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
256	4560	CH3	GGGCAGCCCAGGGAACCTCAGGTCTACGTGCTGCCTCCAAGTCGCGACGAGCTGACCAAGAACCAGGTCTCACT GCTGTGTCTGGTGAAAGGATTCTATCCTTCGATATTGCGTGGAGTGGGAATCTAACGGCCAGCCAGAGAACA ACTACCTGACCTGGCCCCCTGTGCTGGACAGCGATGGGTCCTTCTTCTGTATTCAAAGCTGACAGTGGACAAA
			AGCAGATGGCAGCAGGGAAACGTCTTTAGCTGTTCCGTGATGCACGAAGCCCTGCACAATCATTACACCCAGAA GTCTCTGAGTCTGTCACCTGGC
257	3317	Full	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIKGGGSGGGGSGGGGSEVQLVESGGGLVQPGGSLRLSCAASG FTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSSYNQRFKGRFTLSVDRSKNTLYLQMNSLRAEDTAVYYCAANL GPSFYFDYWGQGTLVTVSSAAEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYVYPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
258	3317	Full	GACATTCAGATGACCCAGAGCCCTAGCTCCCTGAGTGCCTCAGTCGGGGACAGGGTGACTATCACCTGCAAGGC TCCACAGGATGTCAGCACTTGGCGTGGCATGGTACCAGCAGAAGCCAGGGAAAGCACCCAAGCTGCTGATCTACA GCGCCTCCTACAGGTATACAGGCGTGCCATCCCGCTTCTCTGGCAGTGGGTCAGGAACTGACTTTACACTGACT ATTTCTAGTCTGCAGCCCGAAGATTTCGCCACATACTATTGCCAGCAGTACTATATCTACCCTTATACTTTTGG CCAGGGGACCAAAGTGGAGATTAAGGGCGGAGGAGGCTCCGGAGGAGGAGGGTCTGGAGGAGGAGGAAGTGAGG TCCAGCTGGTGGAATCTGGAGGAGGACTGGTGCAGCCAGGAGGGTCCCTGAGGCTGTCTTGTGCCGCTAGTGGC TTCACCTTTACAGACTACACAATGGATTGGGTGCGCCAGGCACCAGGAAAGGGACTGGAATGGGTCGCTGATGT GAACCCTAATAGCGGAGGCTCCATCTACAACCAGCGGTTCAAAGGACGGTTCACCCTGTCAGTGGACCGGAGCA AGAACACCCTGCATCTGCAGATGAACAGCCTGAGAGCCGAGGATACTGCTGTGTACTATTGCGCCAGGAATCCG GGCCCAAGCTTCTACTTTGACTATTGGGGGCAGGGAACACTGGTCACCGTGTCAAGCGCAGCCGAACCCAAATC CTCTGATAAGACTCACACCTGCCCACCTTGTCCAGCTCCAGAGCTGCTGGGAGGACCTAGCGTGTTCCTGTTTC CACCCAAGCCAAAAGACACTCTGATGATTTCTAGAACCCCTGAAGTGACATGTGTGGTCGTGGACGTCAGTCAC GAGGACCCCGAAGTCAAATTCAACTGGTACGTGGATGGCGTCGAGGTGCATAATGCCAAGACCAAACCCCGAGA GGAACAGTACAACTCAACCTACCGGGTCGTGAGCGTCCTGACAGTGCTGCATCAGGACTGGCTGAACGGCAAGG AGTATAAGTGCAAAGTGAGCAACAAGGCTCTGCCTGCACCAATCGAGAAGACCATTTCCAAGGCTAAAGGGCAG CCCCGCGAACCTCAGGTCTACGTGTACCCTCCAAGCCGAGATGAGCTGACAAAAAACCAGGTCTCCCTGACCTG TCTGGTGAAGGGATTTTACCCAAGTGACATCGCAGTGGAGTGGGAATCAAATGGCCAGCCCGAAAACAATTACA AGACCACACCCCCTGTGCTGGACTCTGATGGGAGCTTCTTCCTGTACTCCAAACTGACCGTGGACAAGTCTCGG TGGCAGCAGGGAAACGTCTTTAGCTGTTCCGTGATGCACGAGGCCCTGCACAATCATTACACAGAAATCTCT

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			GAGTCTGTCACCTGGCAAG
259	3317	VL	DIQMTQSPSSLSASVGDRVTITCKASQDVSIGVAWYQQKPGKAPKLLIYSASYRYTGVPSRFSGSGSGTDFTLT ISSLQPEDFATYYCQQYYIYPYTFGQGTKVEIK
260	3317	VL	GACATTCAGATGACCCAGAGCCCTAGCTCCCTGAGTGCCTCAGTCGGGGACAGGGTGACTATCACCTGCAAGGC TTCACAGGATGTCAGCATTGGCGTGGCATGGTACCAGCAGAAGCCAGGGAAAGCACCCAAGCTGCTGATCTACA GCGCCTCCTACAGGTATACAGGCGTGCCATCCCGCTTCTCTGGCAGTGGGTCAGGAACTGACTTTACACTGACT ATTTCTAGTCTGCAGCCCGAAGATTTCGCCACATACTATTGCCAGCAGTACTATATCTACCCTTATACTTTTGG CCAGGGGACCAAAGTGGAGATTAAG
261	3317	L1	QDVSIG
262	3317	L1	CAGGATGTCAGCATTGGC
263	3317	L3	QQYYIYPYT
2 64	3317	L3	CAGCAGTACTATATCTACCCTTATACT
2 65	3317	L2	SAS
266	3317	L2	AGCGCCTCC
267	3317	VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFTDYTMDWVRQAPGKGLEWVADVNPNSGGSIYNQRFKGRFTLSVDR SKNTLYLQMNSLRAEDTAVYYCARNLGPSFYFDYWGQGTLVTVSS
268	3317	VH	GAGGTCCAGCTGGTGGAATCTGGAGGAGGACTGGTGCAGCCAGGAGGGTCCCTGAGGCTGTCTTGTGCCGCTAG TGGCTTCACCTTTACAGACTACACAATGGATTGGGTGCGCCAGGCACCAGGAAAGGGACTGGAATGGGTCGCCG ATGTGAACCCTAATAGCGGAGGCTCCATCTACAACCAGCGGTTCAAAGGACGGTTCACCCTGTCAGTGGACCGG AGCAAGAACACCCTGTATCTGCAGATGAACAGCCTGAGAGCCGAGGATACTGCTGTGTACTATTGCGCCAGGAA TCTGGGCCCAAGCTTCTACTTTGACTATTGGGGCCAGGGAACACTGGTCACTGTGTCAAGC
269	3317	H1	GFTFTDYT
270	3317	H1	GGCTTCACCTTTACAGACTACACA
271	3317	H3	ARNLGPSFYFDY
272	3317	H3	GCCAGGAATCTGGGCCCAAGCTTCTACTTTGACTAT
273	3317	H2	VNPNSGGS
274	3317	H2	GTGAACCCTAATAGCGGAGGCTCC
275	3317	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
276	3317	CH2	GCTCCAGAGCTGCTGGGAGGACCTAGCGTGTTCCTGTTTCCACCCAAGCCAAAAGACACTCTGATGATCTCTAG AACCCCTGAAGTGACATGTGTGGTCGTGGACGTCAGTCACGAGGACCCCGAAGTCAAATTCAACTGGTACGTGG ATGGCGTCGAGGTGCATAATGCCAAGACCAAACCCCGAGAGGAACAGTACAACTCAACCTACCGTGTCGTGAGC GTCCTGACAGTGCTGCATCAGGACTGGCTGAACGGCAAGGAGTATAAGTGCAAAGTGAGCAACAAGGCTCTGCC TGCACCAATCGAGAAGACCATCTCCAAGGCTAAA
277	3317	CH3	GQPREPQVYVYPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFALVSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
278	3317	CH3	GGGCAGCCCCGCGAACCTCAGGTCTACGTGTACCCTCCAAGCCGAGATGAGCTGACAAAAAACCAGGTCTCCCT GACCTGTCTGGTGAAGGGATTTTACCCAAGTGACATCGCAGTGGAGTGGGAATCAAACTGGCCAGCCCGAAAACA ATTATAAGACCACACCCCCTGTGCTGGACTCTGATGGGAGTTTCGCACTGGTCTCCAAACTGACCGTGGACAAG TCTCGGTGGCAGCAGGGAAACGTCTTTAGCTGTTCCGTGATGCACGAGGCCCTGCACAATCATTACACACAGAA ATCTCTGAGTCTGTCACCTGGC
279	1015	Full	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKS CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
280	1015	Full	GAGGTGCAGCTGGTGGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCTCTGCGACTGAGTTGCGCCGCTAC AGGATTCAACATCAAGGACACCTACATTCACTGGGTGCGACAGGCTCCAGGAAAAGGACTGGAGTGGGTGGCCC GAATCTATCCCACTAATGGATACACCCGGTATGCCGACTCCGTGAAGGGGAGGTTTACTATTAGCGCCGATACA TCCAAAAACACTGCTTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACCGCTGTGTACTATTGCAGTCGATG GGGAGGAGACGGATTTCTACGCTATGGATTATTGGGGACAGGGGACCCTGGTGACAGTGAGCTCCGCCTCTACCA AGGGCCCCAGTGTGTTTCCCCTGGCTCCTTCTAGTAAATCCACCTCTGGAGGGACAGCCGCCCTGGGATGTCTG GTGAAGGACTATTTCCCCGAGCCTGTGACCGTGAGTTGGAACTCAGGCGCCCTGACAAGCGGAGTGCACACTTT TCCTGCTGTGCTGCAGTCAAGCGGGCTGTACTCCCTGTCCTCTGTGGTGACAGTGCCAAGTTCAAGCCTGGGCA

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			CACAGACTTA.ATCTGCAACGTGAATCA.AAGCCC.CAAA.ACAAAAGTGGACAAGAAAG.GGAGCCCAAGAGC TGTGA.AAGACCCACACCTGCCCTCCCTGCCCAGCTCCAGAACTGCTGGGAGGACCTAGCGTGTTCCTGTTCC CCC.AAGCCAAAAGACACTC.GA.GA.TCCAGGAC.CCCGAGG.GACC.GCGTGGTGGTGGACGTG.CTCACG AGGACCCCGAAGTGAAGTTCAACTGGTACGTGGATGGCGTGGAAGTGCATAATGCTAAGACAAAACCAAGAGAG GAACAGTACAACTCCACTTA.CGCGTCG.GAGCGTGCTGACCGTGCTGCACCAGGAC.GGC.GAACGGGAAGGA GTA.AAGTGCAAAGTCAGTAATAAGGCCCTGCCTGCTCCAACTCGAAAAAACCATCTCTAAGGCCAAAGGCCAGC CAAGGGAGCCCCAGGTGTACGTGCTGCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTGTCCCTGCTGTGT CTGGTGAAAGGCTTC.ATCC.AG.GA.A..GC.GTGGAG.GGGAATCAAATGGACAGCCAGAGAACAATTACC. GACCTGGCC.CCAGTGCTGGACAGCGATGGCAGCTTCT.CC.GTATTCCAAGCTGACAGTGGATAAA.CTCGA. GGCAGCAGGGGAACG.GTTTAG.TGTCAGTGA.GCATGAAGCCCTGCACAATCA.TACAC.CAGAAGAGCC.G TCCCTGTCTCCCGGCAAA
281	1015	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
282	1015	VH	GAGGTGCAGCTGGTGGAAAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCTCTGCGACTGAGTTGCGCCGCTTC AGGATTCAACATCAAGGACACC.TACA.TCACTGGG.GCGACAGGCTCCAGGAAAAGGACTGGAGTGGGTGGC.C GAATCTATCCCACTAATGGATACACCCGGTATGCCGACTCCGTGAAGGGGAGGTTTACTATAGCGCCGATACA
			TCCAAAAACACTGCTTACCTGCAGATGAACAGCCTGCGAGCCGAAGATACCGCTGTGTACTATTGCAGTCGATG GGGAGGAGACGGATTCTACGC.A.GGA..TA..GGGGACAGGGGACCC.GGTGACAG.GAGC.CC
283	1015	H1	GFNIKDTY
284	1015	H1	GGATTCAACATCAAGGACACCTAC
285	1015	H3	SRWGGDGFYAMDY
286	1015	H3	AGTCGATGGGGAGGAGACGGATTCTACGCTATGGATTAT
287	1015	H2	IYPTNGYT
288	1015	H2	ATCTATCCCACTAATGGATACACC
289	1015	CH1	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKV
290	1015	CH1	GCCTCTACCAAGGGCCCCAGTGTGTTCCCCTGGCTCCTTCTAGTAAATCCACCTCTGGAGGGACAGCCGCTCT GGGATGTCTGGTGAAGGACTATT.CCCCGAGCC.G.GACCG.GAGTTGGAACTCAGGCGCCCTGACAAGCGGAG .GCACACTT..CCTGC.GTGCTGCAG.CAAGCGGGCC.G.AC.CCCTGTCCTCTGTGG.GACAGTGCCAAGTTCA AGCCTGGGCACACAGACTTA.A.CTGCAACGTGAA.CA.AAGCCCTCAAATACAAAAGTGGACAAGAAAGTG
291	1015	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
292	1015	CH2	GCTCCAGAACTGCTGGGAGGACCTAGCGTGTTCCTGTTTCCCCCTAAGCCAAAAGACACCCTGATGATCTCCAG GACCCCCGAGGTGACCTGCGTGGTGGTGGACGTGTCTCACGAGGACCCCGAAGTGAAGTTCAACTGGTACGTGG ATGGCGTGGAAGTGCATAATGCTAAGACAAAAGCCAAGAGAGGAACAGTACAACTCCACTTACCGCGTGGTGAGC GTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGGAAGGAGTATAAGTGCAAAGTCAGTAATAAGGCCCTGCC TGCTCCAATCGAAAAAACCATCTCTAAGGCCAAA
293	1015	CH3	GQPREPQVYVLPPSREELTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
294	1015	CH3	GGCCAGCCAAGGGAGCCCCAGG.GTACGTGCTGCCACCCAGCAGAGACGAACTGACCAAGAACCAGGTGTCCC. GCTGTGTCTGGTGAAAGGCTTCTATCCTAGTGATATTGCTGTGGAGTGGGAATCAAACGGACAGCCAGAGAACA AT.ACCTGACCTGGCC.CCAGTGCTGGACAGCGATGGCAGCTTC.TCCTGTATTCCAAGC.GACAG.GGATAAA TCTCGATGGCAGCAGGGGAACGTGTTTAGTTGTTCAGTGATGCATGAAGCCCTGCACAATCATTACACTCAGAA GAGCCTGTCCCTGTCTCCCGGC
295	5244	Full	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKGGSGGGSGGGSGGGSGGGSEVQLVESGGGLVQPGGSLRLS CAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYY CSRWGGDGFYAMDYWGQGTLVTVSSAAEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYVLPPSREELTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKL TVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
296	5244	Full	GACATTCAGATGACACAGAGCCCCAGCTCCCTGAGTGCTTCAGTCGGCGACAGGGTGACTATCACCTGCCGCGC ATCCCAGGA.GTCAACACCGCTG.GGCA.GGTACCAGCAGAAGCCTGGAAAAGCCCCAAAGCTGCTGATCTACA GCGCTTCCTTCCTGTA.TCTGGCGTGCCAAGTCGG.TTTC.GGAAGTAGATCAGGCACTGACTTCACACTGAC. ATCTCCAGTCTGCAGCCCGAAGATTTTGCCACCTACTATTGCCAGCAGCACTATACCACACCCCCTACATTCGG ACAGGGCAC.AAAGTGGAGA.TAAGGGCGGGTCAGGCGGAGGGAGCGGAGGAGGG.CCGGAGGAGGG.CTGGAG GAGGGAGTGGAGAGGCCAGCTGGTGGAACTGGAGGAGGACTGGTGCAGCCTGGAGGCTCACTGCGACTGAGC

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			TGTGCCGCTTCCGGCTTTAACATCAAAGACACATACATTCATTGGGTCAGGCAGGCACCAGGGAAGGGACTGGA ATGGGTGGCCCGCATCTATCCCACAAATGGGTACACTCGATATGCCGACAGCGTGAAAGGACGGTTTACCATT CTGCTGATACCAGTAAGAACACAGCATACCTGCAGATGAACAGCCTGCGCGCAGAGGATACAGCCGTGTACTA TGCAGTCGATGGGGGGGAGACGGCTTCTACGCCATGGACTACTGGGGCCAGGGGACTCTGGTCACCGTGTCAAG CGCAGCCGAACCTAAACTCCTCTGACAAGACCCACACATGCCCACCCTGTCCTGCTCCAGAGCTGCTGGGAGGAC CATCCGTGTTCCTGTTCCCTCCAAAGCCTAAAGATACACTGATGATTAGCCGCACTCCCGAAGTCACCTGTGTG GTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGGACGGCGTCGAGGTGCATAATGC CAAGACTAAACCAAGAGAGGAACAGTACAATTCAACCTACAGGGTCGTGAGCGTCCTGACAGTGCTGCATCAGG ATTGGCTGAACGGCAAGGAGTATAAGTGCAAAGTCTAACAAGGCCCTGCCCGCCCCTATCGAGAAGACTATT
			AGCAAGGCAAAAGGGCAGCCACGGGAACCCCAGGTCTACGTGCTGCCCCCTAGCAGAGACGAGCTGACCAAAAA CCAGGTCTCCCTGCTGTGTCTGGTGAAGGGCTTCTATCCTAGTGATATCGCTGTGGAGTGGGAATCAAATGGGC AGCCAGAAAACAATTACCTGACATGGCCACCCGTGCTGGACAGCGATGGGTCCTTCTTCTTGTATTCCAAACTG ACTGTGGACAAGTCTAGATGGCAGCAGGGAAACGTCTTCAGCTGTCCGTGATGCACGAGGCCCTGCACAATCA TTACACCCAGAAGTCTCTGAGTCTGTCACCCGGC
297	5244	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK
298	5244	VL	GACATTCAGATGACACAGAGCCCCAGCTCCCTGAGTGCTTCAGTCGGCGACAGGGTGACTATCACCTGCCGCGC ATCCCAGGATGTCAACACCGCTGTGGCATGGTACCAGCAGAAGCCTGGAAAAGCCCCAAAGCTGCTGATCTACA GCGCTTCCTCTGTGTATTCTGGCGTGCCAAGTCGGTTTTCTGGAAGTAGATCAGGCACTGACTTCACACTGACT ATCTCTAGTCTGCAGCCCGAAGATTTTGCCACCTACTATTGCCAGCAGCACTATACCACACCCCCTACATTCGG ACAGGGCACTAAAGTGGAGATTAAG
299	5244	L1	QDVNTA
300	5244	L1	CAGGATGTCAACACCGCT
301	5244	L3	QQHYTTPPT
302	5244	L3	CAGCAGCACTATACCACACCCCCTACA
303	5244	L2	SAS
304	5244	L2	AGCGCTTCC
305	5244	VH	EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT SKNTAYLQMNSLRAEDTAVYYCSRWGGDGFYAMDYWGQGTLVTVSS
306	5244	VH	GAGGTCCAGCTGGTGGAATCTGGAGGAGGACTGGTGCAGCCTGGAGGCTCACTGCGACTGAGCTGTGCCGCTTC CGGCTTTAACATCAAAGACACATACATTCATTGGGTCAGGCAGGCACCAGGGAAGGGACTGGAATGGGTGGCCC GCATCTATCCCACAAATGGGTACACTCGATATGCCGACAGCGTGAAAGGACGGTTTACCATTCTGCTGATACC AGTAAGAACACAGCATACCTGCAGATGAACAGCCTGCGCGCAGAGGATACAGCCGTGTACTATTGCAGTCGATG GGGGGGAGACGGCTTCTACGCCATGGACTATTGGGGCCAGGGGACTCTGGTCACCGTGTCAAGC
307	5244	H1	GFNIKDTY
308	5244	H1	GGCTTTAACATCAAAGACACATAC
309	5244	H3	SRWGGDGFYAMDY
310	5244	H3	AGTCGATGGGGGGGAGACGGCTTCTACGCCATGGATTAT
311	5244	H2	IYPTNGYT
312	5244	H2	ATCTATCCCACAAATGGGTACACT
313	5244	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
314	5244	CH2	GCTCCAGAGTTGTTGGGAGGACCATCCGTGTTCCTGTTTCCTCCAAAGCCTAAAGATACACTGATGATTAGCCG CACTCCCGAAGTCACCTGTGTGGTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGG ACGGCGTCGAGGTGCATAATGCCAAGACTAAACCAAGAGAGGAACAGTACAATTCAACCTACAGGGTCGTGAGC GTCCTGACAGTGCTGCATCAGGATTGGCTGAACGGCAAGGAGTATAAGTGCAAAGTCTAACAAGGCCCTGCC CGCTCCTATCGAGAAGACTATTAGCAAGGCAAAA
315	5244	CH3	GQPREPQVYVLPPSRDELTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
316	5244	CH3	GGGCAGCCACGGGAACCCCAGGTCTACGTGCTGCCCCCTAGCAGAGACGAGCTGACCAAAAACCAGGTCTCCCT GCTGTGTCTGGTGAAGGGCTTCTATCCTAGTGATATCGCTGTGGAGTGGGAATCAAATGGGCAGCCAGAAAACA ACTACCTGACATGGCCACCCGTGCTGGACAGCGATGGGTCCTTCTTCTTGTATTCCAAACTGACTGTGGACAAG TCTAGATGGCAGCAGGGAAACGTCTTCAGCTGTCCGTGATGCACGAGGCCCTGCACAATCATTACACCCAGAA GTCTCTGAGTCTGTCACCCGGC
317	-2	Full	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			KVNNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
318	-2	Full	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC AAGTCAGGACGTTAACACCGCTAGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTACT CTGCATCCTTTTTGTACAGTGGGGTCCCATCAAGGTTCAGTGGCAGTCGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGCATTACACTACCCCACCCACTTTCGG CCAAGGGACCAAAGTGGAGATCAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGC AGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGG AAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTA CAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCC ATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
319	-2	VL	DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK
320	-2	VL	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGC AAGTCAGGACGTTAACACCGCTAGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTACT CTGCATCCTTTTTGTACAGTGGGGTCCCATCAAGGTTCAGTGGCAGTCGATCTGGGACAGATTTCACTCTCACC ATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGCATTACACTACCCCACCCACTTTCGG CCAAGGGACCAAAGTGGAGATCAAA
321	-2	L1	QDVNTA
322	-2	L1	CAGGACGTTAACACCGCT
323	-2	L3	QQHYTTPPT
324	-2	L3	CAACAGCATTACACTACCCCACCCACT
325	-2	L2	SAS
326	-2	L2	TCTGCATCC
327	-2	CL	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
328	-2	CL	CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCCTCTGT TGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGG GTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTG AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCAC AAAGAGCTTCAACAGGGGAGAGTGT
329	4372	Full	EPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYVLPPSRDELTKNQV SLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPG
330	4372	Full	GAACCTAAATCGAGCGACAAGACCCACACATGCCCCCCTTGTCCAGCTCCAGAACTGCTGGGAGGACCAAGCGT GTTCCTGTTCCACCCAAGCCCAAAGATACACTGATGATCAGCCGAACTCCCGAGGTCACCTGCGTGGTCGTGG ACGTGTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGGACGGCGTCGAAGTGCATAATGCAAAGACT AAACCACGGGAGGAACAGTACAACTCTACATATAGAGTCGTGAGTGTCCTGACTGTGCTGCATCAGGATTGGCT GAACGGCAAAGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCCTGCTCCAATCGAGAAAACTATTAGTAAGG CAAAAGGGCAGCCCAGGAACCTCAGGTCTACGTGCTGCCTCCAAGTCGCGACGAGCTGACCAAGAACCAGGTC TCACTGCCTGTGCTGGTGAAAGGATTCTATCCTTCCGATATTGCCGTGGAGTGGGAATCTAATGGCCAGCCAGA GAACAATTACCTGACCTGGCCCCCTGTGCTGGACAGCGACGGGTCCTTCTTTCTGTACTCAAAGCTGACAGTGG ACAAAAGCAGATGGCAGCAGGGAAACGTCTTTAGCTGTTCCGTGATGCACGAAGCCCTGCACAATCATTACACCC CAGAAGTCTCTGAGTCTGTCACCTGGC
331	4372	CH2	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVS VLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
332	4372	CH2	GCTCCAGAACTGCTGGGAGGACCAAGCGTGTTCCTGTTTCCACCCAAGCCCAAAGATACACTGATGATCAGCCG AACTCCCGAGGTCACCTGCGTGGTCGTGGACGTGTCCCACGAGGACCCCGAAGTCAAGTTCAACTGGTACGTGG ACGGCGTCGAAGTGCATAATGCAAAGACTAAACCACGGGAGGAACAGTACAACTCTACATATAGAGTCGTGAGT GTCCTGACTGTGCTGCATCAGGATTGGCTGAACGGCAAAGAGTATAAGTGCAAAGTGTCTAATAAGGCCCTGCC TGCTCCAATCGAGAAAACTATTAGTAAGGCAAAA
333	4372	CH3	GQPREPQVYVLPPSRDELTKNQVSLLCLVKGFYPSDIAVEWESNGQPENNYLTWPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
334	4372	CH3	GGGCAGCCCAGGGAACCTCAGGTCTACGTGCTGCCTCCAAGTCGCGACGAGCTGACCAAGAACCAGGTCTCACT GCCTGTGTCTGGTGAAAGGATTCTATCCTTCCGATATTGCCGTGGAGTGGGAATCTAATGGCCAGCCAGAGAACA ATTACCTGACCTGGCCCCCTGTGCTGGACAGCGATGGGTCCTTCTTCTGTATTCAAAGCTGACAGTGGACAAA AGCAGATGGCAGCAGGGAAACGTCTTTAGCTGTTCCGTGATGCACGAAGCCCTGCACAATCATTACACCCAGAA

SEQ ID NO.	Clone	Desc.	Sequence (amino acid or
			GTCTCGAGCTCTGTCACCTGGC

SEQ ID NO:	*Pertuzumab WT COR*	sequences
335	CDR-H2	VNPNSGGS
336	CDR-H3	ARNLGPSFYFDY
337	CDR-H1	GFTFTDYT
338	CDR-L2	SAS
339	CDR-L3	QQYYIYPYT
340	CDR-L1	QDVSIG
SEQ ID NO:	*Trastuzumab WT CDR*	sequences
341	CDR-H2	IYPTNGYT
342	CDR-H3	SRWGGDGFYAMDY
343	CDR-H1	GFNIKDTY
344	CDR-L2	SAS
345	CDR-L3	QQHYTTPPT
346	CDR-L1	QDVNTA

Pertuzumab variant CDR-L3: QQYYIYPAT
Clone 3382, variant 10000 (SEQ ID NO: 347)

Pertuzumab variant CDR-H1: GFTFADYT
Clone 6586, variant 10000 (SEQ ID NO:348)

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PATENTKRAV

1. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst hos et menneske,

hvor antigenbindingskonstruktet omfatter et første antigenbindingspolypeptidkonstrukt, der monovalent og specifikt binder et HER2- (human epidermal vækstfaktorreceptor 2) ECD2- (ekstracellulært domæne 2) antigen på en HER2-udtrykkende celle og et andet antigenbindingspolypeptidkonstrukt, der monovalent og specifikt binder et HER2-ECD4- (ekstracellulært domæne 4) antigen på en HER2-udtrykkende celle,

hvor det første antigenbindingspolypeptidkonstrukt er operativt bundet til et første linker-polypeptid og det andet antigenbindingspolypeptidkonstrukt er operativt bundet til et andet linker-polypeptid, hvor første og anden linker-polypeptider er kovalent bundet til hinanden, og hvor linker-polypeptiderne er kovalent bundet til et humant IgG1-Fc-område,

hvor det første antigenbindingspolypeptidkonstrukt er et Fab, hvor Fab-linker-IgG1-Fc omfatter en tungkæde omfattende SEQ ID NO: 97 og en letkæde omfattende SEQ ID NO: 69, og

hvor det andet antigenbindingspolypeptidkonstrukt er et scFv, hvor scFv-linker-IgG1-Fc omfatter SEQ ID NO: 295, og

hvor mennesket administreres mindst 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 eller 20 doser af antigenbindingskonstruktet, og/eller hvor hver dosis administreres mindst på hver dag 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 eller 20.

2. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge krav 1, hvor antigenbindingskonstruktet administreres til mennesket ved en dosis på mindst 0,3, 0,5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 eller 20 mg/kg.

3. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge krav 1 eller krav 2, hos et menneske, hvor tumoren er en lungetumor eventuelt HER2-lav, ikke-HER2-gen-amplificeret ikke-pladeepitel ikke-småcellet lungecancer (NSCLC), en hoved- og halstumor eventuelt et HER2-lavt, ikke-HER2-gen-amplificeret pladecellekarcinom i hoved og hals, en brysttumor eventuelt en HER2 1+, ER+/PR-brystcancer med en luminal B molekylær klassifikation, en pancreastumor eventuelt en HER2-negativ pancreascancer bestemt ved immunhistokemi eller en gastrisk cancer eventuelt en HER2 3+-gastrisk cancer.

4. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge krav 1 hos et menneske, hvor tumoren er en HER2-lav, ikke-HER2-gen-amplificeret ikke-pladeepitel ikke-småcellet lungecancer (NSCLC), hvor antigenbindingskonstruktet administreres intravenøst til mennesket ved en dosis på 15 mg/kg på dag 1 efterfulgt af en dosis på 10 mg/kg på dag 4, 8, 11, 15, 18, 22 og 25.

5. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge krav 1 hos et menneske, hvor tumoren er et HER2-lavt, ikke-HER2-gen-amplificeret pladecellekarcinom i hoved og hals, hvor antigenbindingskonstruktet administreres intravenøst til mennesket én gang ved en dosis på 15 mg/kg på dag 1 efterfulgt af en dosis på 10 mg/kg på dag 4, 8, 11, 15, 18, 22 og 25, eventuelt hvor cisplatin administreres intraperitonealt til mennesket ved en dosis på 2 mg/kg på dag 1, 3, 5, 7, 9 og 11.

6. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge krav 1 hos et menneske, hvor tumoren er en HER2 1+, ER++/PR-brystcancer med en luminal B molekylær klassifikation, hvor antigenbindingskonstruktet administreres intravenøst til mennesket ved en dosis på 3 mg/kg eller 10 mg/kg eller 30 mg/kg på dag 1, 4, 8, 11, 15, 18, 22, 25, 28 og 32.

7. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge krav 1 hos et menneske, hvor tumoren er en HER2-negativ pancreascancer bestemt ved immunhistokemi, hvor antigenbindingskonstruktet administreres intravenøst til mennesket ved en dosis på 30 mg/kg, to gange om ugen i fire uger, eventuelt hvor nab-paclitaxel administreres intravenøst til mennesket ved en dosis på 30 mg/kg på dag 2, 9 og 16.

8. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge krav 1 hos et menneske, hvor tumoren er en HER2 3+-gastrisk cancer, hvor antigenbindingskonstruktet administreres intravenøst til mennesket ved en dosis på 30 mg/kg, to gange om ugen i fire uger.

9. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge et hvilket som helst foregående krav, hvor antigenbindingskonstruktet konjugeres til et lægemiddel.

10. Antigenbindingskonstruktet til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge krav 9, hvor lægemidlet er maytansin (DM1).

11. Antigenbindingskonstruktet til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge krav 10, hvor konstruktet konjugeres til DM1 via en SMCC-linker.

12. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge et hvilket som helst foregående krav, hvor antigenbindingskonstruktet er formuleret i en farmaceutisk sammensætning med en farmaceutisk bærer, eventuelt hvor den farmaceutiske bærer omfatter en buffer, en antioxidant, et molekyle med lav molekylvægt, et lægemiddel, et protein, en aminosyre, et kulhydrat, et lipid, et chelaterende middel, et stabiliseringsmiddel eller et hjælpestof.

13. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge et hvilket som helst af kravene 1-3, eller 9-12, hvor antigenbindingskonstruktet administreres ved injektion eller infusion, eventuelt hvor administrationen er intravenøs.

14. Antigenbindingskonstrukt til anvendelse i en fremgangsmåde til hæmning af tumorvækst ifølge et hvilket som helst foregående krav, og som endvidere omfatter administration til mennesket med et supplerende middel, eventuelt et kemoterapeutisk middel, eventuelt hvor det supplerende middel er én eller flere af bleomycin, carboplatin, cisplatin, nab-paclitaxel, docetaxel, doxorubicin, erlotinib, fluoruracil, gemcitabin, methotrexat, pemetrexed, topotecan, vinorelbin, capecitabin, navelbin eller paclitaxel.

DRAWINGS

Drawing

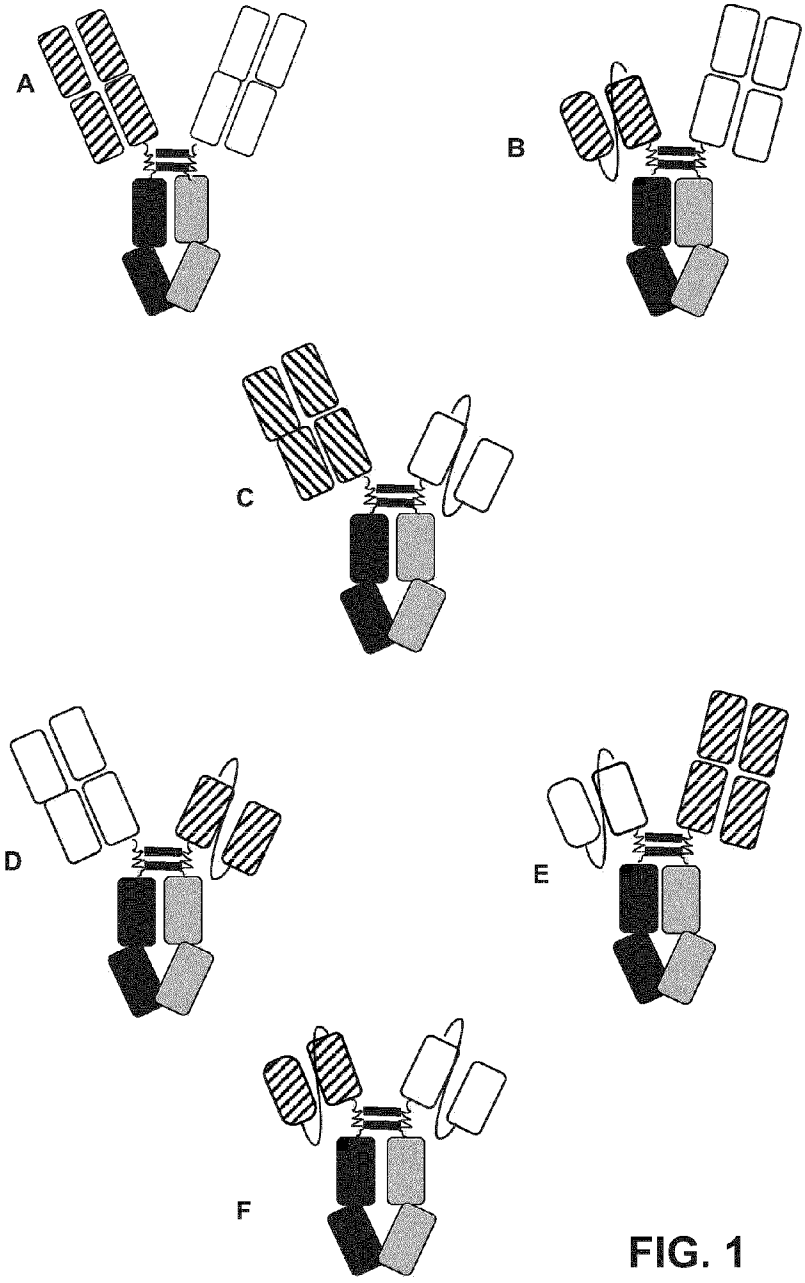


FIG. 1

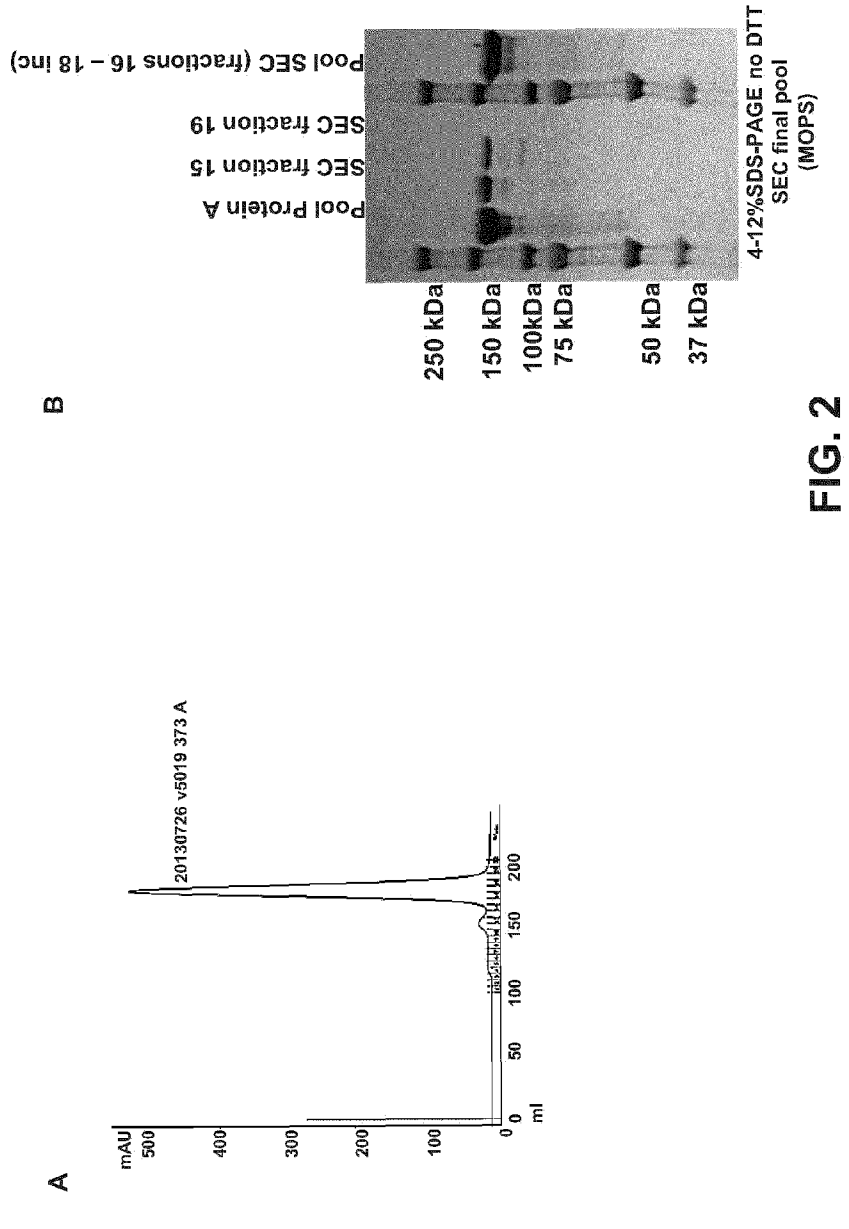

A
mAU
500
400
300
200
100
0
ml
0
50
100
150
200
20130726 v5019 373 A
B
250 kDa
150 kDa
100kDa
75 kDa
50 kDa
37 kDa
4-12%SDS-PAGE no DTT
SEC final pool
(MOPS)
Pool Protein A
SEC fraction 15
SEC fraction 19
Pool SEC (fractions 16 – 18 inc)

FIG. 2

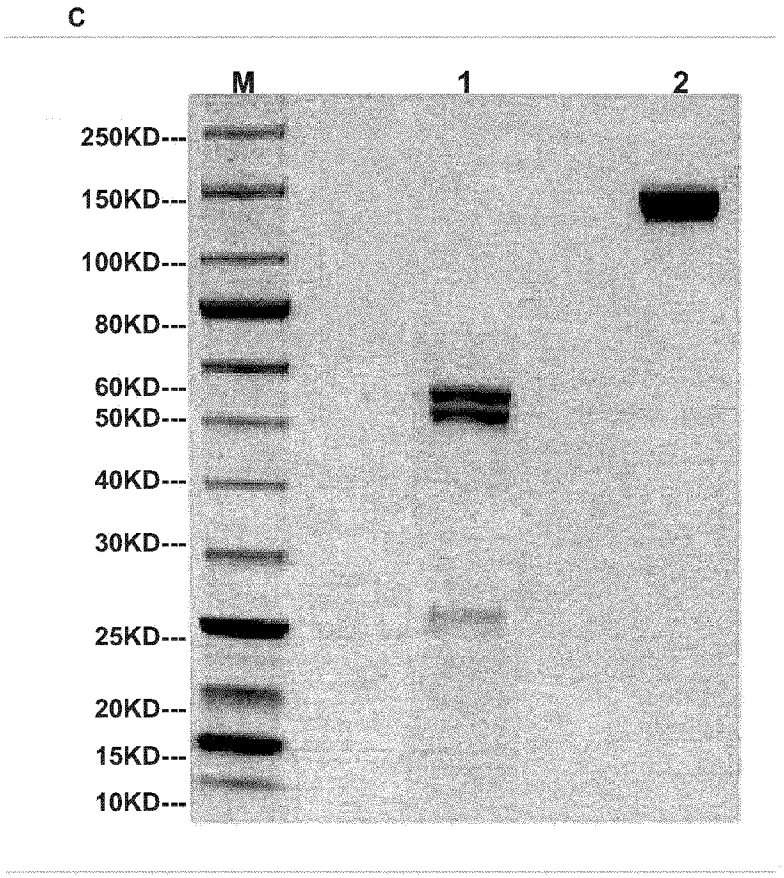
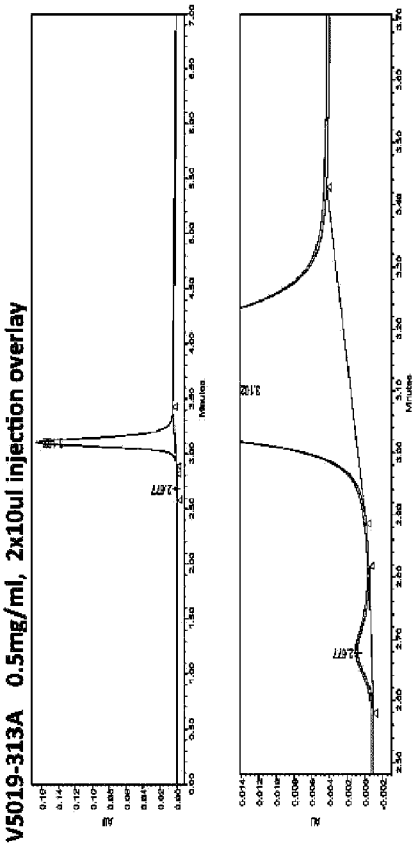
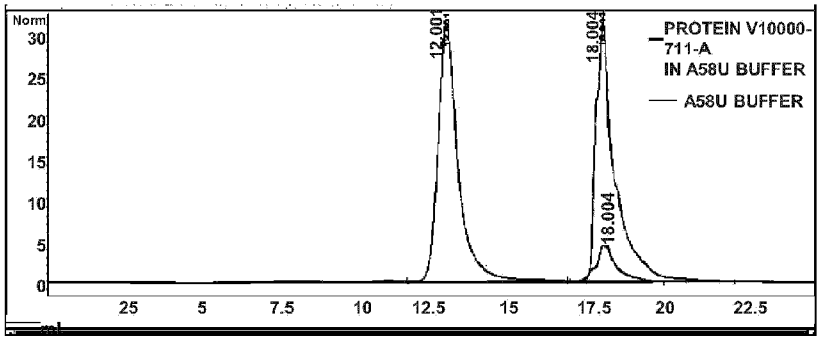

C
M
1
2
250KD---
150KD---
100KD---
80KD---
60KD---
50KD---
40KD---
30KD---
25KD---
20KD---
15KD---
10KD---

FIG. 2 (Cont'd...)



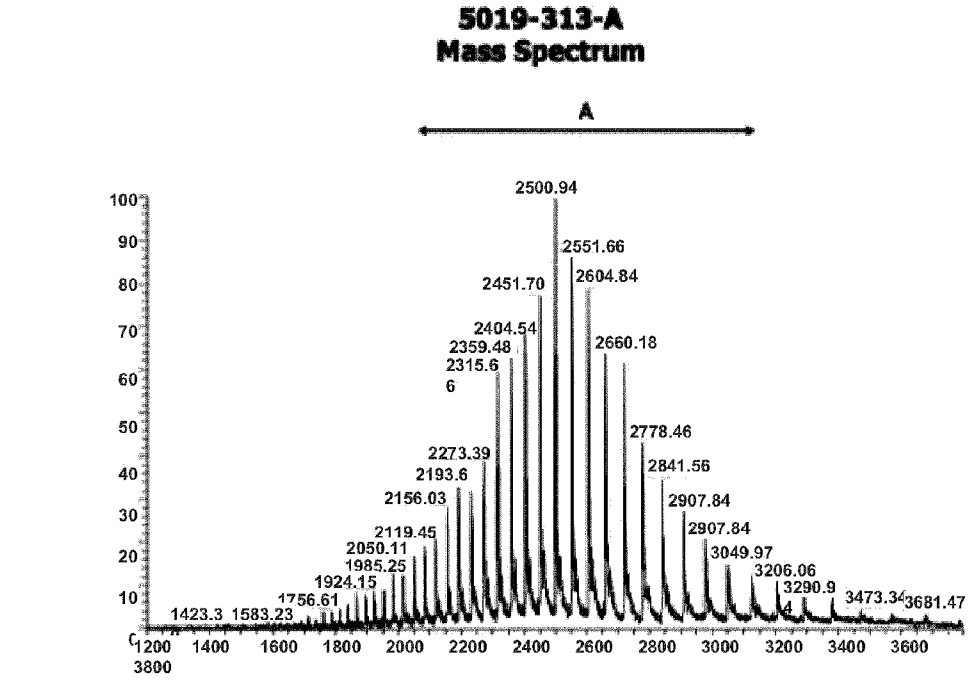
v5019-313A	est MW	log MW	Ret time	% peak area
peak 1	300902	5.478426	2.677	0.83
	298938	5.475581	2.680	0.82
peak 2	118957	5.075391	3.102	99.17
	118957	5.075391	3.102	99.18

FIG. 3A



Peak #	RT (min)	Type	Height	Area	Area%
1	8.022	MF R	0.183	22.103	1.508
2	11.059	MF R	0.179	9.708	0.662
3	12.961	MF R	30.701	1416.498	96.619
4	15.419	FM R	0.299	17.759	1.211

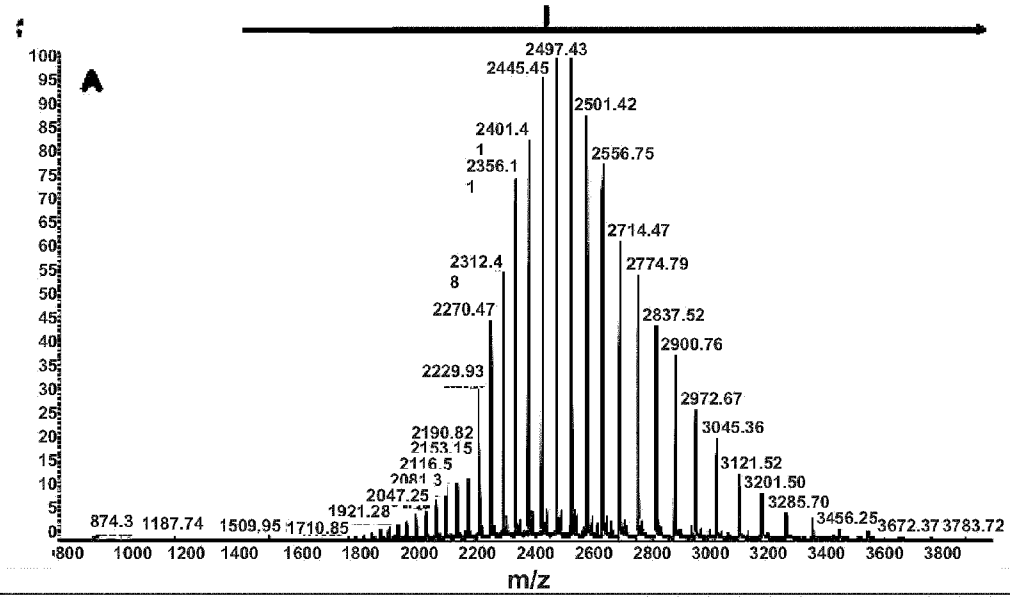
FIG. 3B



Sample	Desired heterodimer species	Higher-mass homodimer species	Lower-mass homodimer species	Higher-mass half-antibody species	Lower-mass half-antibody species	Other species
5019-3313-A	100	0.0	0.0	0.0	0.0	0.0

FIG. 4A

10000-719-A
Mass Spectrum



Sample	Desired heterodimer species	Higher-mass homodimer species	Lower-mass homodimer species	Higher-mass half-antibody species	Lower-mass half-antibody species	Other species
10000-719-A	97.9	1.0	0.7	0.3	0.0	0.0

FIG. 4B

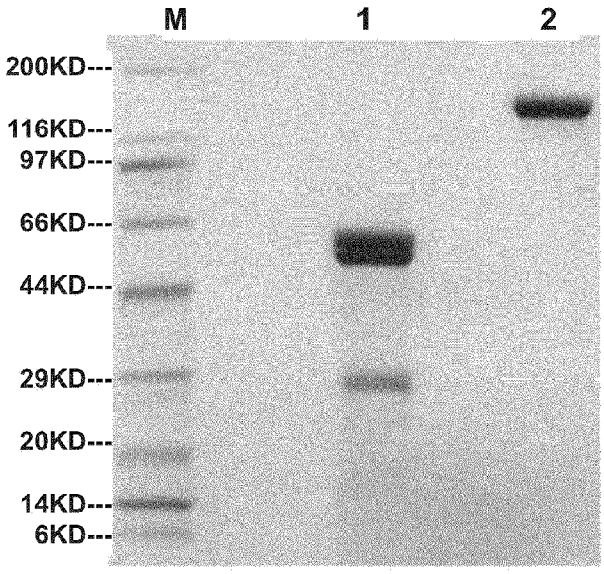
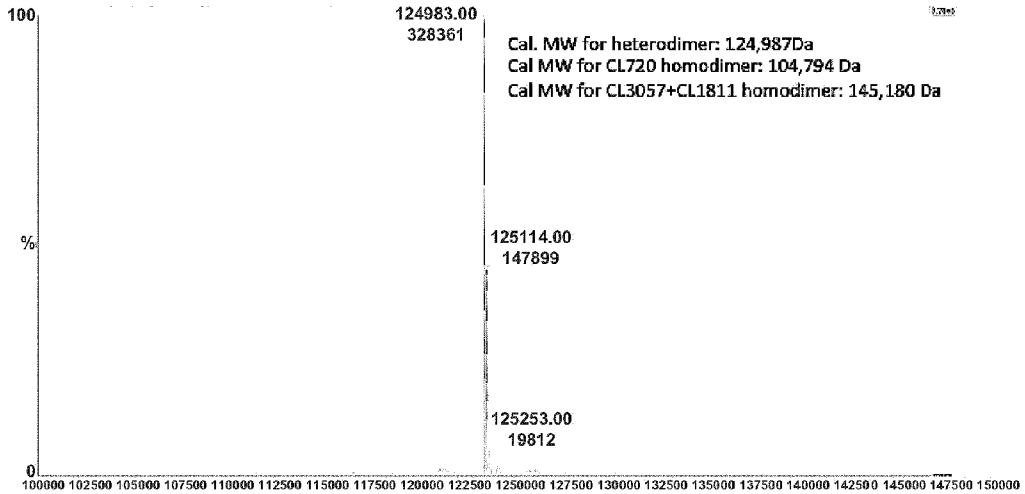


FIG. 5A



	Amount of Each Species as a Percentage of All Quantified				
	Heterodimer	CH-A Homodimer	CH-B Homodimer	CH-B Monomer	CH-A Half-antibody
v5019	99.4	0.3	0.1	0	0.2

*: Lysine and O-glycan side peaks were not included in this calculation

FIG. 5B

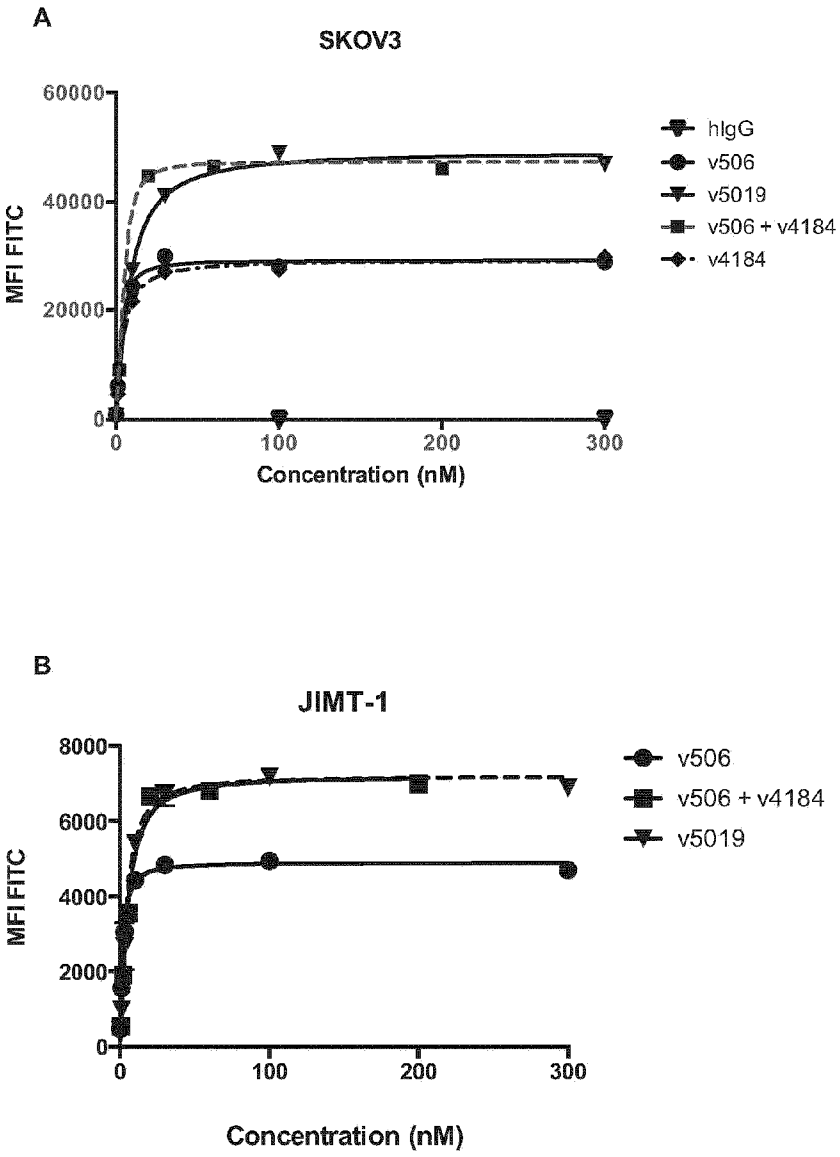
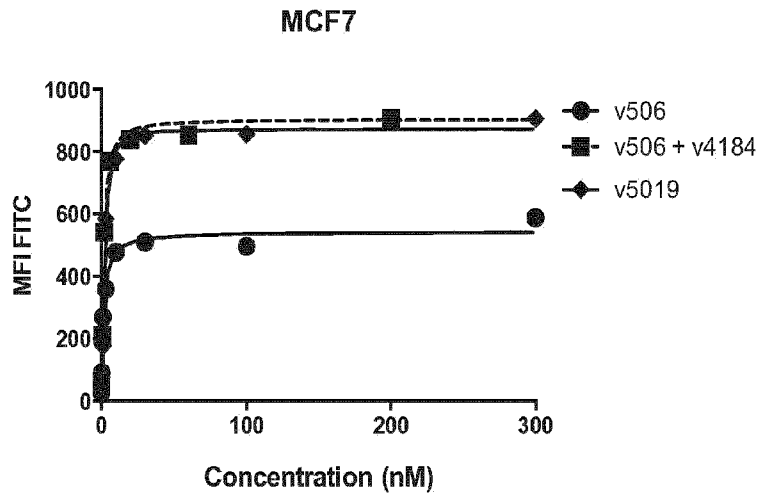

A
SKOV3
60000
40000
20000
0
MFI FITC
0
100
200
300
Concentration (nM)
hIgG
v506
v5019
v506 + v4184
v4184
B
JIMT-1
8000
6000
4000
2000
0
MFI FITC
0
100
200
300
Concentration (nM)
v506
v506 + v4184
v5019

FIG. 6

C



D

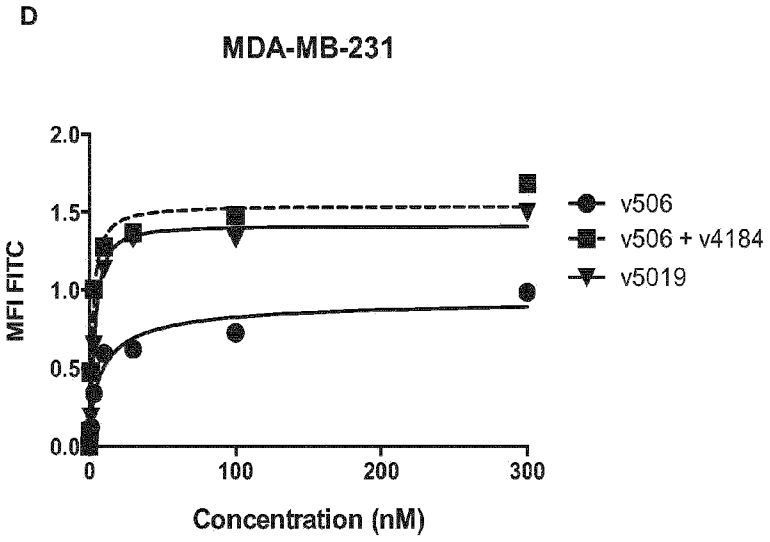
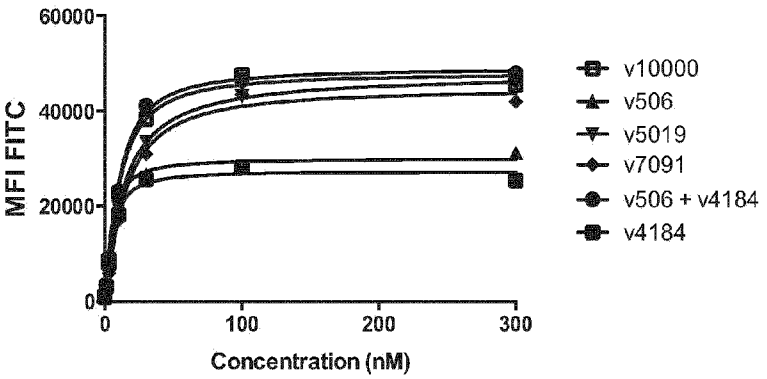


FIG. 6 (Cont'd...)

E



F

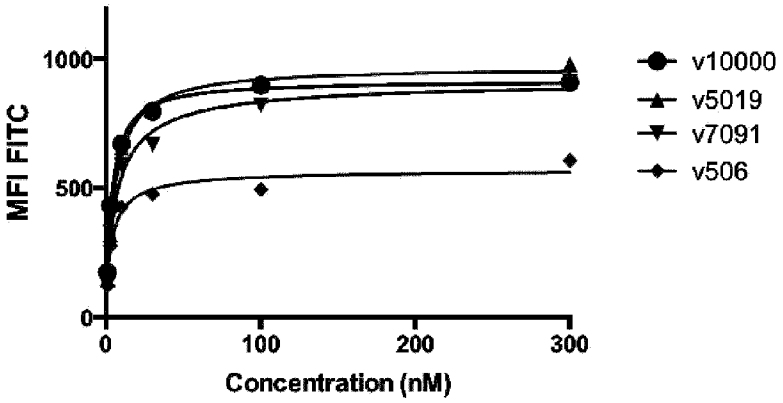


FIG. 6 (Cont'd...)

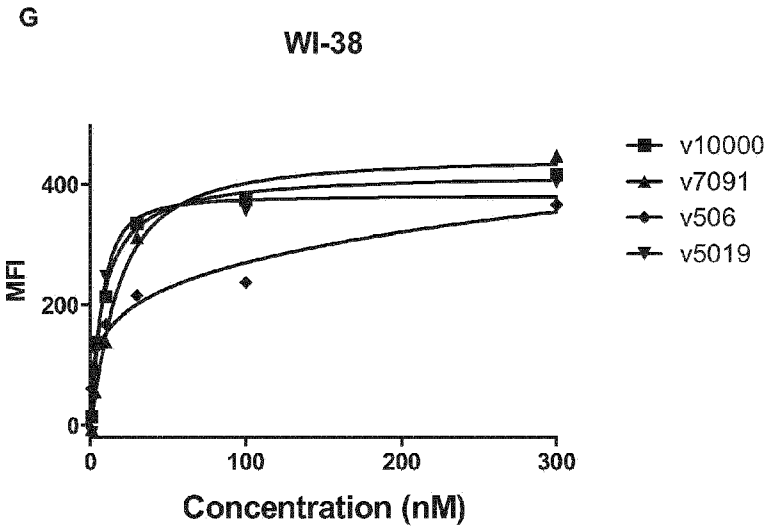
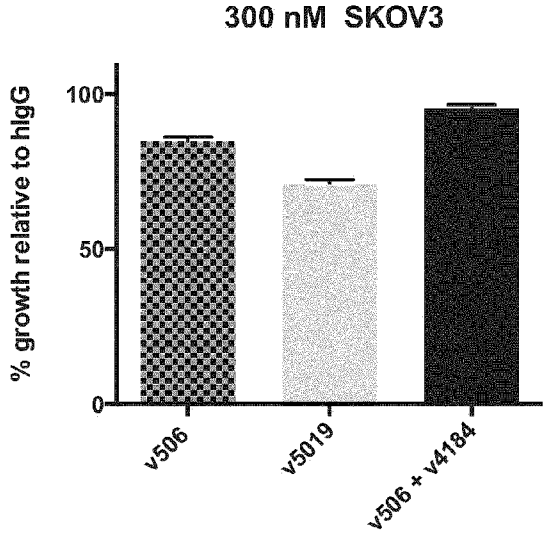

G
WI-38
MFI
400
200
0
0
100
200
300
Concentration (nM)
v10000
v7091
v506
v5019

FIG. 6 (Cont'd...)

A


300 nM SKOV3
% growth relative to hIgG
100
50
0
v506
v5019
v506 + v4184

B

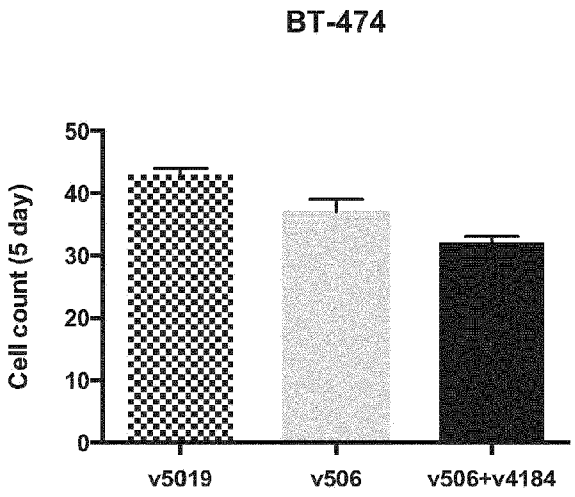

BT-474
Cell count (5 day)
50
40
30
20
10
0
v5019
v506
v506+v4184

FIG. 7

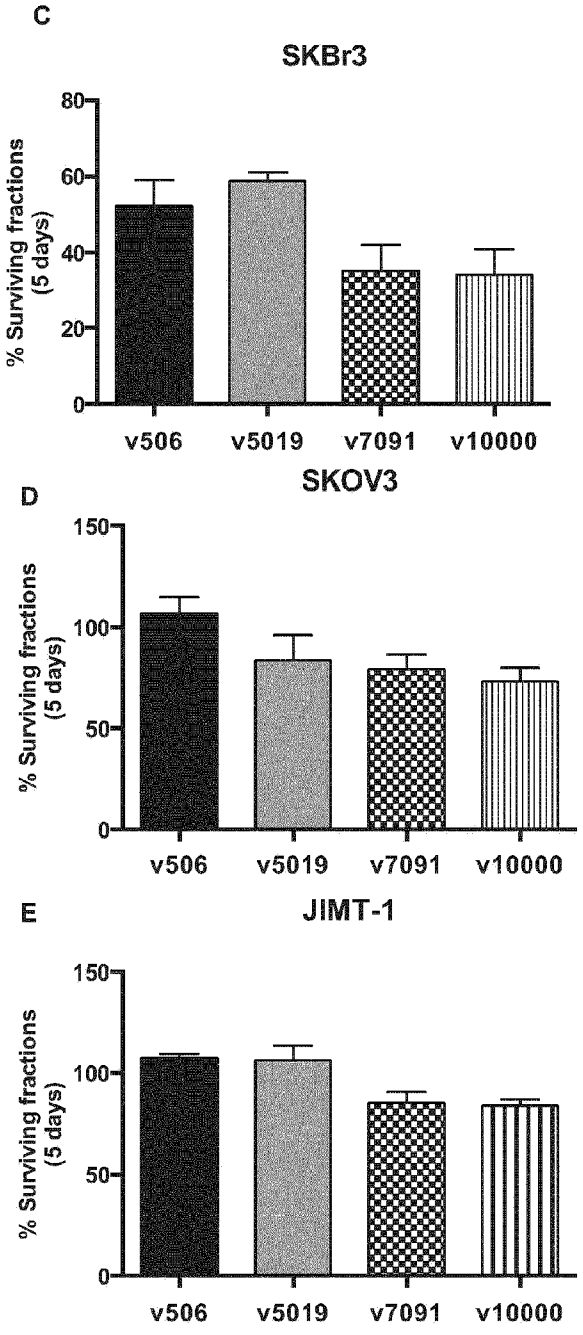

C
SKBr3
% Surviving fractions (5 days)
80
60
40
20
0
v506
v5019
v7091
v10000
D
SKOV3
% Surviving fractions (5 days)
150
100
50
0
v506
v5019
v7091
v10000
E
JIMT-1
% Surviving fractions (5 days)
150
100
50
0
v506
v5019
v7091
v10000

FIG. 7
(Cont'd...)

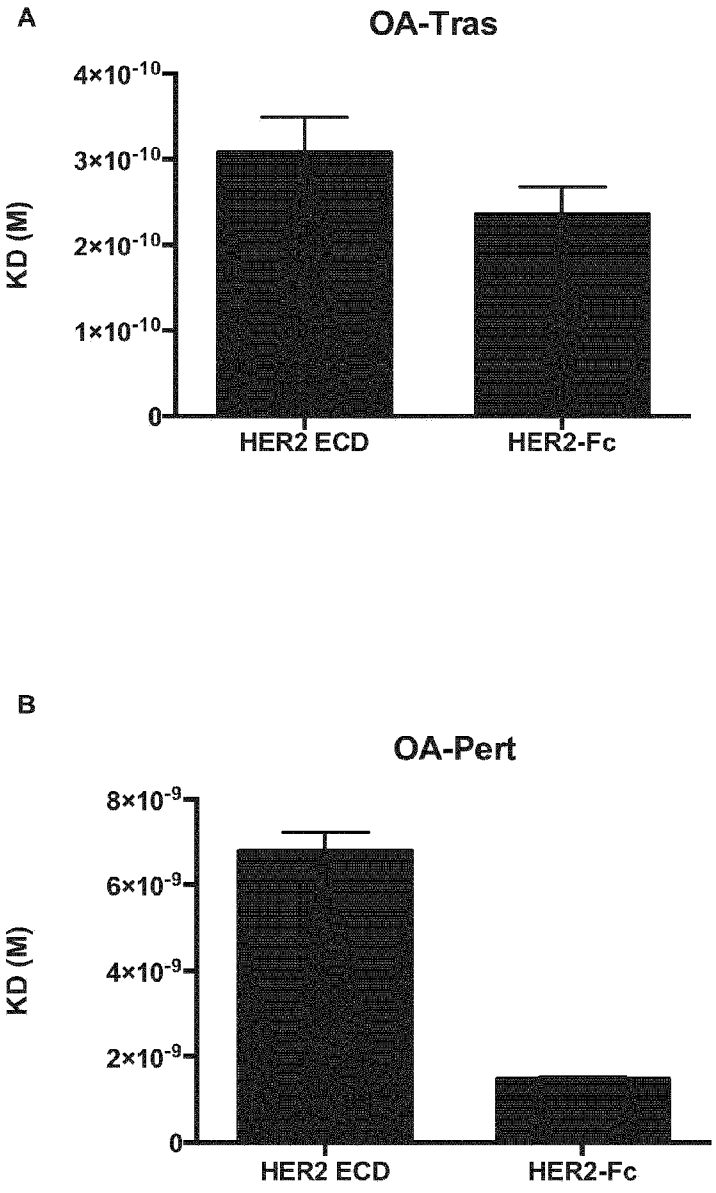

A
OA-Tras
KD (M)
4×10-10
3×10-10
2×10-10
1×10-10
0
HER2 ECD
HER2-Fc
B
OA-Pert
KD (M)
8×10-9
6×10-9
4×10-9
2×10-9
0
HER2 ECD
HER2-Fc

FIG. 8

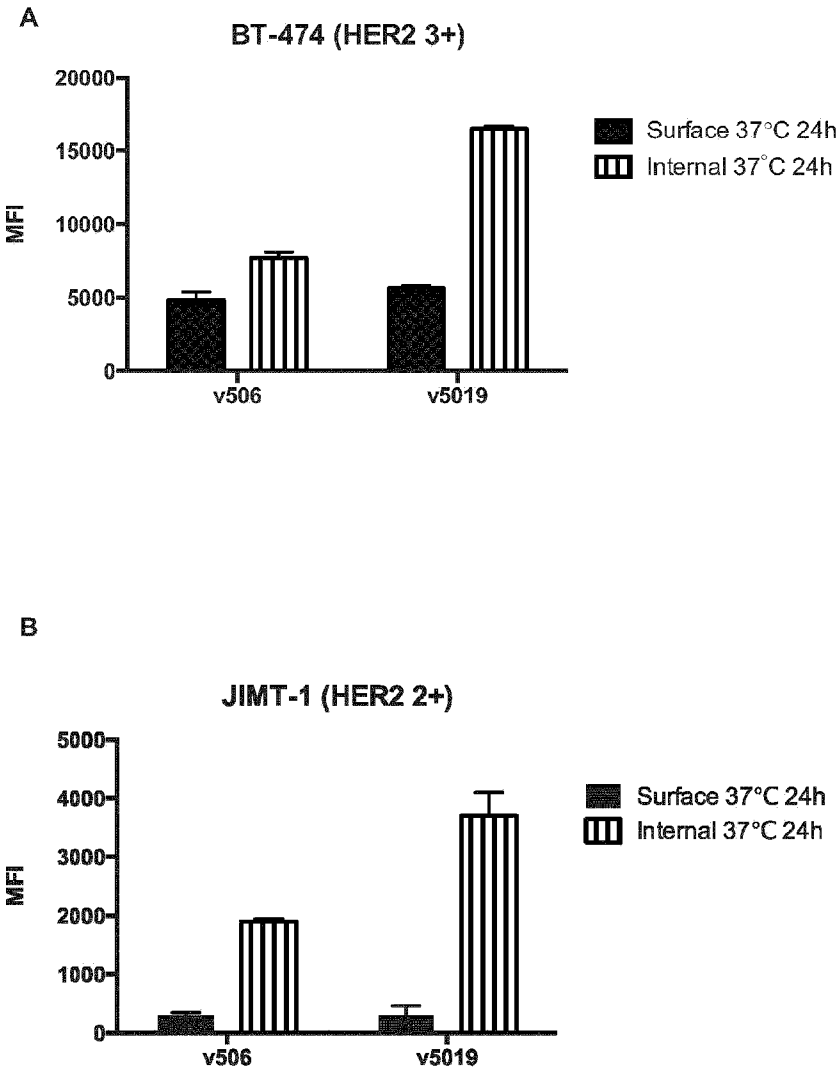

A
BT-474 (HER2 3+)
MFI
20000
15000
10000
5000
0
v506
v5019
Surface 37°C 24h
Internal 37°C 24h
B
JIMT-1 (HER2 2+)
MFI
5000
4000
3000
2000
1000
0
v506
v5019
Surface 37°C 24h
Internal 37°C 24h

FIG. 9

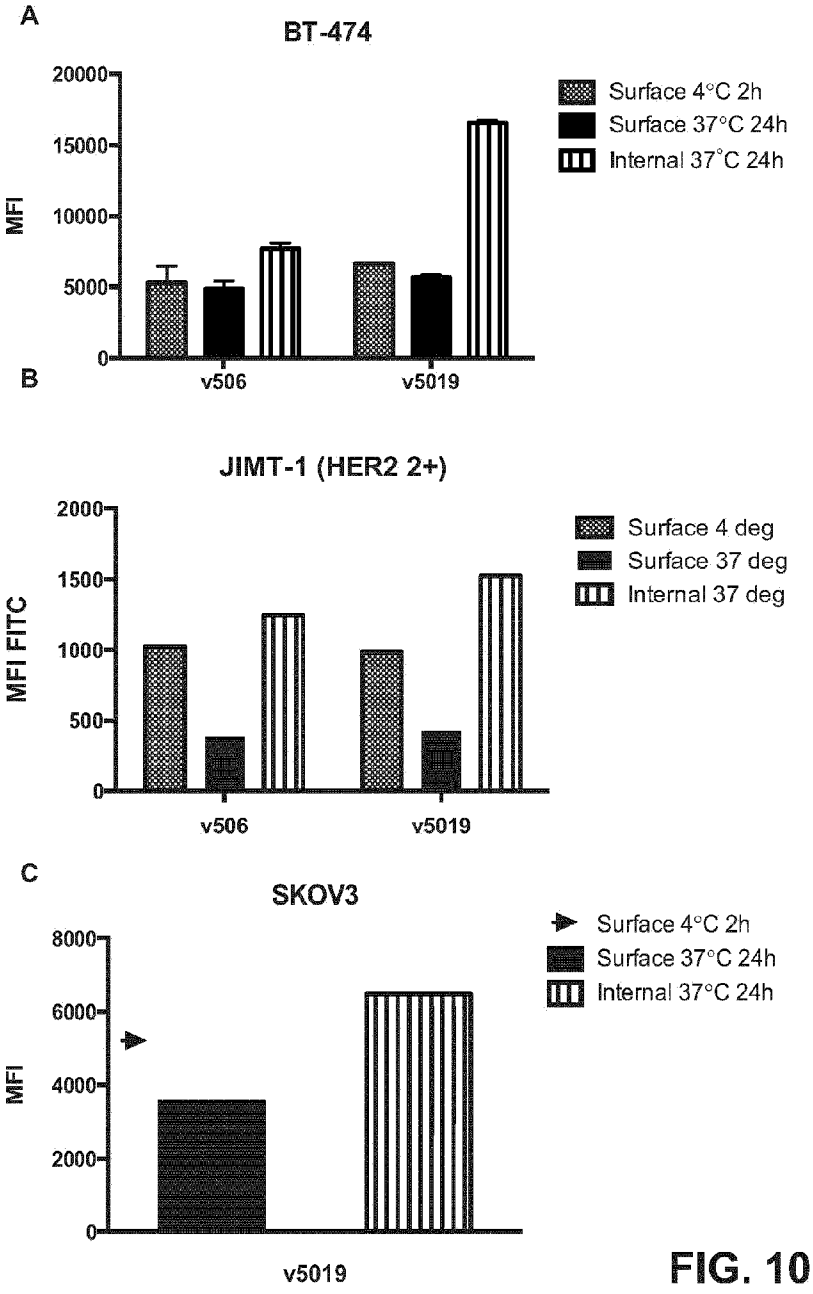

A
BT-474
20000
15000
10000
5000
0
MFI
Surface 4°C 2h
Surface 37°C 24h
Internal 37°C 24h
v506
v5019
B
JIMT-1 (HER2 2+)
2000
1500
1000
500
0
MFI FITC
Surface 4 deg
Surface 37 deg
Internal 37 deg
v506
v5019
C
SKOV3
8000
6000
4000
2000
0
MFI
Surface 4°C 2h
Surface 37°C 24h
Internal 37°C 24h
v5019

FIG. 10

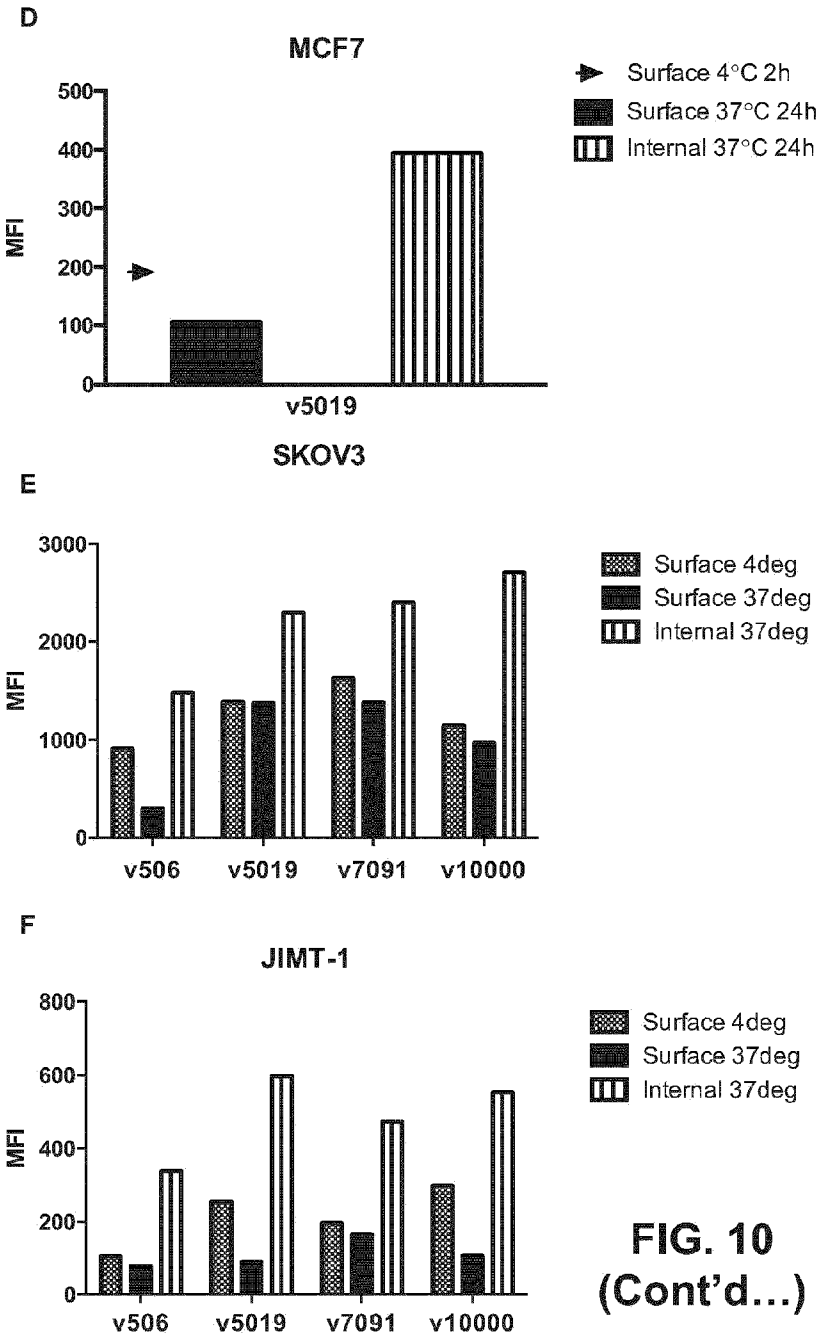

D
MCF7
Surface 4°C 2h
Surface 37°C 24h
Internal 37°C 24h
MFI
500
400
300
200
100
0
v5019
SKOV3
E
3000
2000
1000
0
Surface 4deg
Surface 37deg
Internal 37deg
v506
v5019
v7091
v10000
F
JIMT-1
800
600
400
200
0

FIG. 10
(Cont'd...)

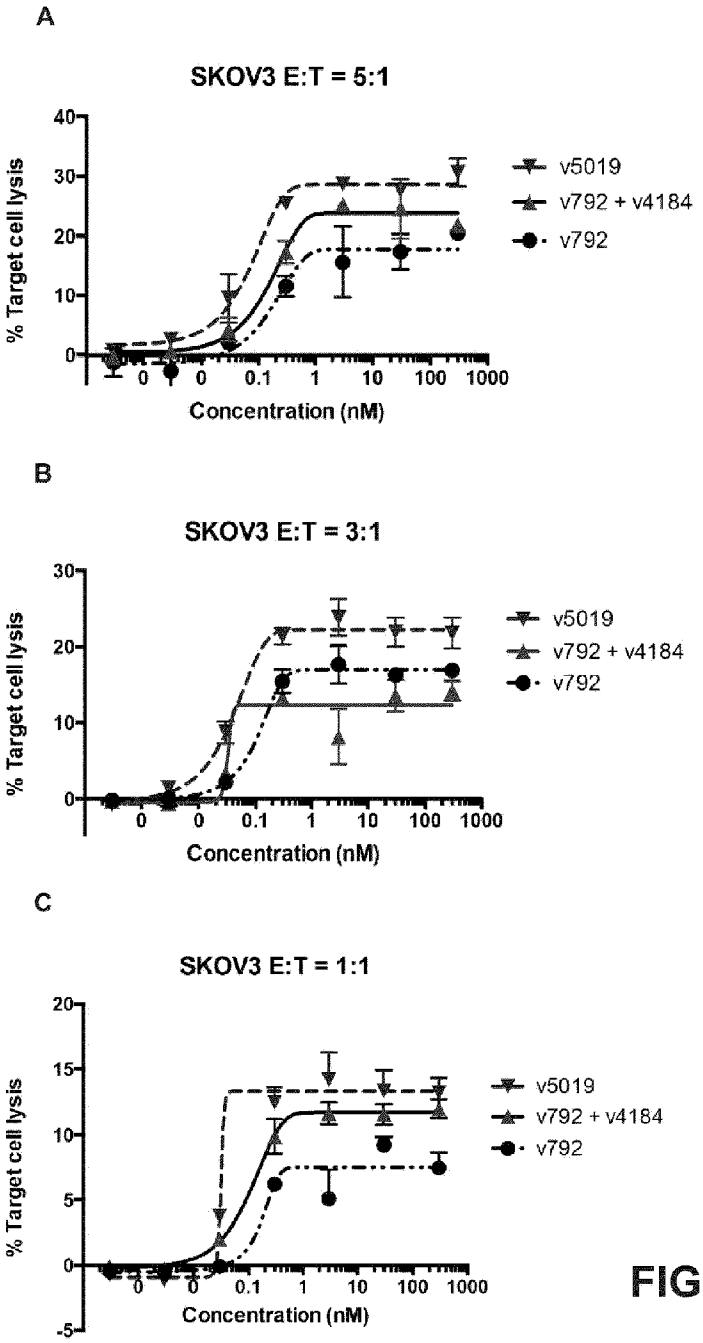

A
SKOV3 E:T = 5:1
% Target cell lysis
Concentration (nM)
v5019
v792 + v4184
v792
B
SKOV3 E:T = 3:1
% Target cell lysis
Concentration (nM)
v5019
v792 + v4184
v792
C
SKOV3 E:T = 1:1
% Target cell lysis
Concentration (nM)
v5019
v792 + v4184
v792

FIG. 11

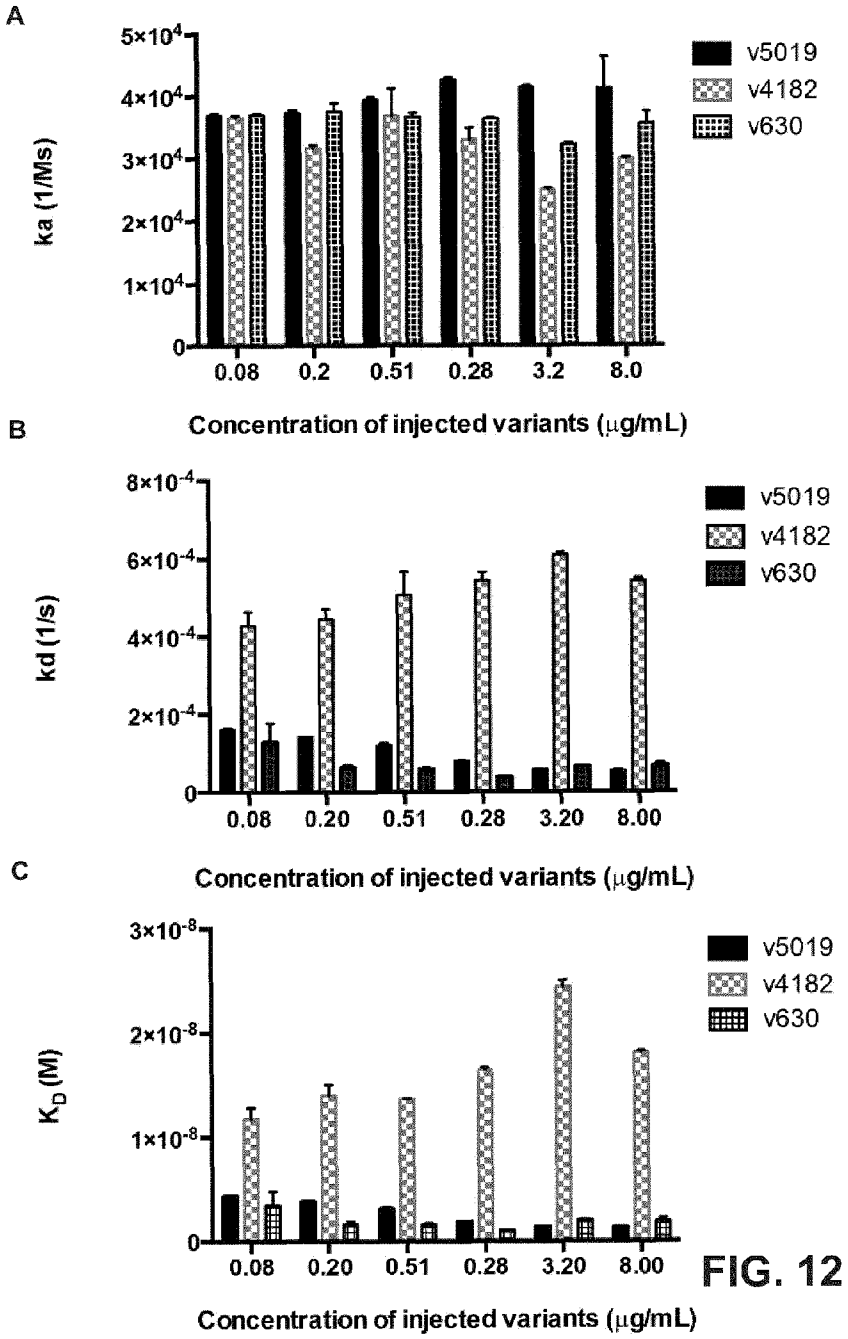

A
ka (1/Ms)
5×10^4
4×10^4
3×10^4
2×10^4
1×10^4
0
0.08
0.2
0.51
0.28
3.2
8.0
v5019
v4182
v630
Concentration of injected variants (µg/mL)
B
kd (1/s)
8×10^-4
6×10^-4
4×10^-4
2×10^-4
0
0.08
0.20
0.51
0.28
3.20
8.00
v5019
v4182
v630
Concentration of injected variants (µg/mL)
C
K_D (M)
3×10^-8
2×10^-8
1×10^-8
0
0.08
0.20
0.51
0.28
3.20
8.00
v5019
v4182
v630
Concentration of injected variants (µg/mL)

FIG. 12

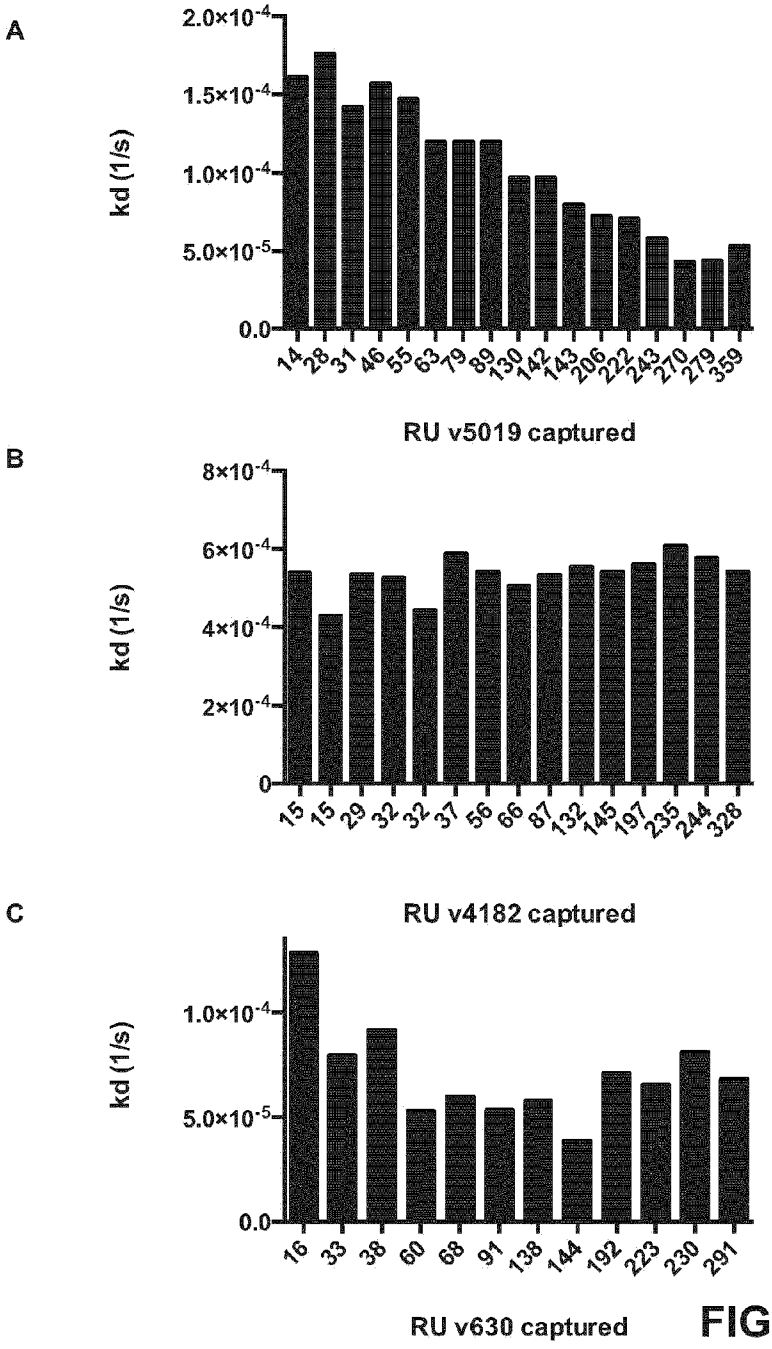
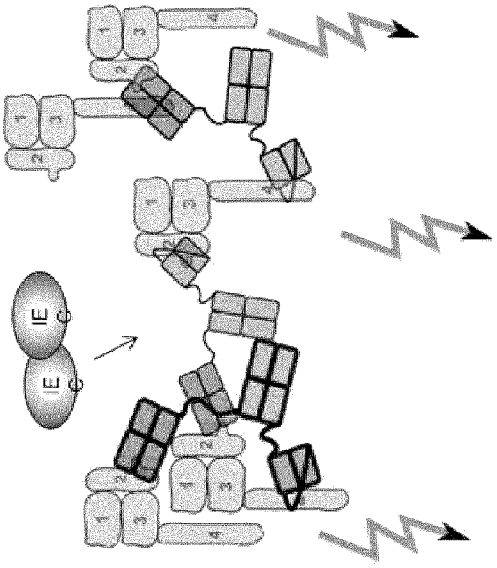

A
kd (1/s)
2.0×10-4
1.5×10-4
1.0×10-4
5.0×10-5
0.0
14 28 31 46 55 63 79 89 130 142 143 206 222 243 270 279 359
RU v5019 captured
B
kd (1/s)
8×10-4
6×10-4
4×10-4
2×10-4
0
15 15 29 32 32 37 56 66 87 132 145 197 235 244 328
RU v4182 captured
C
kd (1/s)
1.0×10-4
5.0×10-5
0.0
16 33 38 60 68 91 138 144 192 223 230 291
RU v630 captured

FIG. 13



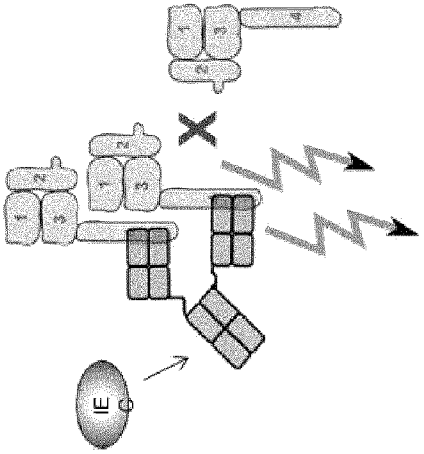


FIG. 14

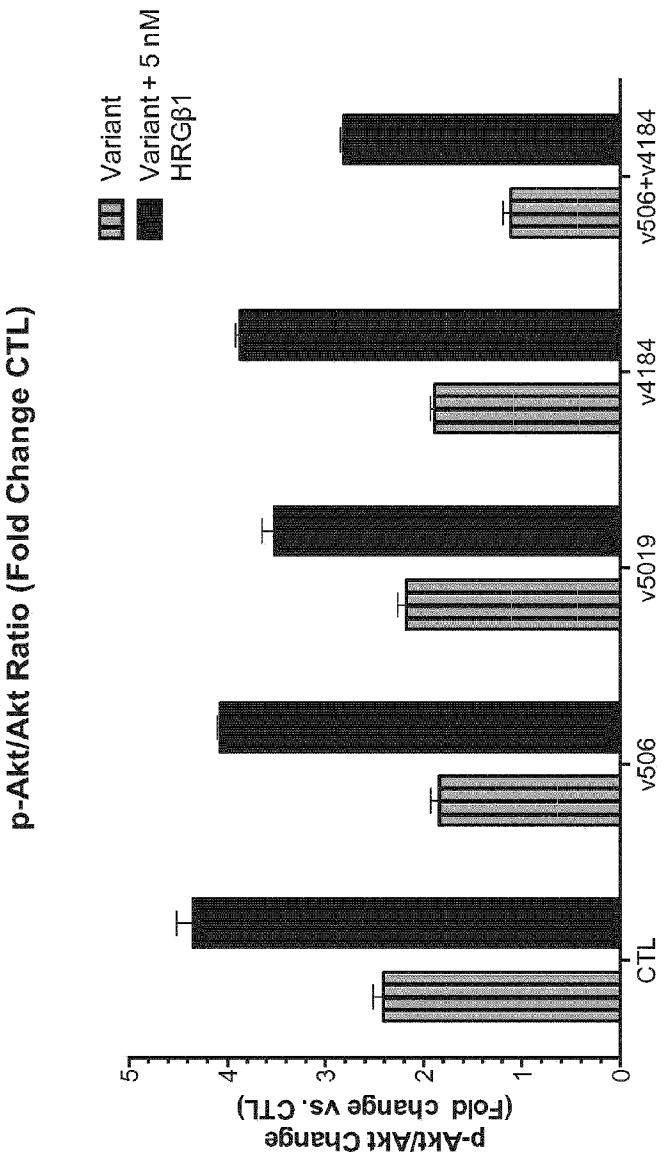

p-Akt/Akt Ratio (Fold Change CTL)
Variant
Variant + 5 nM HRGβ1
p-Akt/Akt Change (Fold change vs. CTL)
0
1
2
3
4
5
CTL
v506
v5019
v4184
v506+v4184

FIG. 15

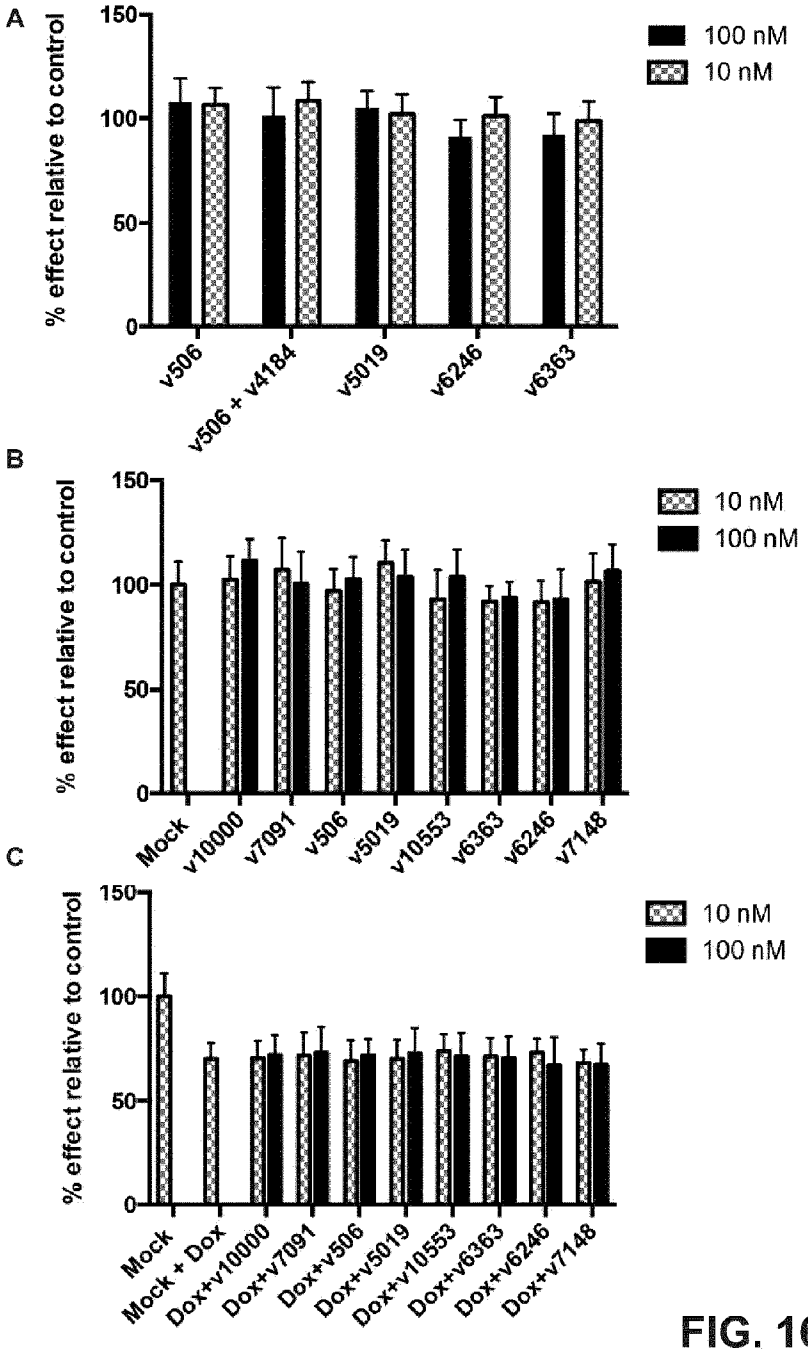
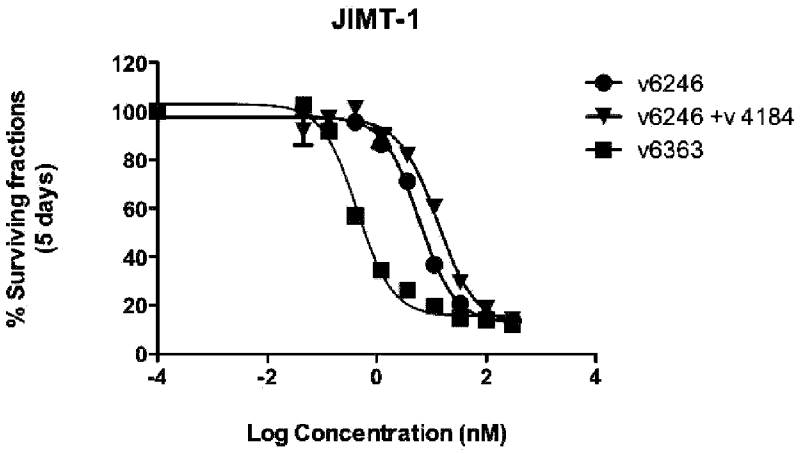

A
% effect relative to control
150
100
50
0
100 nM
10 nM
v506
v506 + v4184
v5019
v6246
v6363
B
% effect relative to control
150
100
50
0
10 nM
100 nM
Mock
v10000
v7091
v506
v5019
v10553
v6363
v6246
v7148
C
% effect relative to control
150
100
50
0
10 nM
100 nM
Mock
Mock + Dox
Dox+v10000
Dox+v7091
Dox+v506
Dox+v5019
Dox+v10553
Dox+v6363
Dox+v6246
Dox+v7148

FIG. 16

A


JIMT-1
% Surviving fractions (5 days)
120
100
80
60
40
20
0
-4
-2
0
2
4
Log Concentration (nM)
v6246
v6246 +v 4184
v6363

B

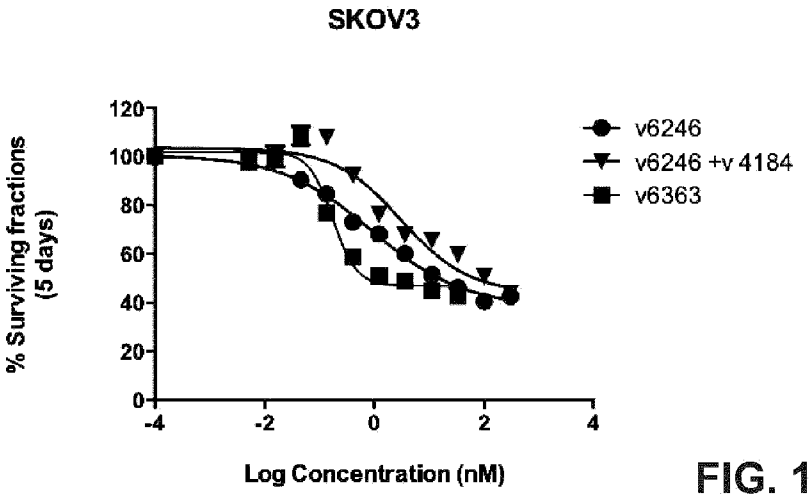
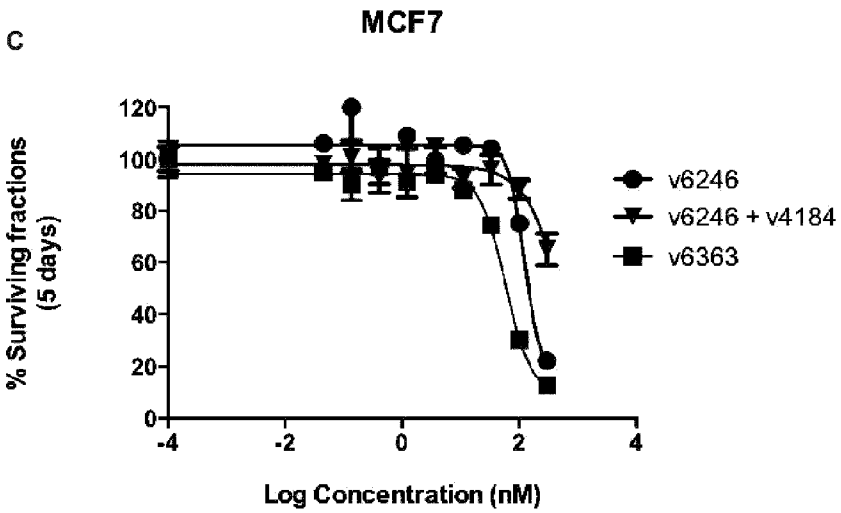

SKOV3
% Surviving fractions (5 days)
120
100
80
60
40
20
0
-4
-2
0
2
4
Log Concentration (nM)
v6246
v6246 +v 4184
v6363

FIG. 17

C



D

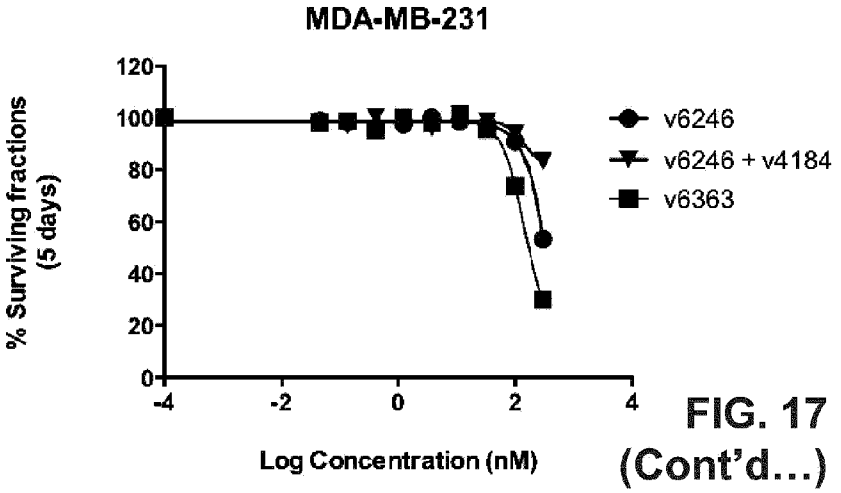
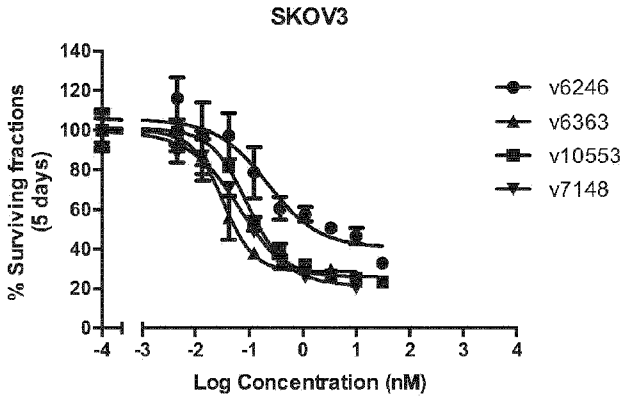
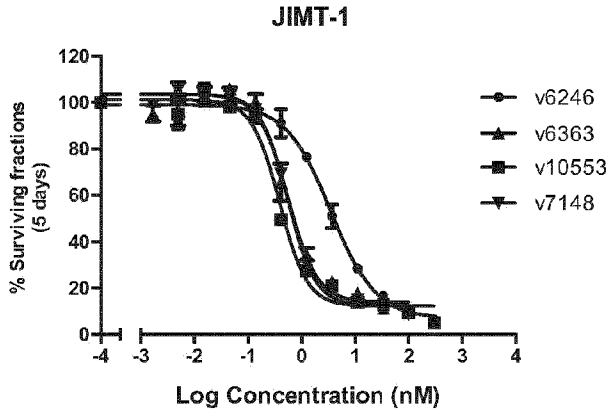


FIG. 17 (Cont'd...)

E



F



G

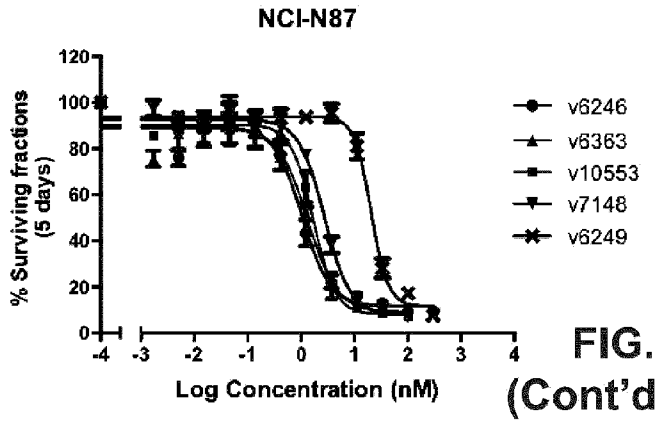


FIG. 17 (Cont'd...)

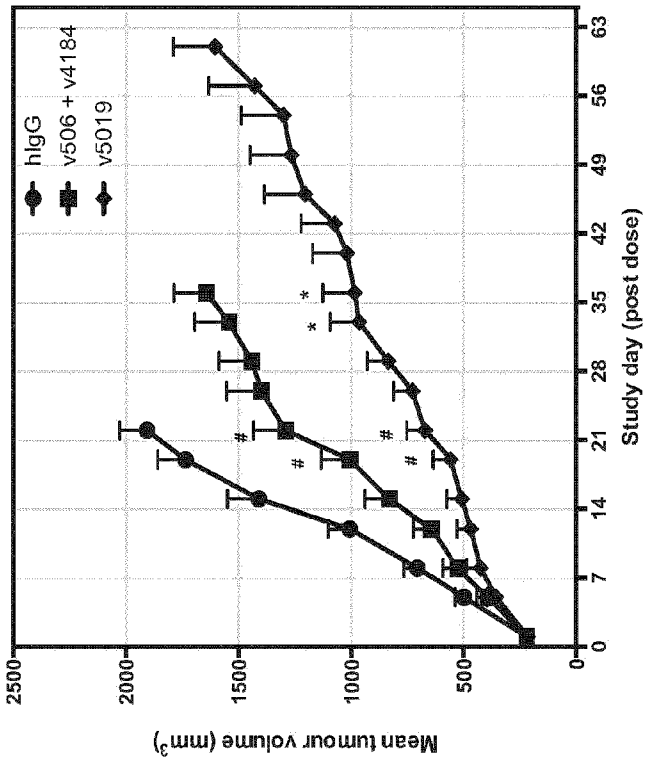

hIgG
v506 + v4184
v5019
Mean tumour volume (mm3)
2500
2000
1500
1000
500
0
0
7
14
21
28
35
42
49
56
63
Study day (post dose)
#
*
ANOVA
*, p<0.05 vs v506
#, p<0.05 vs IgG

FIG. 18A

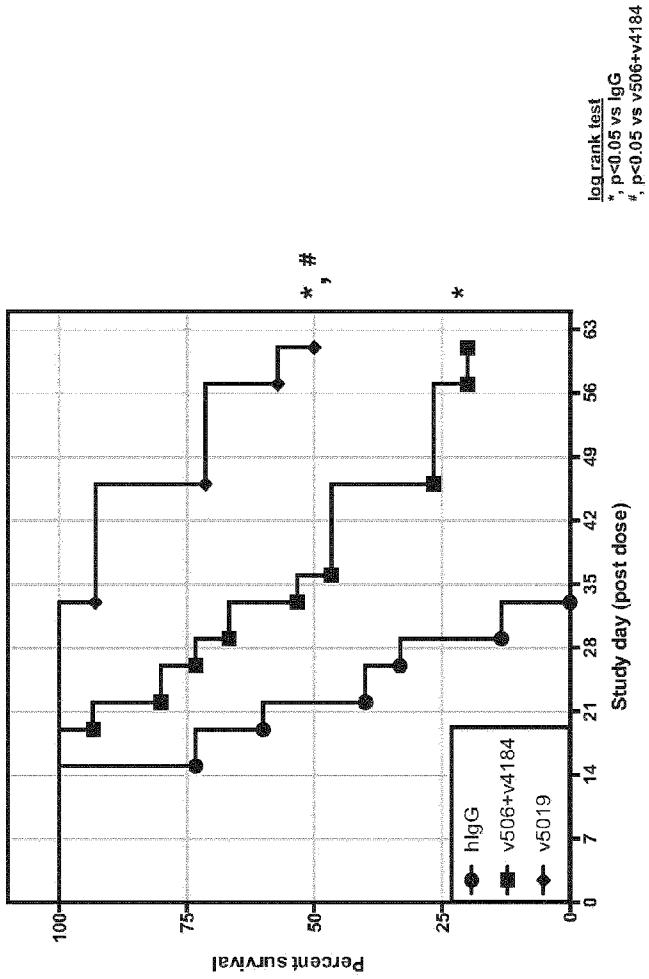

log rank test
*, p<0.05 vs IgG
#, p<0.05 vs v506+v4184
* , #
*
Percent survival
100
75
50
25
0
0
7
14
21
28
35
42
49
56
63
Study day (post dose)
hIgG
v506+v4184
v5019

FIG. 18B

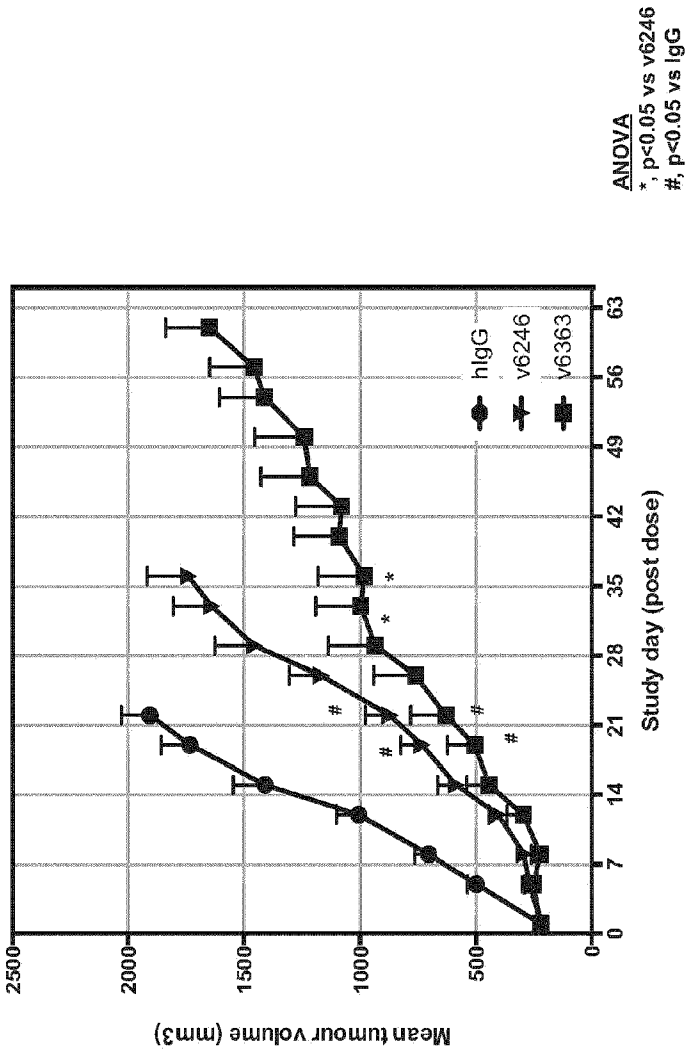

ANOVA
*, p<0.05 vs v6246
#, p<0.05 vs IgG
hIgG
v6246
v6363
Mean tumour volume (mm3)
Study day (post dose)
0
500
1000
1500
2000
2500
0
7
14
21
28
35
42
49
56
63

FIG. 19A

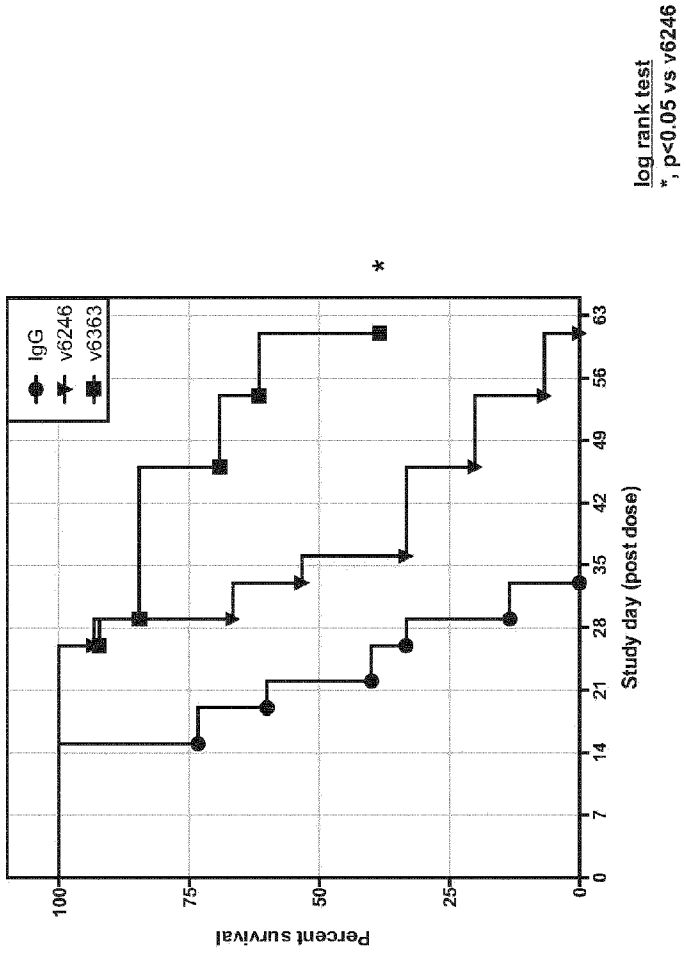

log rank test
*, p<0.05 vs v6246
*
IgG
v6246
v6363
Percent survival
100
75
50
25
0
0
7
14
21
28
35
42
49
56
63
Study day (post dose)

FIG. 19B

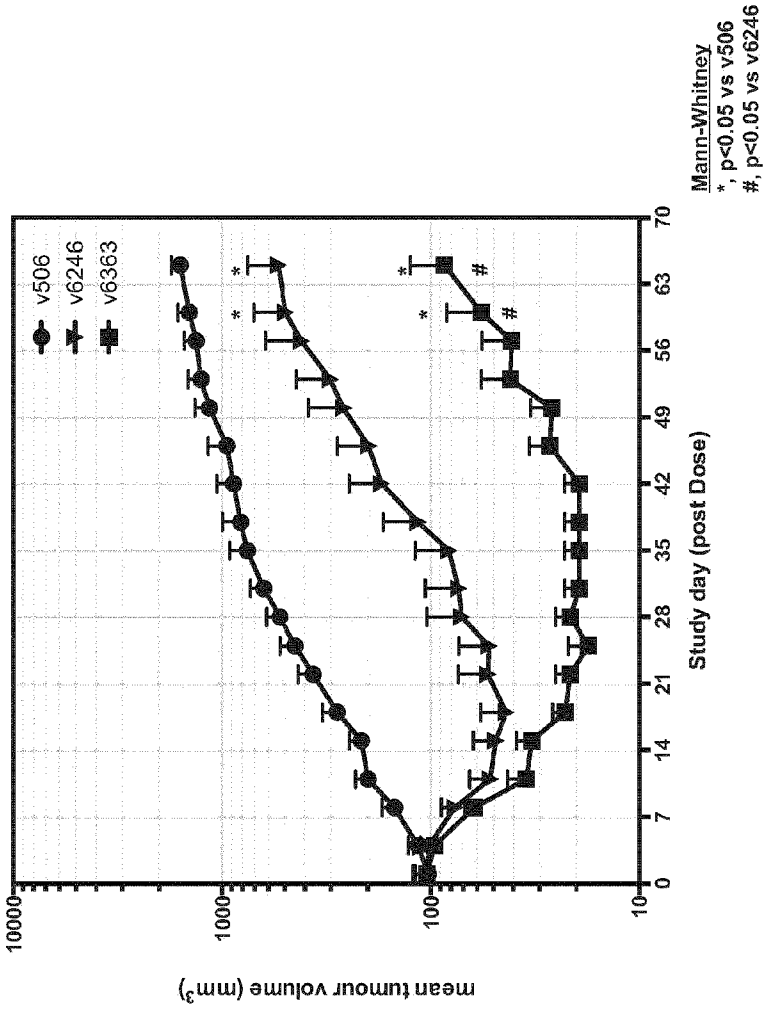

v506
v6246
v6363
mean tumour volume (mm3)
10000
1000
100
10
0
7
14
21
28
35
42
49
56
63
70
Study day (post Dose)
Mann-Whitney
*, p<0.05 vs v506
#, p<0.05 vs v6246

FIG. 20

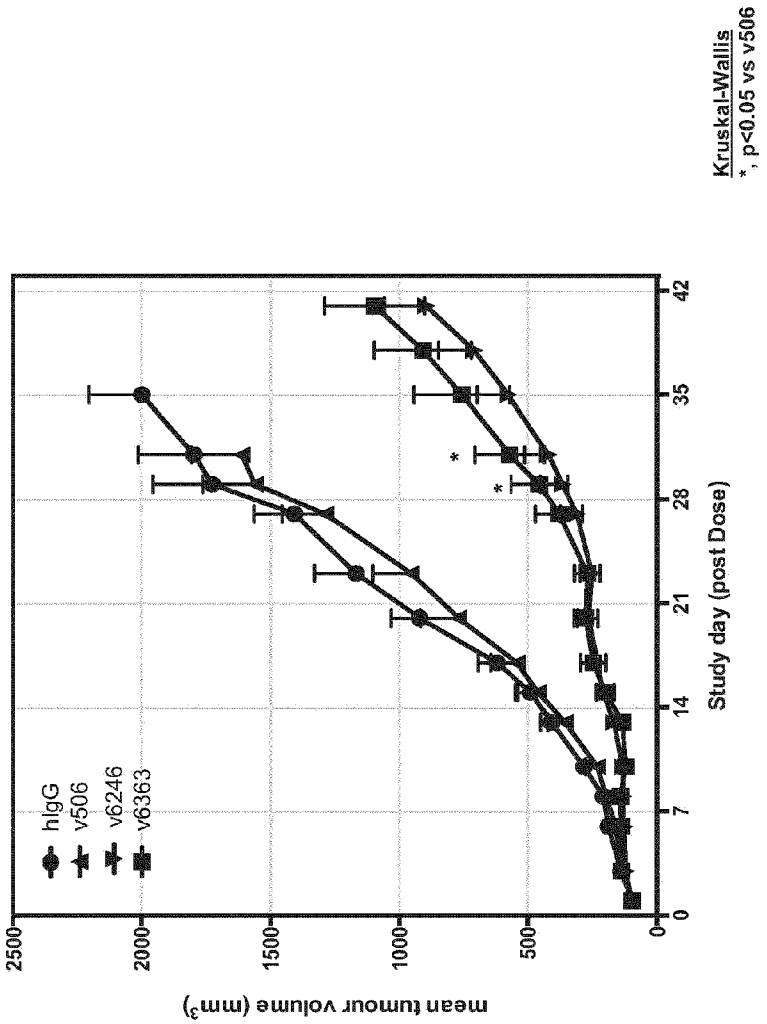

Kruskal-Wallis
*, p<0.05 vs v506
hIgG
v506
v6246
v6363
mean tumour volume (mm³)
Study day (post Dose)
0
500
1000
1500
2000
2500
0
7
14
21
28
35
42
*
*

FIG. 21

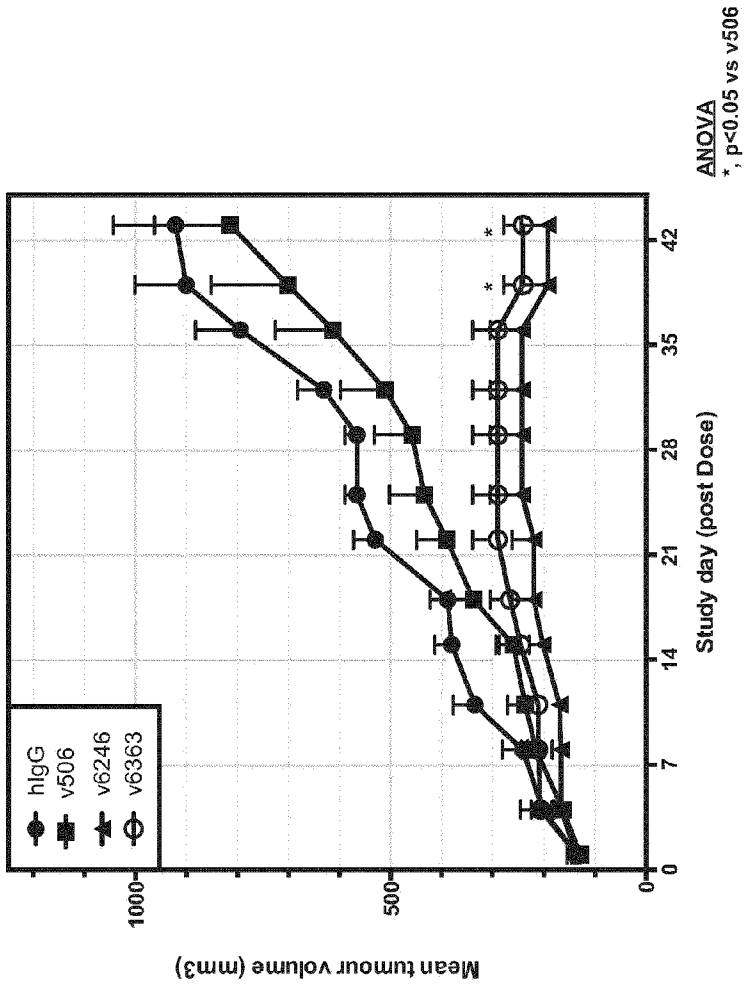

hIgG
v506
v6246
v6363
Mean tumour volume (mm3)
1000
500
0
0
7
14
21
28
35
42
Study day (post Dose)
ANOVA
*, p<0.05 vs v506
*
*

FIG. 22

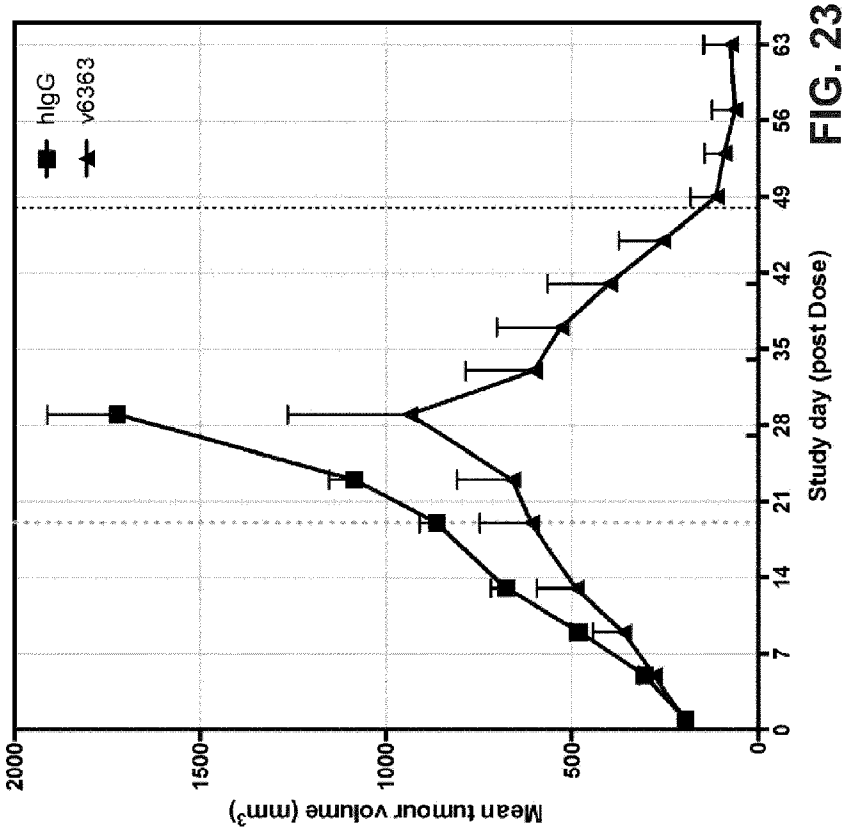

hIgG
v6363
Mean tumour volume (mm³)
2000
1500
1000
500
0
0
7
14
21
28
35
42
49
56
63
Study day (post Dose)

FIG. 23

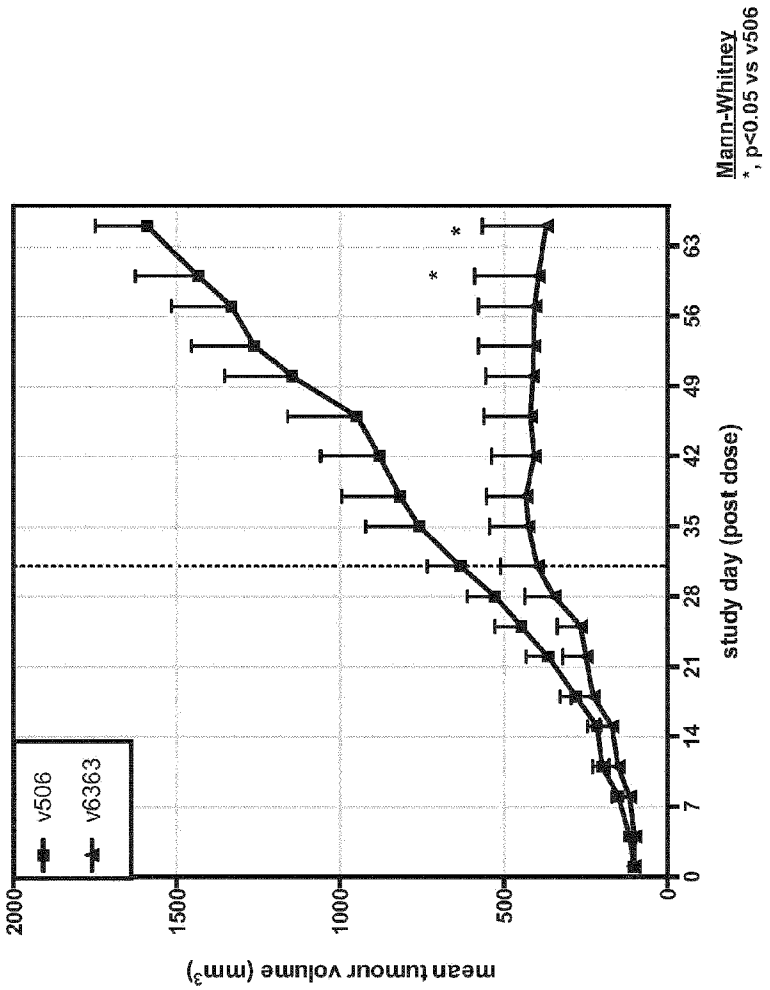

mean tumour volume (mm³)
2000
1500
1000
500
0
0
7
14
21
28
35
42
49
56
63
study day (post dose)
v506
v6363
*
*
Mann-Whitney
*, p<0.05 vs v506

FIG. 24

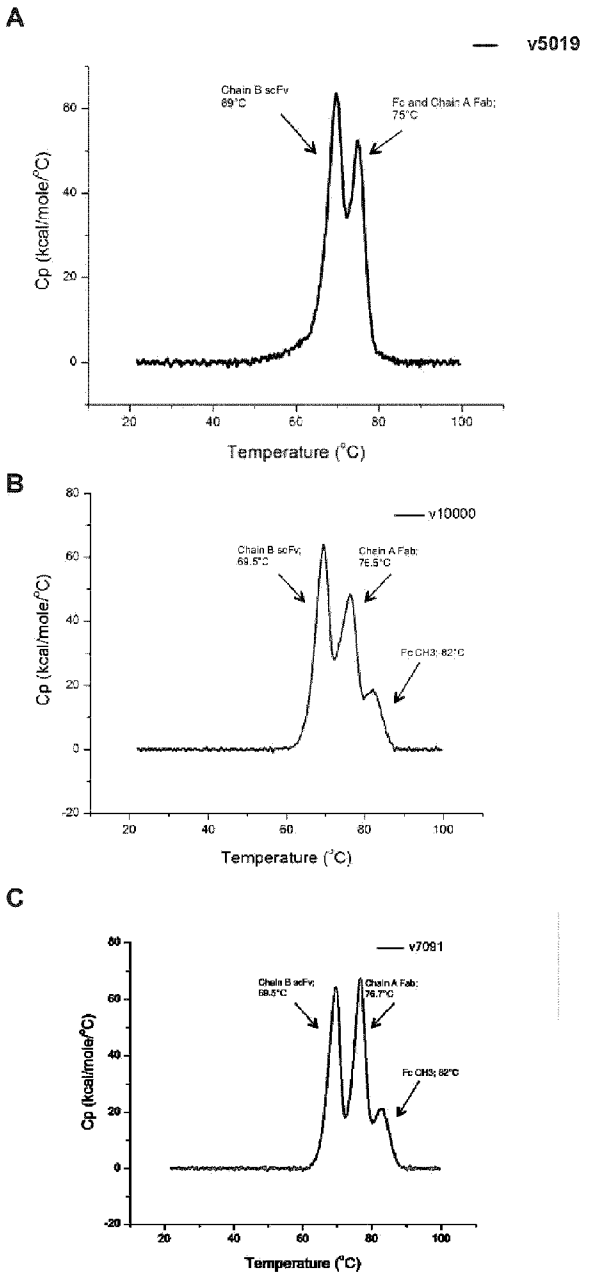
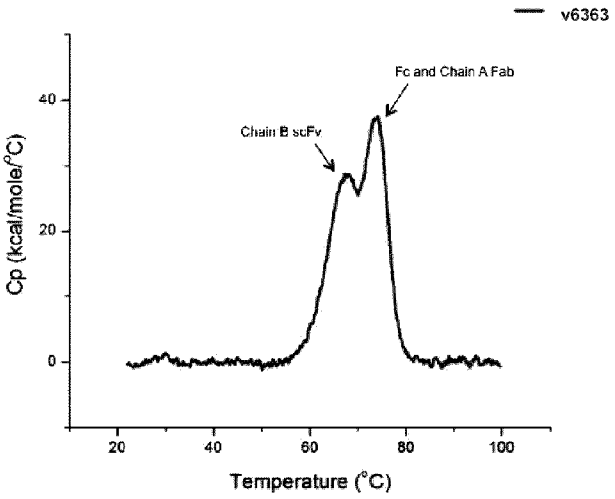
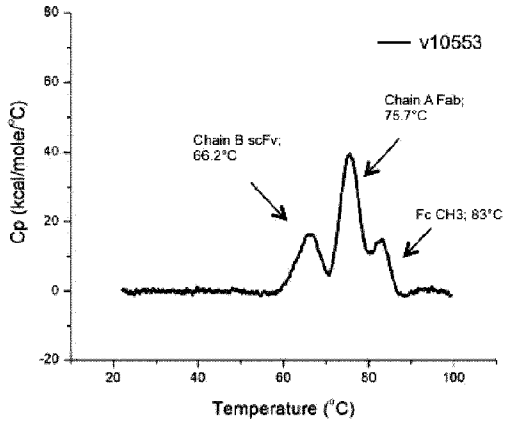

A
v5019
Chain B scFv
69°C
Fc and Chain A Fab;
75°C
Cp (kcal/mole/°C)
Temperature (°C)
B
v10000
Chain B scFv;
69.5°C
Chain A Fab;
76.5°C
Fc CH3; 82°C
Cp (kcal/mole/°C)
Temperature (°C)
C
v7091
Chain B scFv;
69.5°C
Chain A Fab;
76.7°C
Fc CH3; 82°C
Cp (kcal/mole/°C)
Temperature (°C)

FIG. 25

A


v6363
Fc and Chain A Fab
Chain B scFv
Cp (kcal/mole/°C)
Temperature (°C)

B


v10553
Chain A Fab;
75.7°C
Chain B scFv;
66.2°C
Fc CH3; 83°C
Cp (kcal/mole/°C)
Temperature (°C)

C

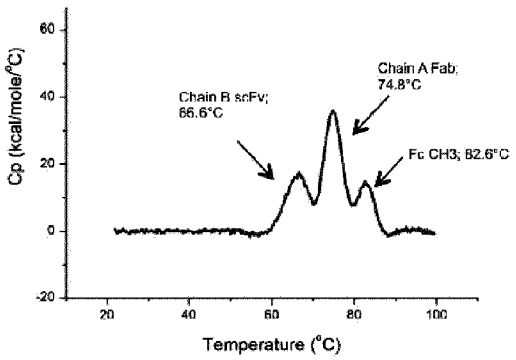
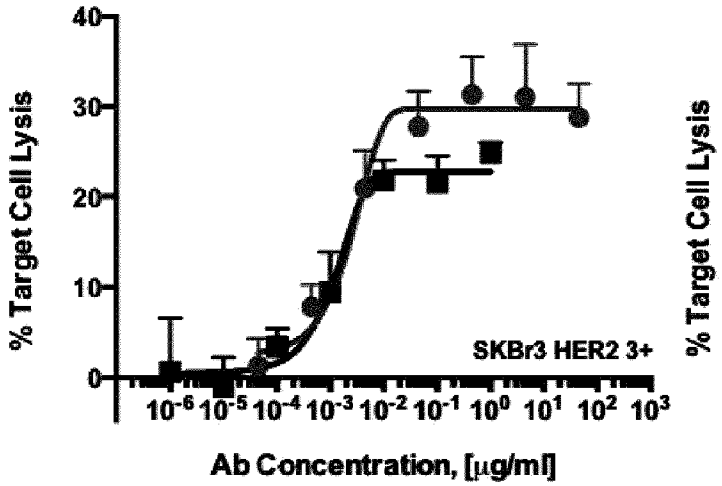

Chain A Fab;
74.8°C
Chain B scFv;
66.6°C
Fc CH3; 82.6°C
Cp (kcal/mole/°C)
Temperature (°C)

FIG. 26

A


% Target Cell Lysis
40
30
20
10
0
SKBr3 HER2 3+
10^{-6} 10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
Ab Concentration, [μg/ml]
% Target Cell Lysis

B

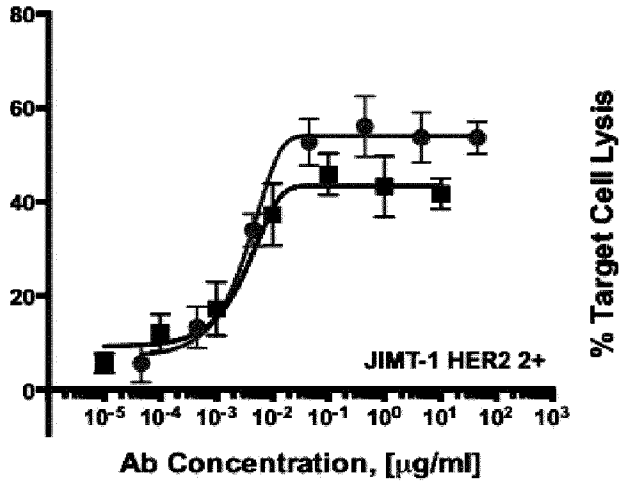
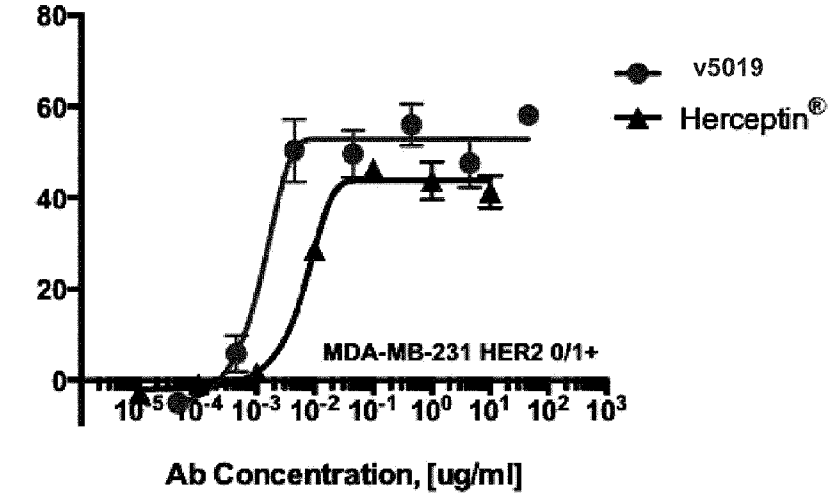

80
60
40
20
0
JIMT-1 HER2 2+
10^{-5} 10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2} 10^{3}
Ab Concentration, [μg/ml]
% Target Cell Lysis

FIG. 27

C



D

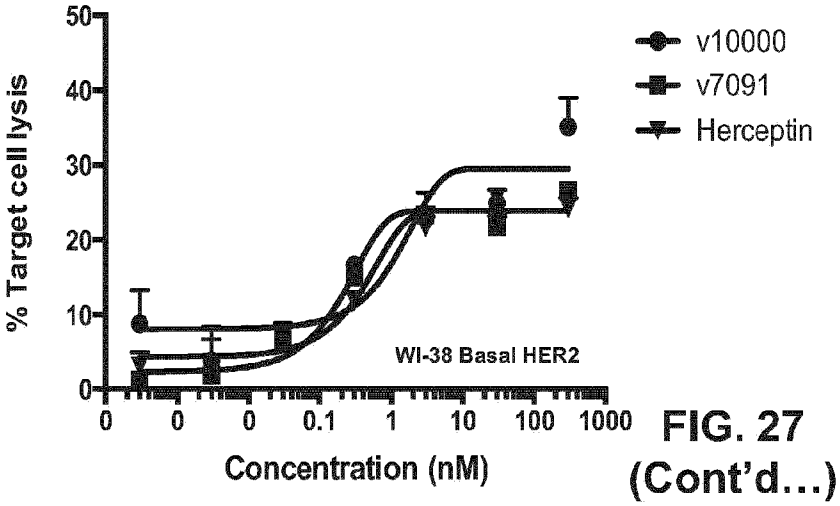
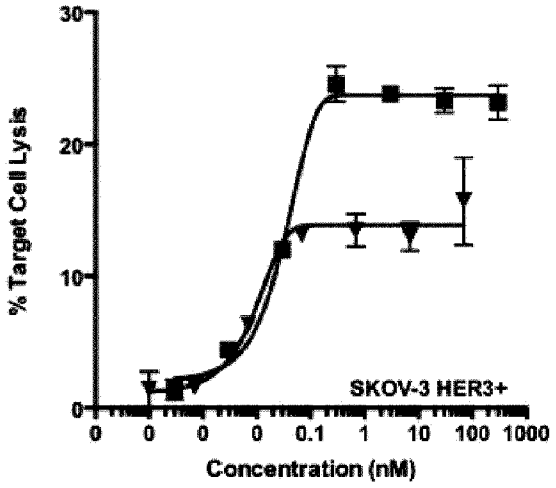


FIG. 27
(Cont'd...)

A


% Target Cell Lysis
30
20
10
0
SKOV-3 HER3+
0 0 0 0 0.1 1 10 100 1000
Concentration (nM)

B

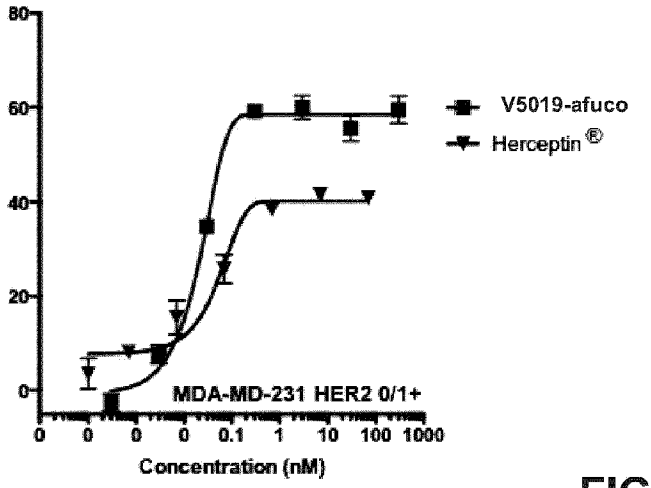

80
60
40
20
0
V5019-afuco
Herceptin®
MDA-MD-231 HER2 0/1+
0 0 0 0 0.1 1 10 100 1000
Concentration (nM)

FIG. 28

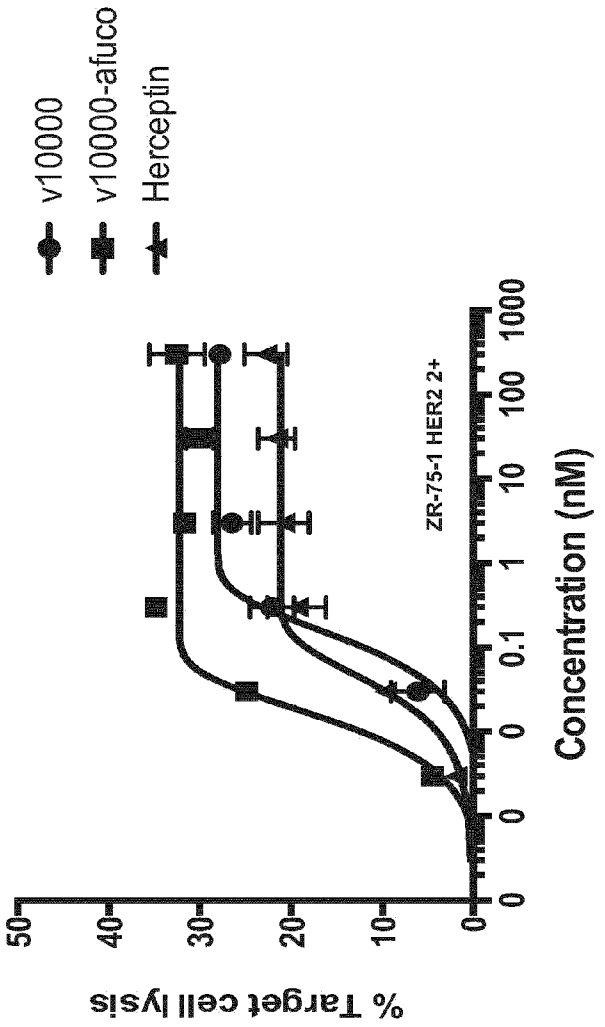

v10000
v10000-afuco
Herceptin
ZR-75-1 HER2 2+
% Target cell lysis
0
10
20
30
40
50
0
0
0
0.1
1
10
100
1000
Concentration (nM)

FIG. 28C

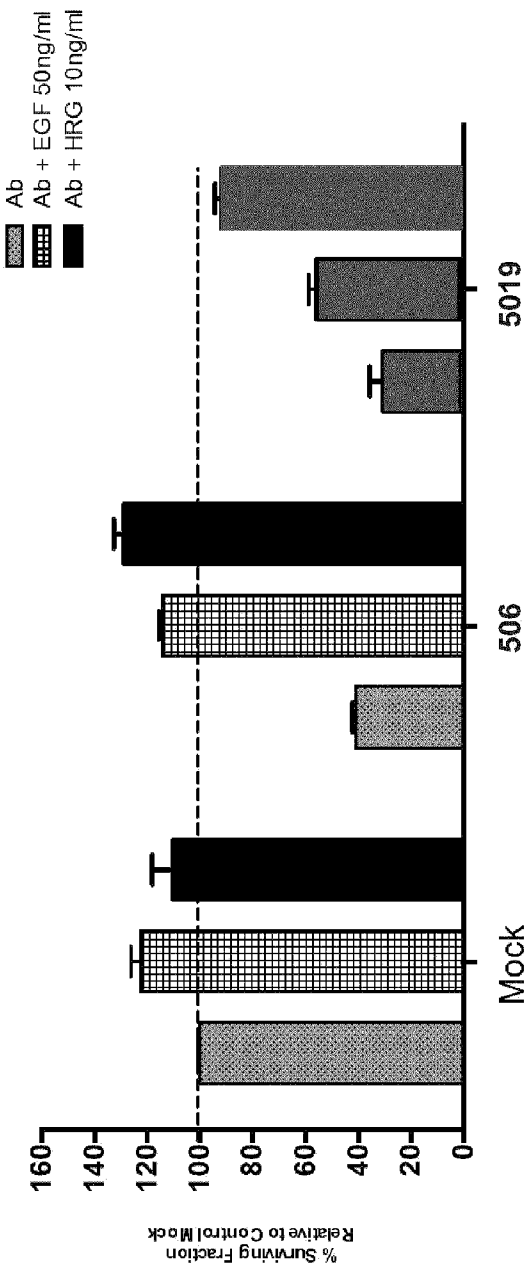

Ab
Ab + EGF 50ng/ml
Ab + HRG 10ng/ml
% Surviving Fraction
Relative to Control Mock
160
140
120
100
80
60
40
20
0
Mock
506
5019

FIG. 29

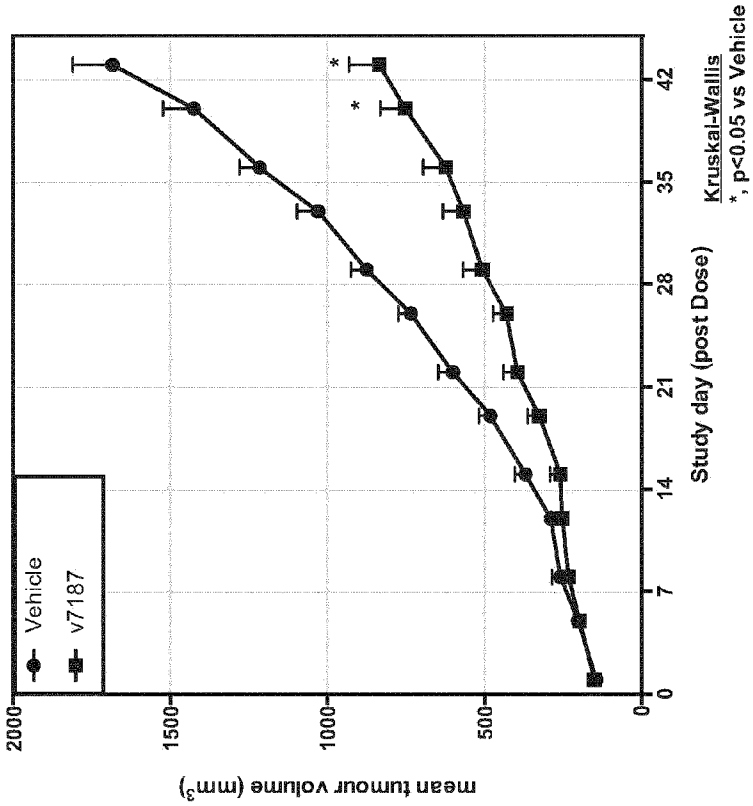
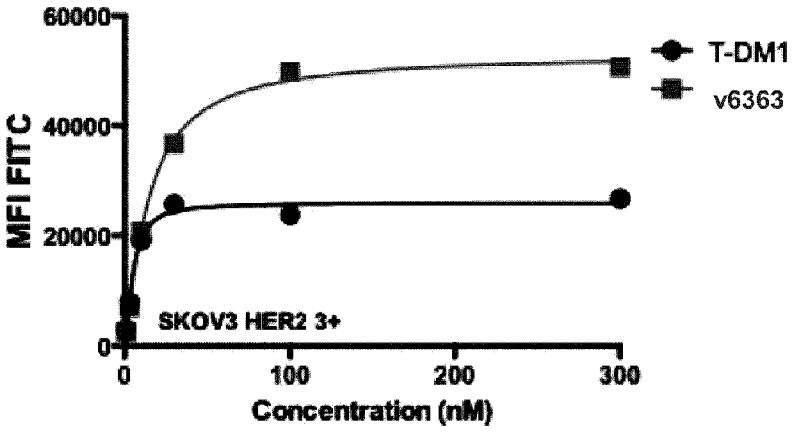

mean tumour volume (mm3)
2000
1500
1000
500
0
0
7
14
21
28
35
42
Study day (post Dose)
Vehicle
v7187
*
*
Kruskal-Wallis
*, p<0.05 vs Vehicle

FIG. 30

A


60000
40000
20000
0
MFI FITC
SKOV3 HER2 3+
0
100
200
300
Concentration (nM)
T-DM1
v6363

B

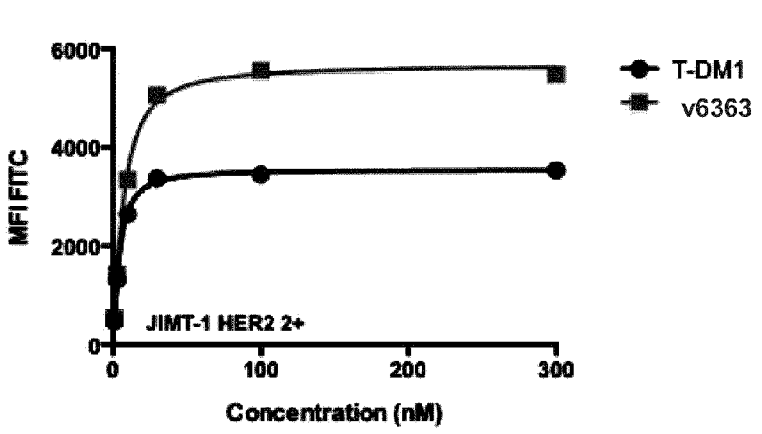
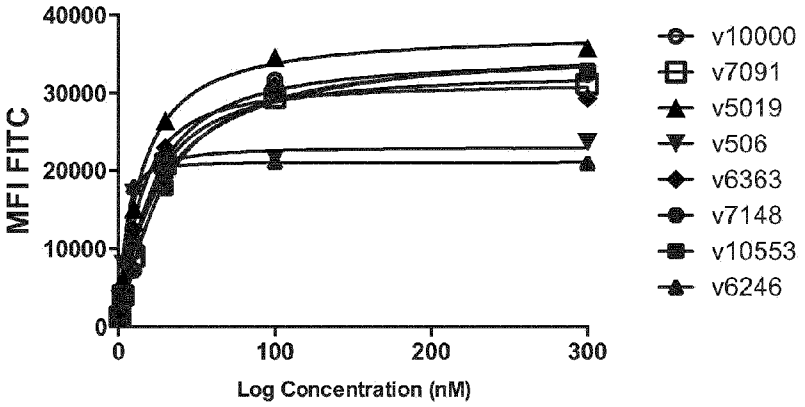

6000
4000
2000
0
MFI FITC
JIMT-1 HER2 2+
0
100
200
300
Concentration (nM)
T-DM1
v6363

FIG. 31

C



D

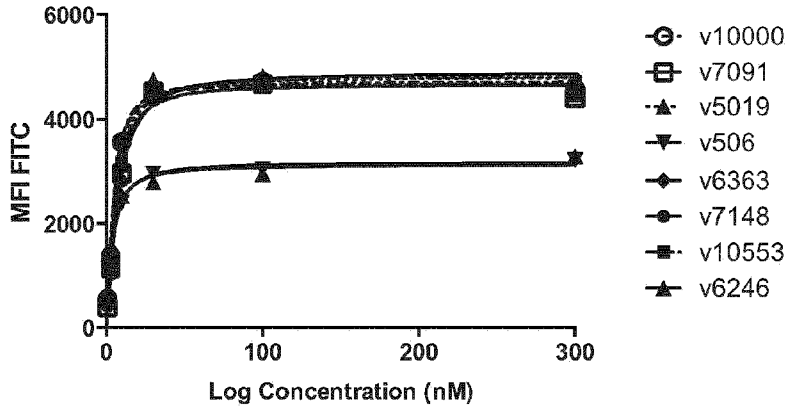


FIG. 31 (Cont'd...)

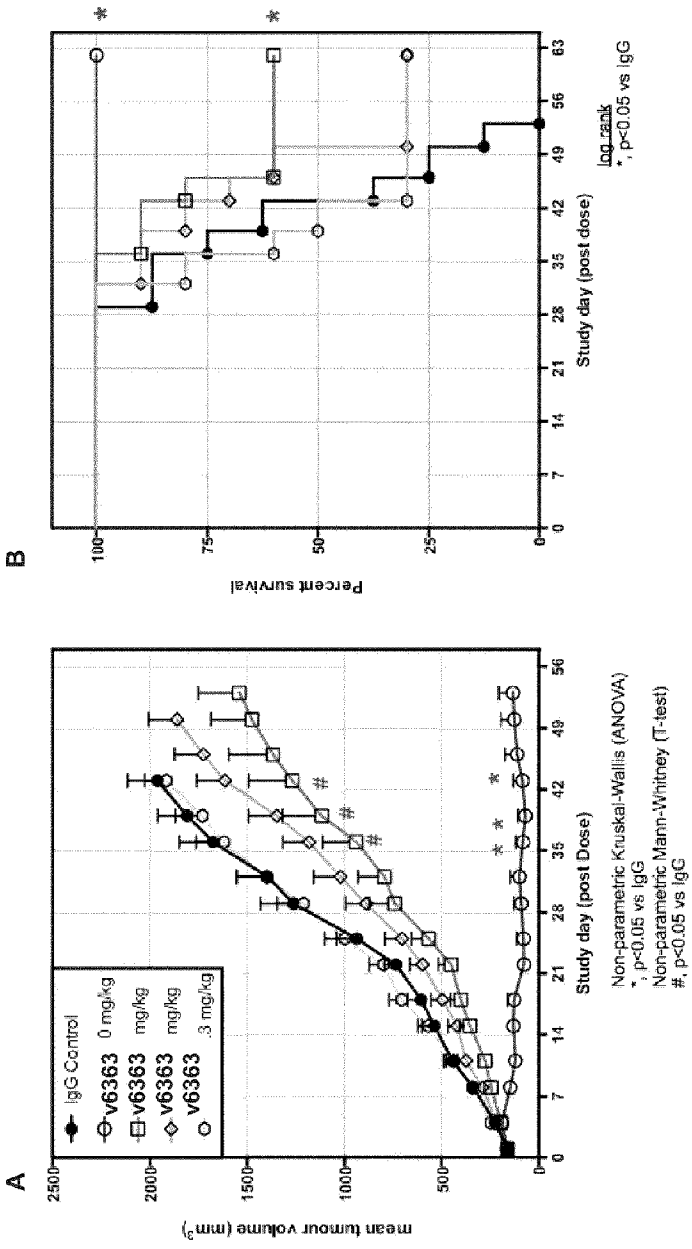

A
mean tumor volume (mm3)
2500
2000
1500
1000
500
0
0
7
14
21
28
35
42
49
56
Study day (post Dose)
IgG Control
v6363 0 mg/kg
v6363 mg/kg
v6363 mg/kg
v6363 .3 mg/kg
Non-parametric Kruskal-Wallis (ANOVA)
*, p<0.05 vs IgG
Non-parametric Mann-Whitney (T-test)
#, p<0.05 vs IgG
B
Percent survival
100
75
50
25
0
0
7
14
21
28
35
42
49
56
63
Study day (post dose)
log rank
*, p<0.05 vs IgG

FIG. 32

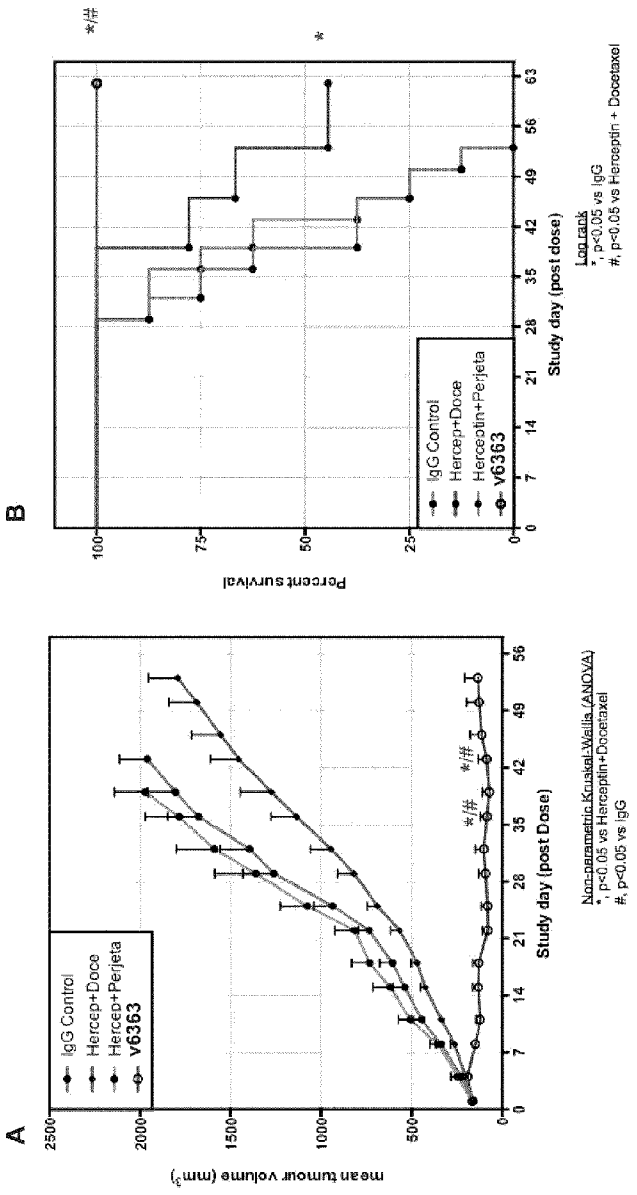

A
mean tumour volume (mm³)
2500
2000
1500
1000
500
0
0 7 14 21 28 35 42 49 56
Study day (post Dose)
IgG Control
Hercep+Doce
Hercep+Perjeta
v6363
*/#
*/#
Non-parametric Kruskal-Wallis (ANOVA)
*, p<0.05 vs Herceptin+Docetaxel
#, p<0.05 vs IgG
B
Percent survival
100
75
50
25
0
0 7 14 21 28 35 42 49 56 63
Study day (post dose)
IgG Control
Hercep+Doce
Herceptin+Perjeta
v6363
*/#
*
Log rank
*, p<0.05 vs IgG
#, p<0.05 vs Herceptin + Docetaxel

FIG. 33

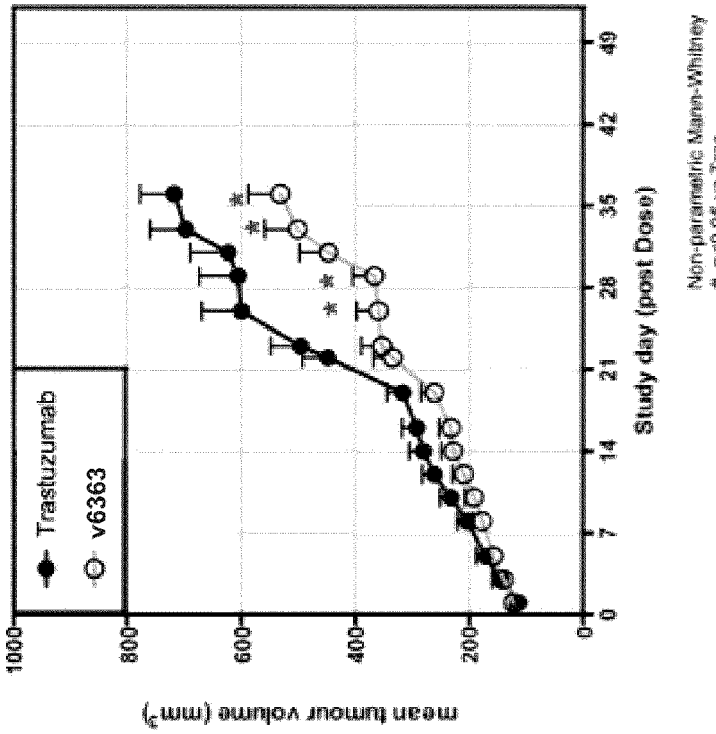

Trastuzumab
v6363
mean tumour volume (mm³)
Study day (post Dose)
0
7
14
21
28
35
42
49
200
400
600
800
1000
Non-parametric Mann-Whitney
#, p<0.05 vs Tras

FIG. 34

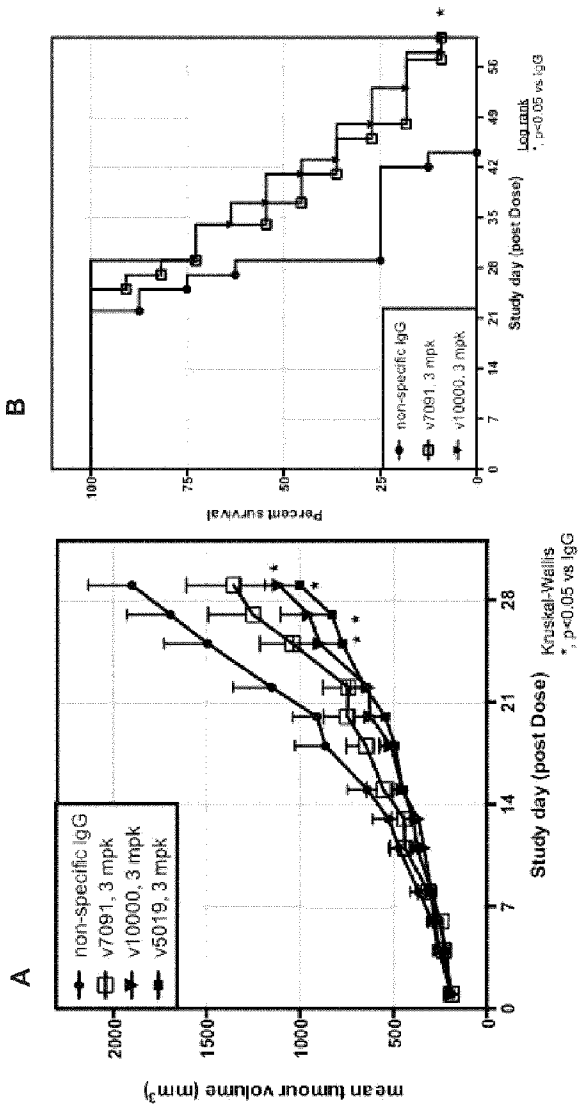

A
mean tumour volume (mm³)
2000
1500
1000
500
0
0
7
14
21
28
Study day (post Dose)
non-specific IgG
v7091, 3 mpk
v10000, 3 mpk
v5019, 3 mpk
Kruskal-Wallis
*, p<0.05 vs IgG
B
Percent survival
100
75
50
25
0
0
7
14
21
28
35
42
49
56
Study day (post Dose)
non-specific IgG
v7091, 3 mpk
v10000, 3 mpk
Log rank
*, p<0.05 vs IgG

FIG. 35

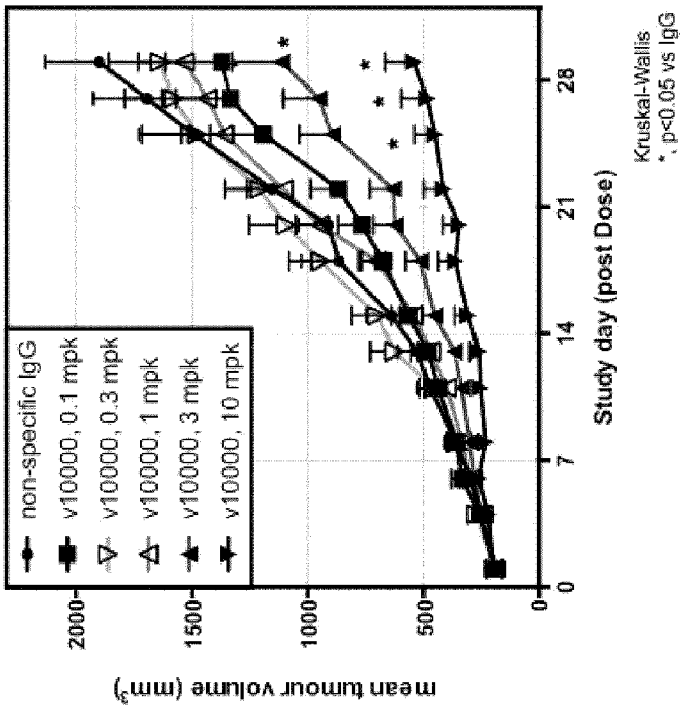
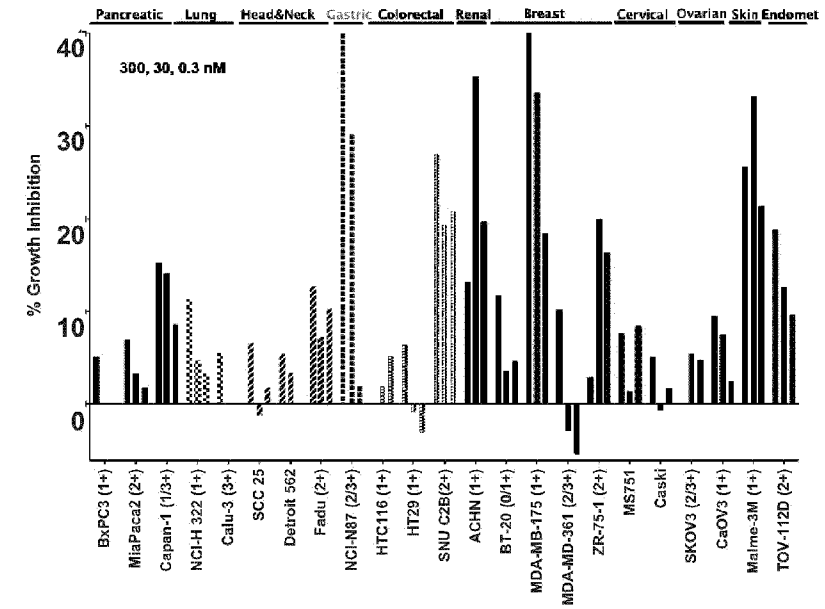

mean tumour volume (mm³)
2000
1500
1000
500
0
0
7
14
21
28
Study day (post Dose)
non-specific IgG
v10000, 0.1 mpk
v10000, 0.3 mpk
v10000, 1 mpk
v10000, 3 mpk
v10000, 10 mpk
Kruskal-Wallis
*, p<0.05 vs IgG

FIG. 36

A


Pancreatic
Lung
Head&Neck
Gastric
Colorectal
Renal
Breast
Cervical
Ovarian
Skin
Endomet
300, 30, 0.3 nM
% Growth Inhibition
40
30
20
10
0
BxPC3 (1+)
MiaPaca2 (2+)
Capan-1 (1/3+)
NCI-H 322 (1+)
Calu-3 (3+)
SCC 25
Detroit 562
Fadu (2+)
NCI-N87 (2/3+)
HTC116 (1+)
HT29 (1+)
SNU C2B(2+)
ACHN (1+)
BT-20 (0/1+)
MDA-MB-175 (1+)
MDA-MD-361 (2/3+)
ZR-75-1 (2+)
MS751
Caski
SKOV3 (2/3+)
CaOV3 (1+)
Malme-3M (1+)
TOV-112D (2+)

B

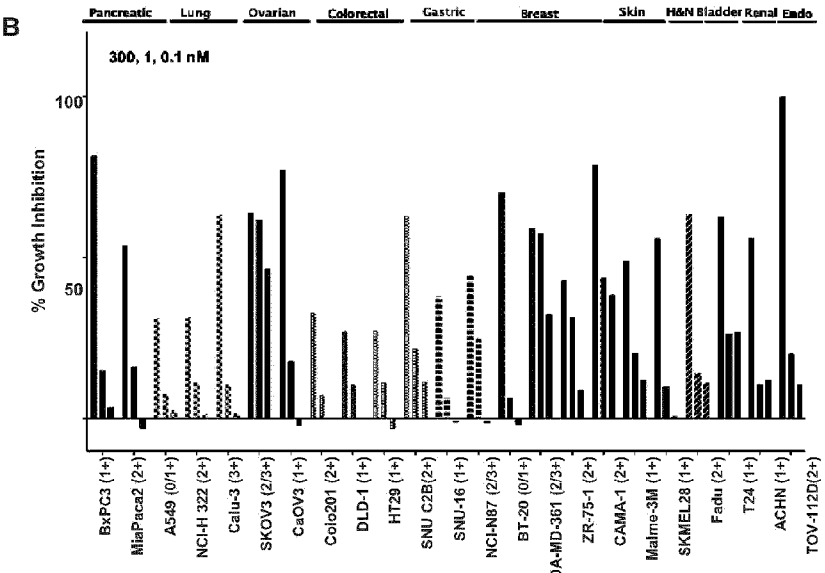

Pancreatic
Lung
Ovarian
Colorectal
Gastric
Breast
Skin
H&N
Bladder
Renal
Endo
300, 1, 0.1 nM
% Growth Inhibition
100
50
BxPC3 (1+)
MiaPaca2 (2+)
A549 (0/1+)
NCI-H 322 (2+)
Calu-3 (3+)
SKOV3 (2/3+)
CaOV3 (1+)
Colo201 (2+)
DLD-1 (1+)
HT29 (1+)
SNU C2B(2+)
SNU-16 (1+)
NCI-N87 (2/3+)
BT-20 (0/1+)
MDA-MD-361 (2/3+)
ZR-75-1 (2+)
CAMA-1 (2+)
Malme-3M (1+)
SKMEL28 (1+)
Fadu (2+)
T24 (1+)
ACHN (1+)
TOV-112D(2+)

FIG. 37

Cell Line	Cell Line Description	IHC Receptor			V1000	V10553	Ref
		HER2	EGFR	HER3	activity	activity	
BxPC3	human pancreas adenocarcinoma	1	2	1	–	+	1,2,3
Capan-1	human pancreatic adenocarcinoma	1/3	1	0	–	+	1,4,5
MiaPaca2	human pancreas carcinoma	2	1/2	0	–	+	3,4
SW 1990	human pancreas adenocarcinoma, metastatic	2	1	0	-	+	2,4
Panc1	human pancreas carcinoma	1	1/2		-	+	4
A549	human lung carcinoma	0/1	1		-	+	6,7
Calu-3	human lung adenocarcinoma	3	2	1	–	+	6,8,9
Calu-6	human lung anaplastic carcinoma	0			-	+	6
NCI-H2126	human adenocarcinoma; non-small cell lung cancer				-	+	10
NCI-H322	human Caucasian bronchioalveolar carcinoma	2	2		–	+	6,7,11
Detroit 562	human pharyngeal carcinoma				–	+	12
SCC-15	human tongue squamous cell carcinoma		2		-	+	12
SCC-25	human tongue squamous cell carcinoma		2		–	+	12
FaDu	squamous cell carcinoma, pharynx	2	2		–	+	
Colo201	human colorectal adenocarcinoma	2	1		-	+	13
DLD-1	human colorectal adenocarcinoma, Dukes' type C	1	0/1		-	+	14
HCT116	human colorectal carcinoma	1	0/1		-	+	14
HT 29	human colorectal adenocarcinoma;	1	0				14
SNU-C2B	humancecum colorectal carcinoma	2*			–	+	
SNU-1	human gastric carcinoma	0			-	+	15
SNU-16	human gastric carcinoma	1			-	+	15
NCI-N87	human gastric carcinoma	3	2	1	–	+	15
MDAMB175	human breast ductal carcinoma, ER+	1	1	0/1	–	+	8,16
MDAMB361	human breast adenocarcinoma, ER+, HER2 amp	2/3	1	1	–	+	9,15,17
ZR-75-1	human breast duct epithelial ductal carcinoma, ER+ luminal A	2	1	1	-	+	9
BT-20	human breast carcinoma, Basal A TNBC	0/1	2	1	–	+	18
BT549	human breast ductal carcinoma, Basal B, Mesenchymal-like TNBC, ER-	0	0/1	0	-	+	18
CAMA-1	human breast adenocarcinoma, ER+	2	0	1	-	+	
MDAMB453	human breast metastatic carcinoma, ER-, HER2amp luminal A TNBC	0	0/1	0	-	+	18
T47D	human breast ductal carcinoma, ER+	1	0	1	-	+	19
SK-UT-1	human uterus mesodermal tumor (mixed) grade III				-	+	
TOV-112D	human primary malignant adenocarcinoma; endometrioid carcinoma	2	1	2	–	+	20
A431	human skin epidermoid carcinoma	1	3		-	+	21
Malme-3M	human malignant melanoma, metastatic lung	1	1	1	–	+	9, 22
SKMEL28	human malignant melanoma	1	0		-	+	22
Caski	human cervix carcinoma	1				+	23
MS751	human cervix epidermoid carcinoma				–	+	
T24	human urinary bladder carcinoma	1	0		-	+	19,21,24
ACHN	human renal cell adenocarcinoma	1	2	0/1	–	+	9, 25
CaOV3	human ovary adenocarcinoma	1	1		–	+	26
Ovcar-3	human ovary adenocarcinoma	1/2	2	2	-	+	20, 26
SKOV3	human ovary adenocarcinoma	2/3	2	0/1	-	+	

FIG. 38

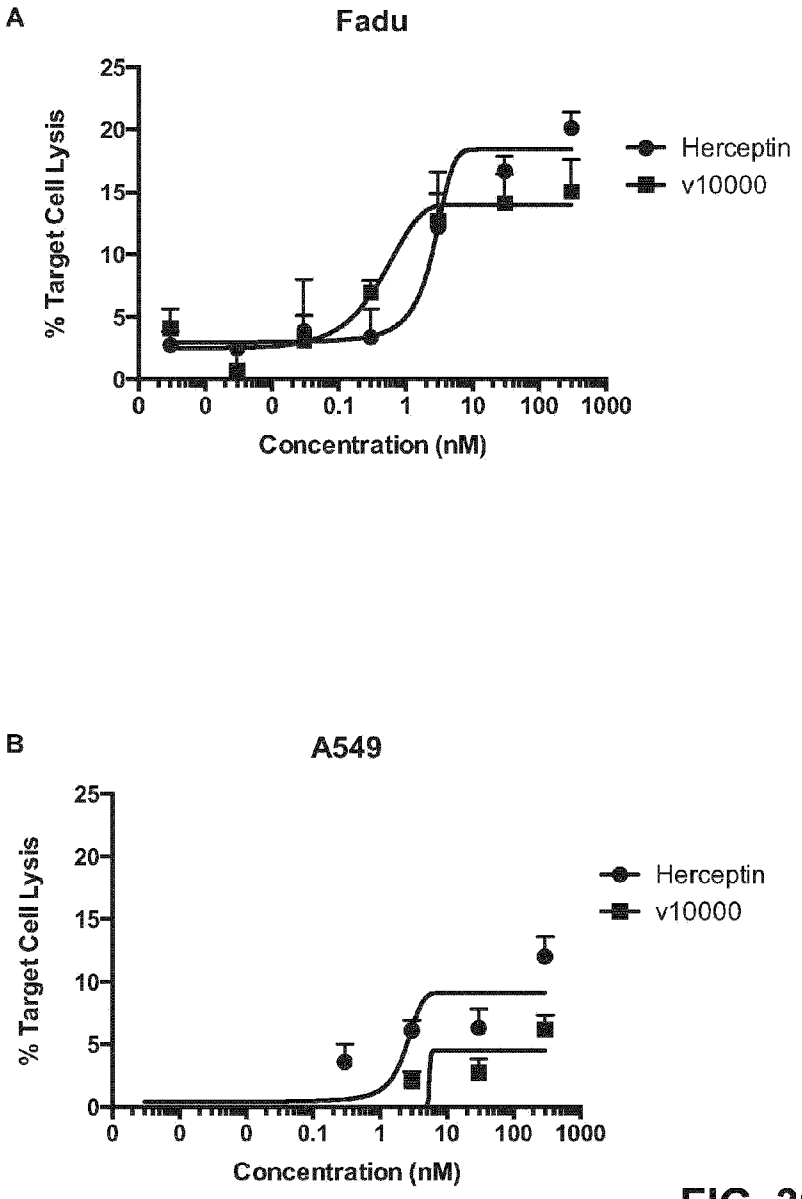
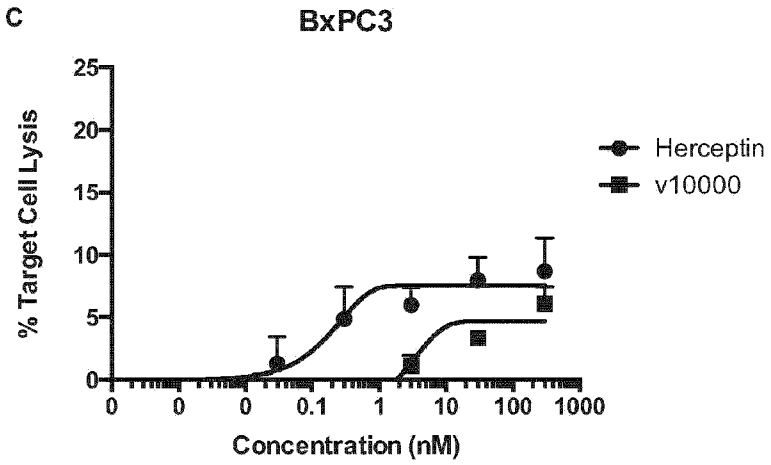

A
Fadu
% Target Cell Lysis
25
20
15
10
5
0
0 0 0 0.1 1 10 100 1000
Concentration (nM)
Herceptin
v10000
B
A549
% Target Cell Lysis
25
20
15
10
5
0
0 0 0 0.1 1 10 100 1000
Concentration (nM)
Herceptin
v10000

FIG. 39


C
BxPC3
% Target Cell Lysis
25
20
15
10
5
0
0 0 0 0.1 1 10 100 1000
Concentration (nM)
Herceptin
v10000

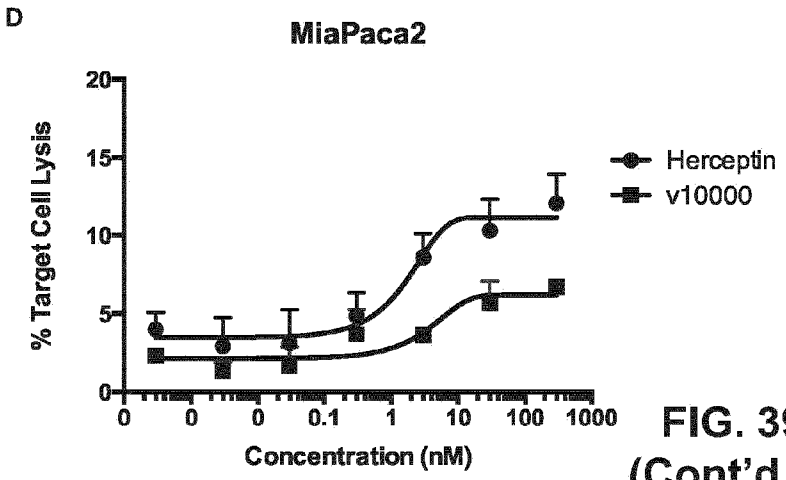

D
MiaPaca2
% Target Cell Lysis
20
15
10
5
0
0 0 0 0.1 1 10 100 1000
Concentration (nM)
Herceptin
v10000

FIG. 39
(Cont'd...)

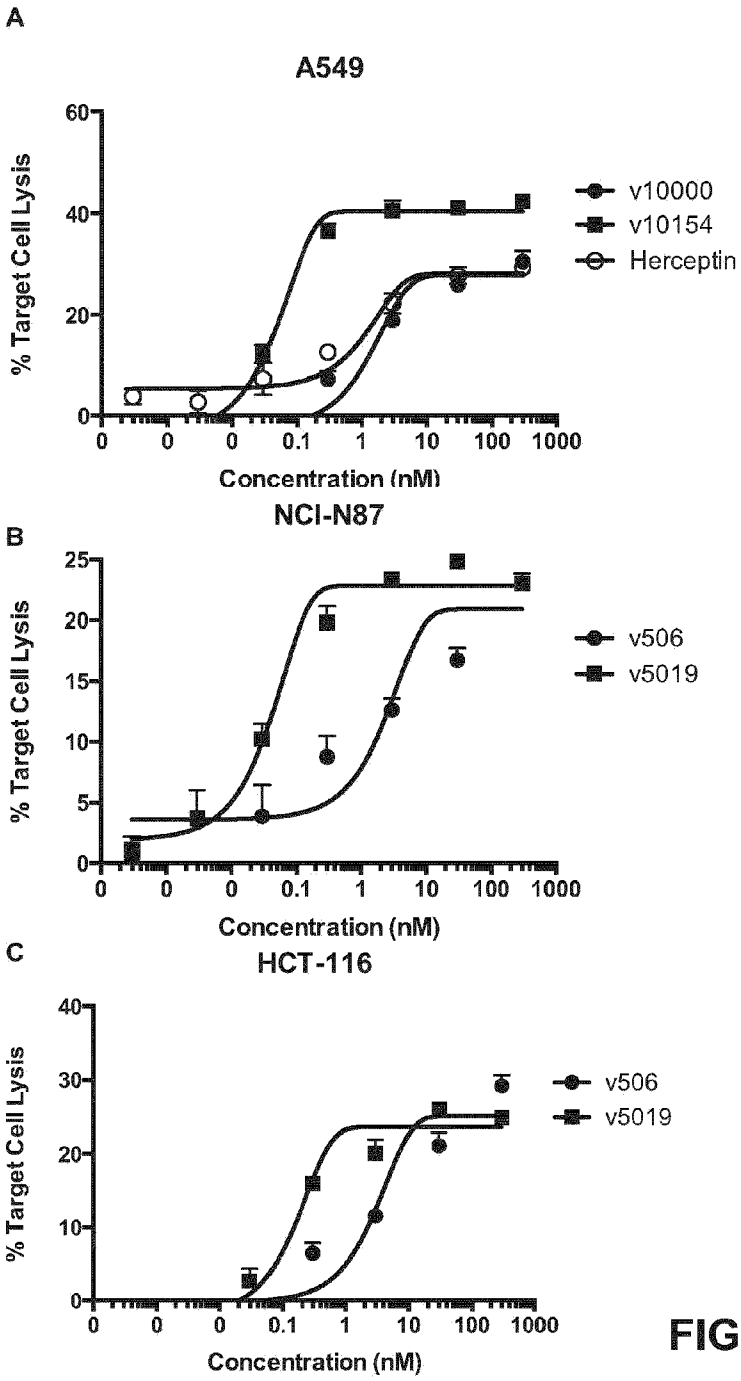
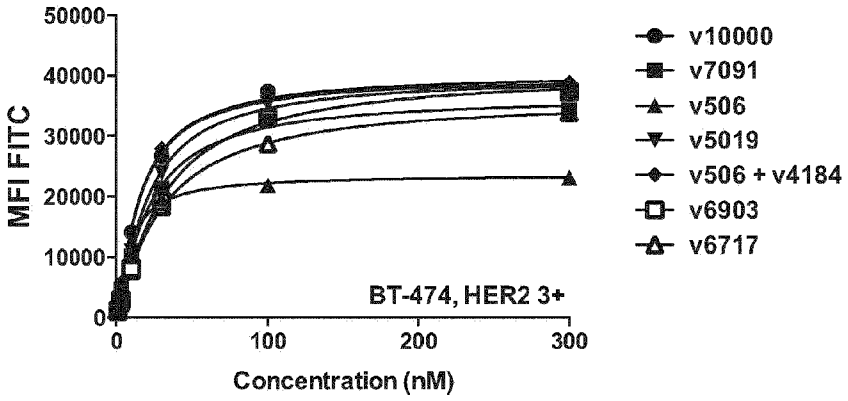

A
A549
% Target Cell Lysis
60
40
20
0
0 0 0 0.1 1 10 100 1000
Concentration (nM)
v10000
v10154
Herceptin
B
NCI-N87
% Target Cell Lysis
25
20
15
10
5
0
0 0 0 0.1 1 10 100 1000
Concentration (nM)
v506
v5019
C
HCT-116
% Target Cell Lysis
40
30
20
10
0
0 0 0 0.1 1 10 100 1000
Concentration (nM)
v506
v5019

FIG. 40

A


50000
40000
30000
20000
10000
0
MFI FITC
BT-474, HER2 3+
0
100
200
300
Concentration (nM)
v10000
v7091
v506
v5019
v506 + v4184
v6903
v6717

B

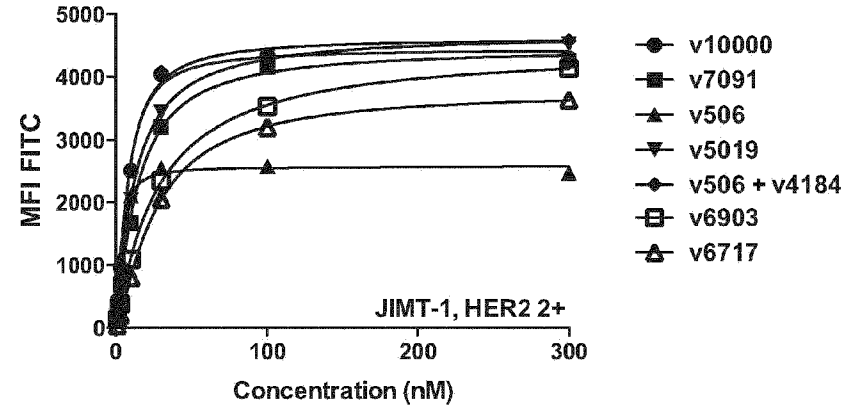
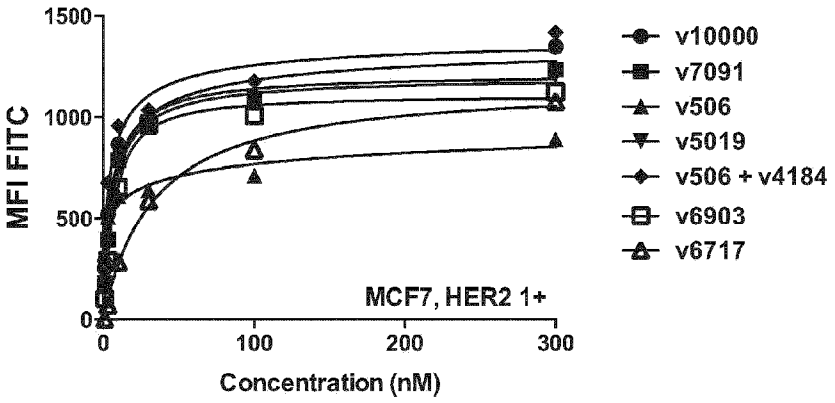

5000
4000
3000
2000
1000
0
MFI FITC
JIMT-1, HER2 2+
0
100
200
300
Concentration (nM)
v10000
v7091
v506
v5019
v506 + v4184
v6903
v6717

FIG. 41

C



D

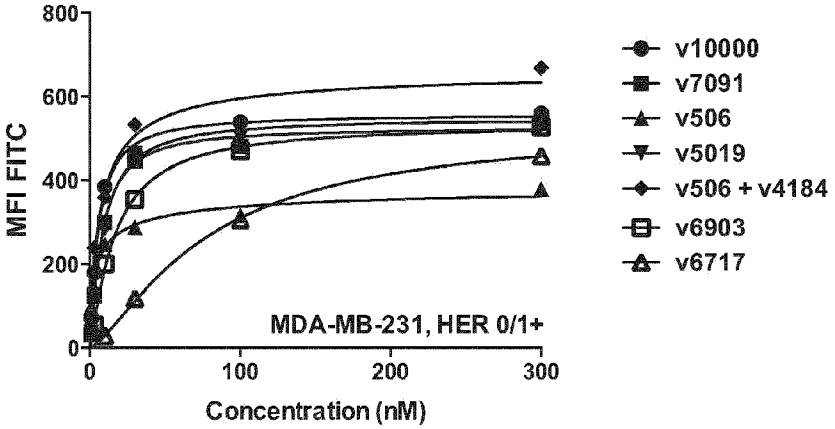


FIG. 41 (Cont'd...)

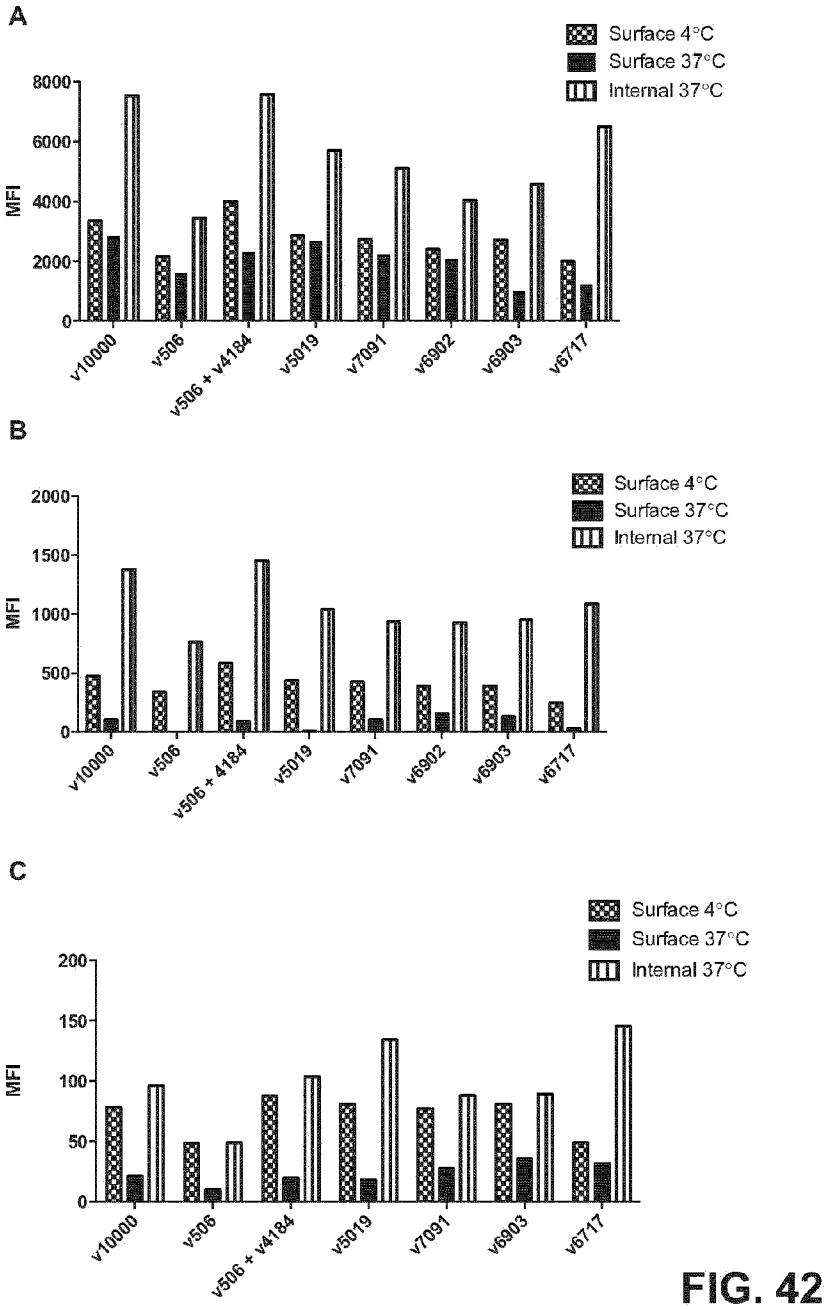
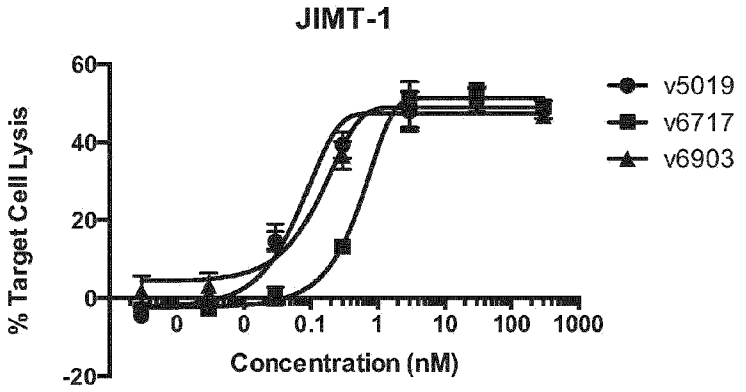

A
Surface 4°C
Surface 37°C
Internal 37°C
MFI
8000
6000
4000
2000
0
v10000
v506
v506 + v4184
v5019
v7091
v6902
v6903
v6717
B
Surface 4°C
Surface 37°C
Internal 37°C
MFI
2000
1500
1000
500
0
v10000
v506
v506 + 4184
v5019
v7091
v6902
v6903
v6717
C
Surface 4°C
Surface 37°C
Internal 37°C
MFI
200
150
100
50
0
v10000
v506
v506 + v4184
v5019
v7091
v6903
v6717

FIG. 42

A


JIMT-1
% Target Cell Lysis
60
40
20
0
-20
0
0
0.1
1
10
100
1000
Concentration (nM)
v5019
v6717
v6903

B

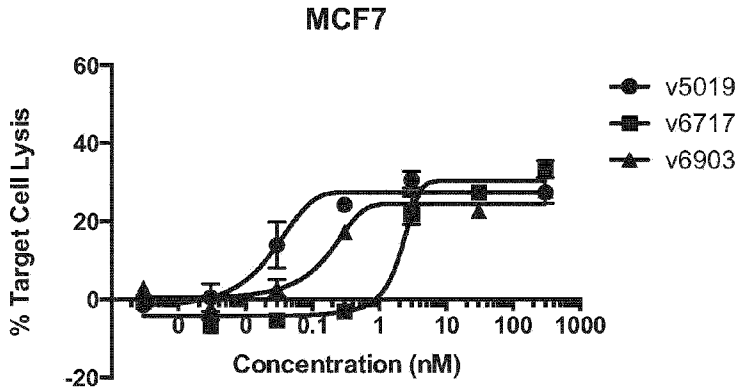

MCF7
% Target Cell Lysis
60
40
20
0
-20
0
0
0.1
1
10
100
1000
Concentration (nM)
v5019
v6717
v6903

FIG. 43

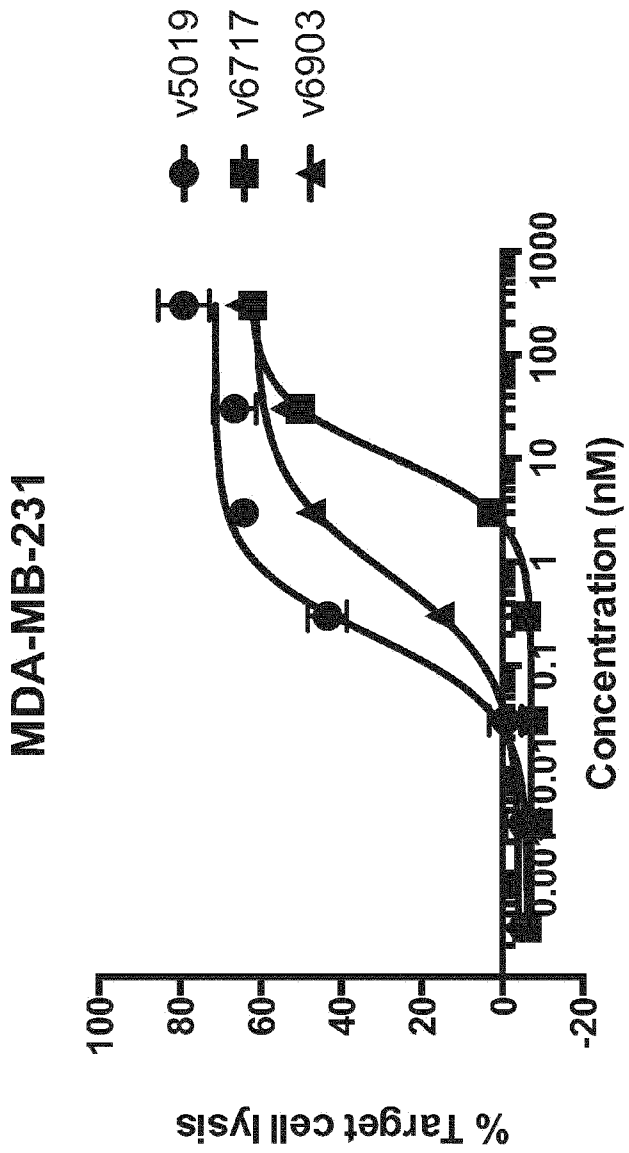

MDA-MB-231
v5019
v6717
v6903
% Target cell lysis
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
1000
Concentration (nM)

FIG. 43C


Control
+ 10ng/ml rhHRG-β1
+ 50ng/ml hEGF
% Surviving Fraction
Relative to Control Mock
160
140
120
100
80
60
40
20
0
mock
V506
V5019
V10000
V7091
V506 + V4184
V6903
V6717

FIG. 44

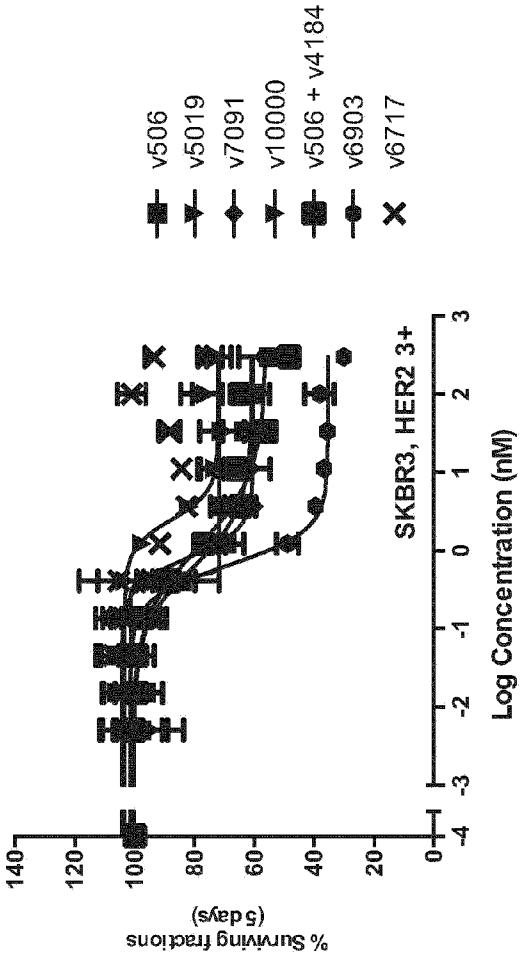

v506
v5019
v7091
v10000
v506 + v4184
v6903
v6717
SKBR3, HER2 3+
% Surviving fractions (5 days)
Log Concentration (nM)
140
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
3

FIG. 45

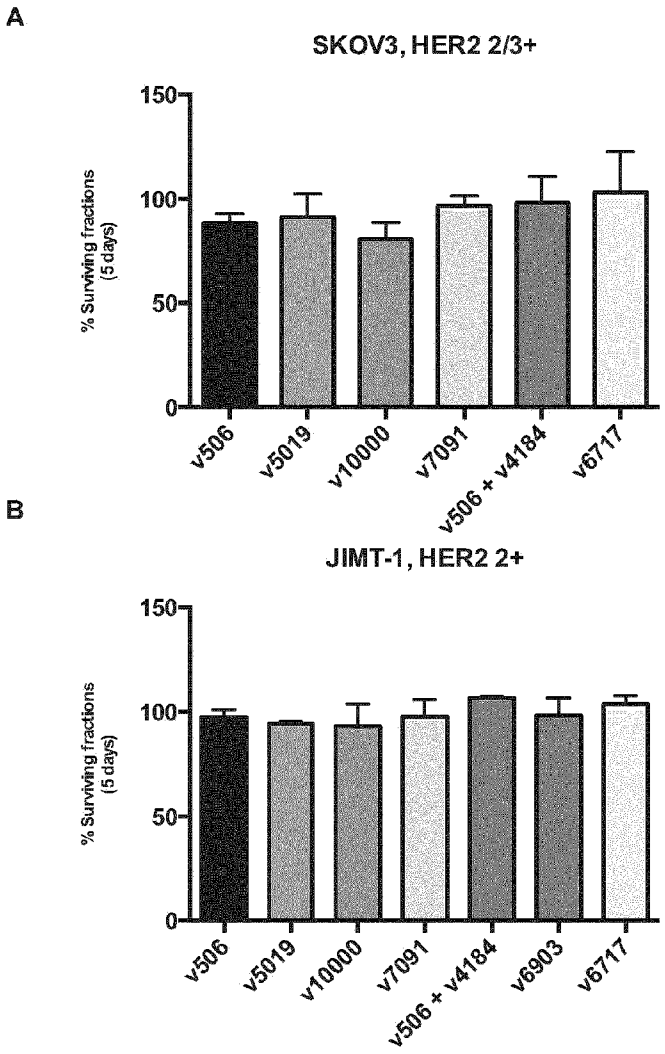

A
SKOV3, HER2 2/3+
% Surviving fractions (5 days)
150
100
50
0
v506
v5019
v10000
v7091
v506 + v4184
v6717
B
JIMT-1, HER2 2+
% Surviving fractions (5 days)
150
100
50
0
v506
v5019
v10000
v7091
v506 + v4184
v6903
v6717

FIG. 46

C

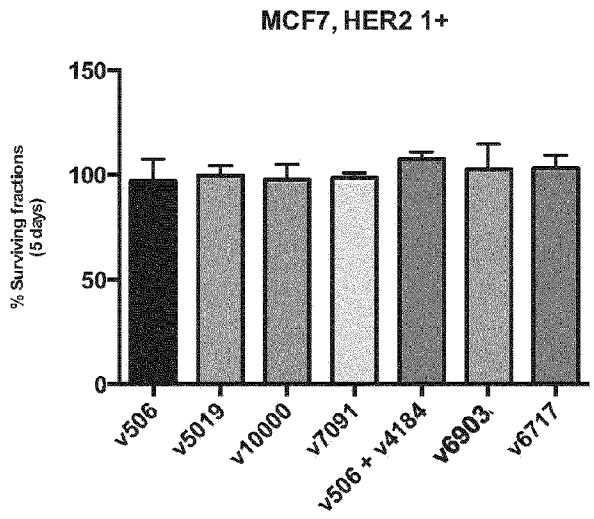
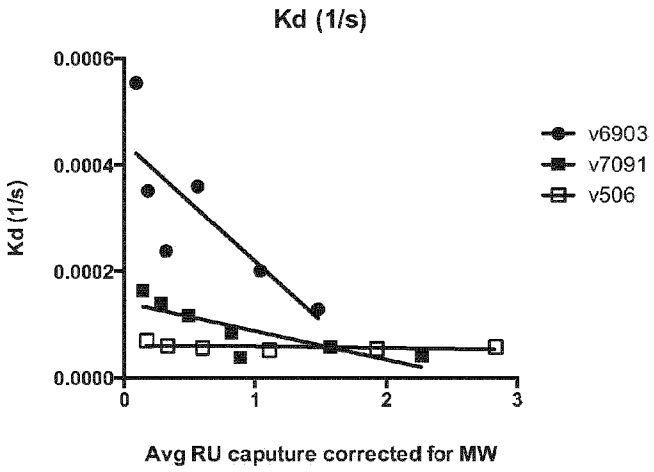


FIG. 46 (Cont'd...)

A


Kd (1/s)
0.0006
0.0004
0.0002
0.0000
Kd (1/s)
0
1
2
3
Avg RU caputure corrected for MW
v6903
v7091
v506

B

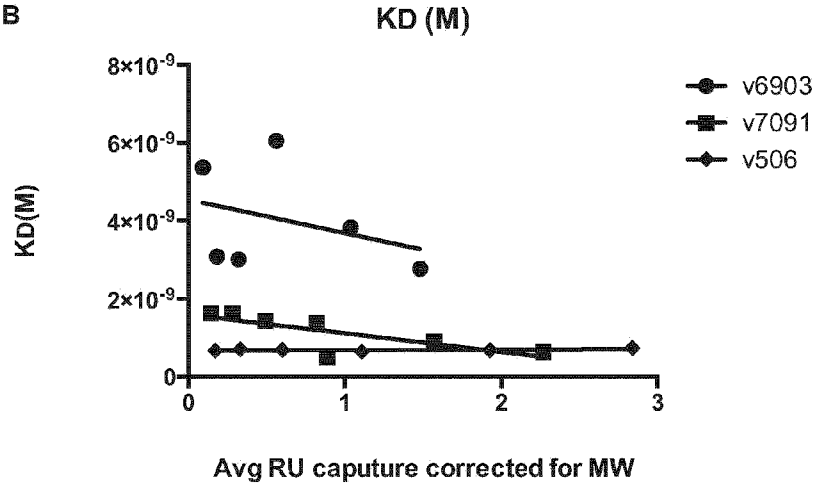

KD (M)
8×10-9
6×10-9
4×10-9
2×10-9
0
KD(M)
0
1
2
3
Avg RU caputure corrected for MW
v6903
v7091
v506

FIG. 47

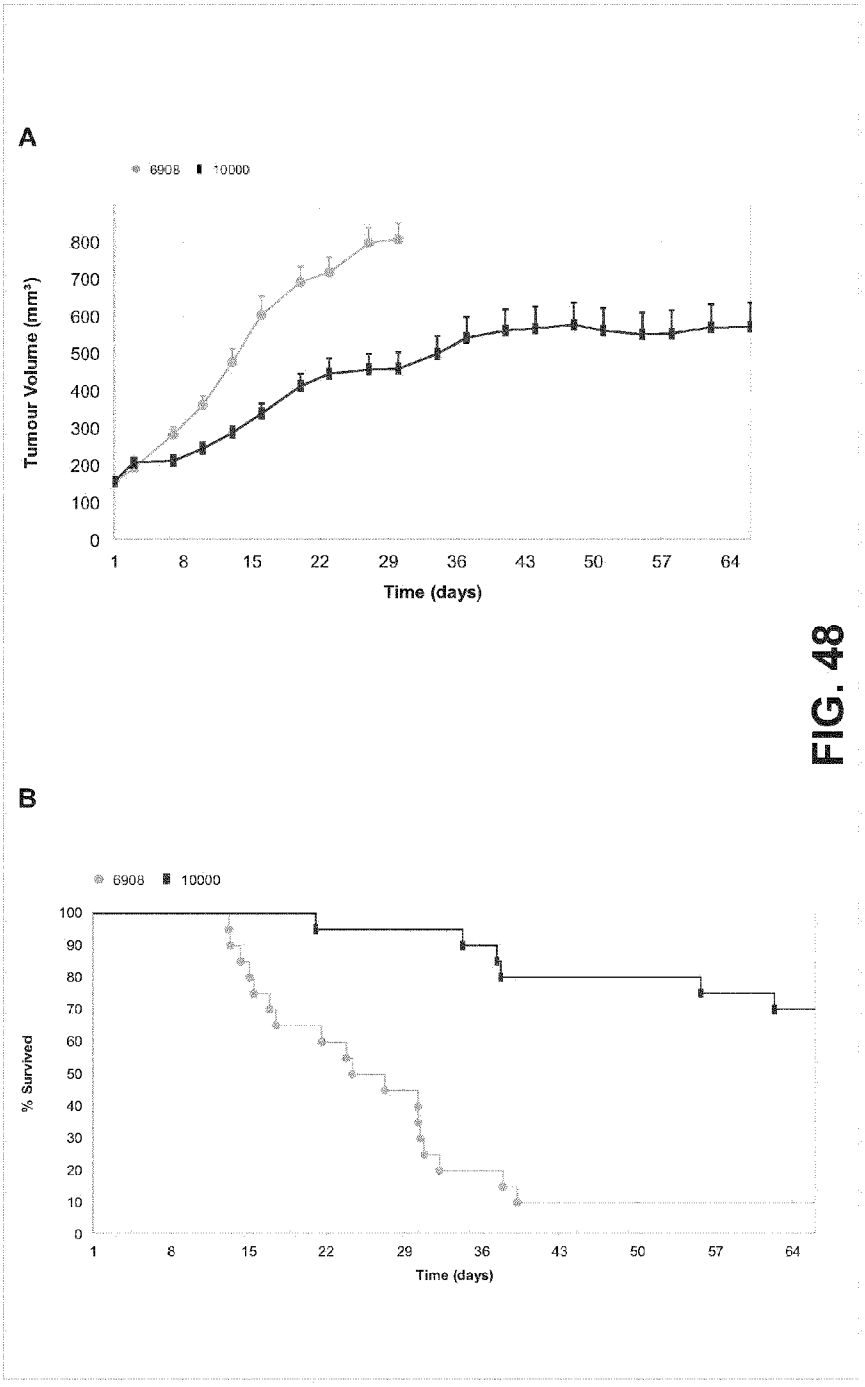

A
6908
10000
Tumour Volume (mm³)
800
700
600
500
400
300
200
100
0
1
8
15
22
29
36
43
50
57
64
Time (days)
B
6908
10000
% Survived
100
90
80
70
60
50
40
30
20
10
0
1
8
15
22
29
36
43
50
57
64
Time (days)

FIG. 48

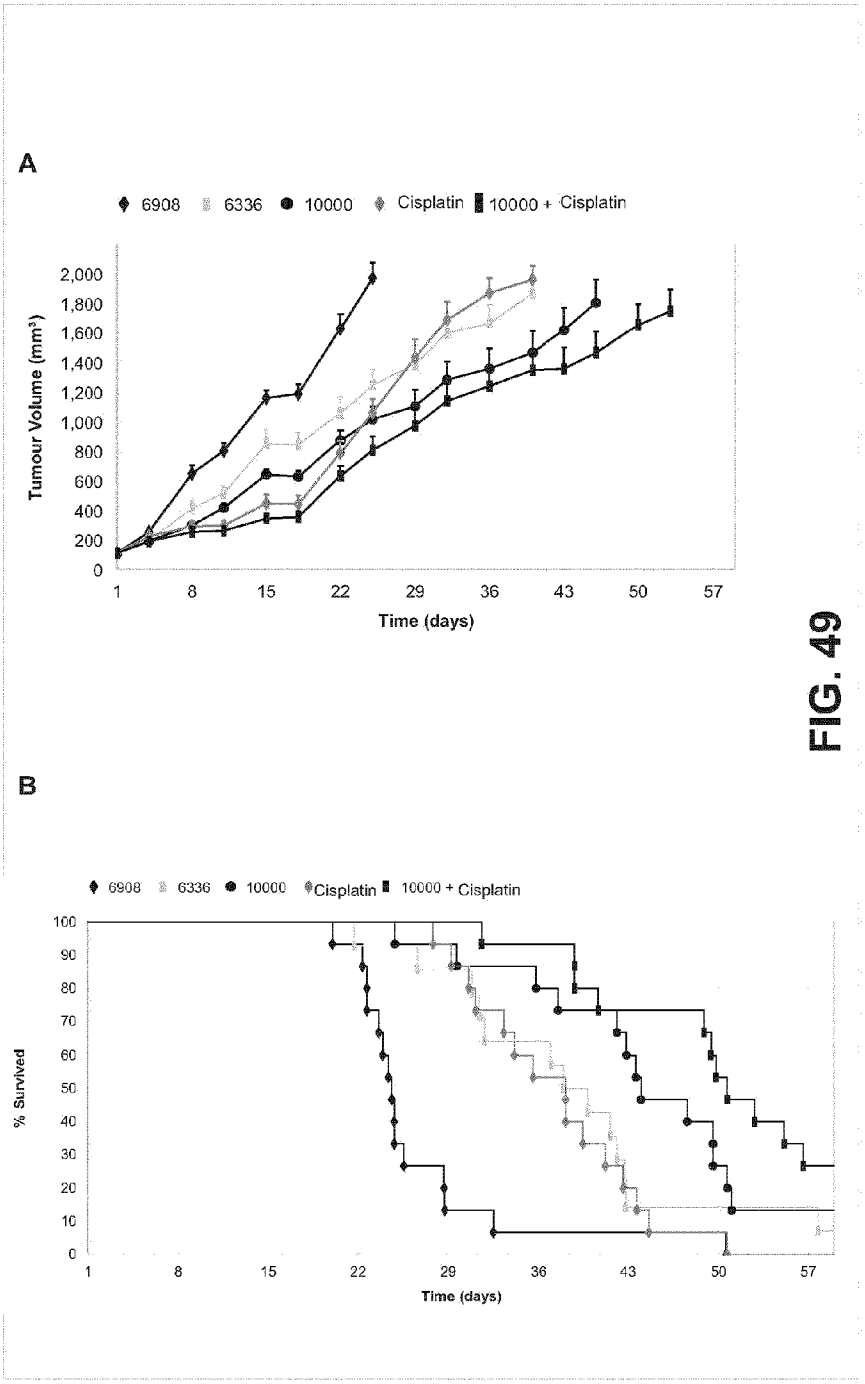

A
6908
6336
10000
Cisplatin
10000 + Cisplatin
Tumour Volume (mm³)
2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0
1
8
15
22
29
36
43
50
57
Time (days)
B
6908
6336
10000
Cisplatin
10000 + Cisplatin
% Survived
100
90
80
70
60
50
40
30
20
10
0
1
8
15
22
29
36
43
50
57
Time (days)

FIG. 49

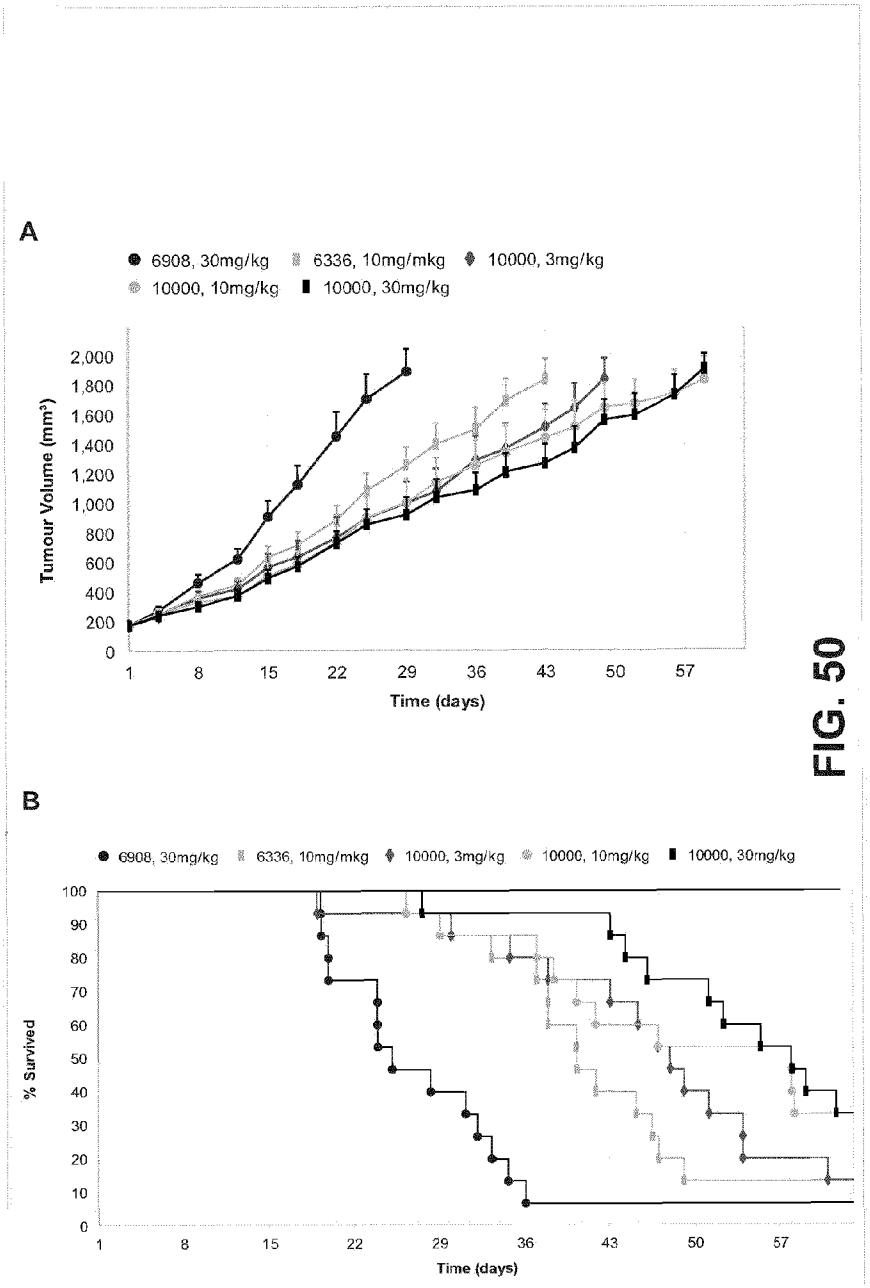

A
6908, 30mg/kg
6336, 10mg/mkg
10000, 3mg/kg
10000, 10mg/kg
10000, 30mg/kg
Tumour Volume (mm³)
2,000
1,800
1,600
1,400
1,200
1,000
800
600
400
200
0
1
8
15
22
29
36
43
50
57
Time (days)
B
6908, 30mg/kg
6336, 10mg/mkg
10000, 3mg/kg
10000, 10mg/kg
10000, 30mg/kg
% Survived
100
90
80
70
60
50
40
30
20
10
0
1
8
15
22
29
36
43
50
57
Time (days)

FIG. 50

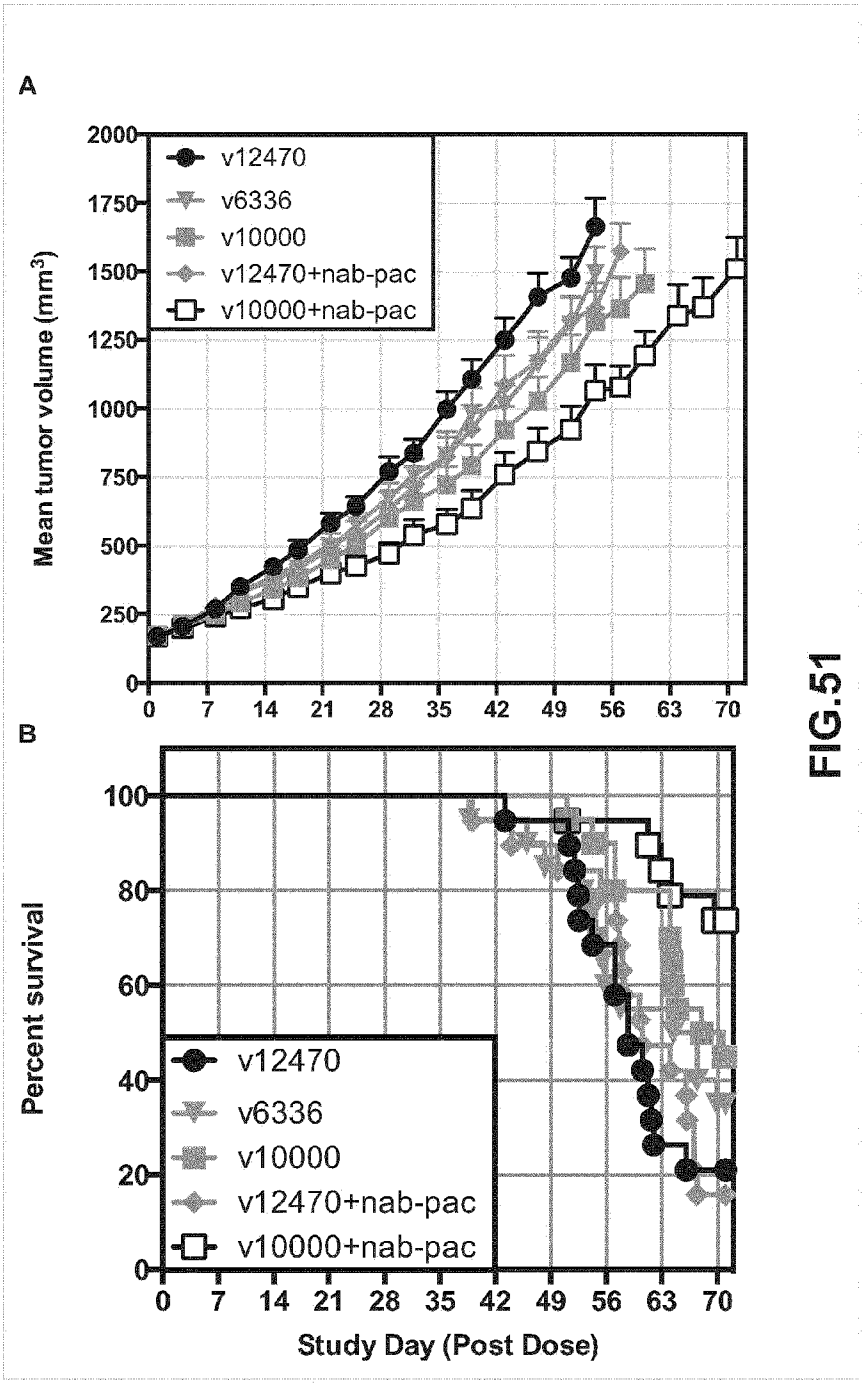

A
Mean tumor volume (mm3)
2000
1750
1500
1250
1000
750
500
250
0
0 7 14 21 28 35 42 49 56 63 70
v12470
v6336
v10000
v12470+nab-pac
v10000+nab-pac
B
Percent survival
100
80
60
40
20
0
0 7 14 21 28 35 42 49 56 63 70
Study Day (Post Dose)
v12470
v6336
v10000
v12470+nab-pac
v10000+nab-pac

FIG.51

A

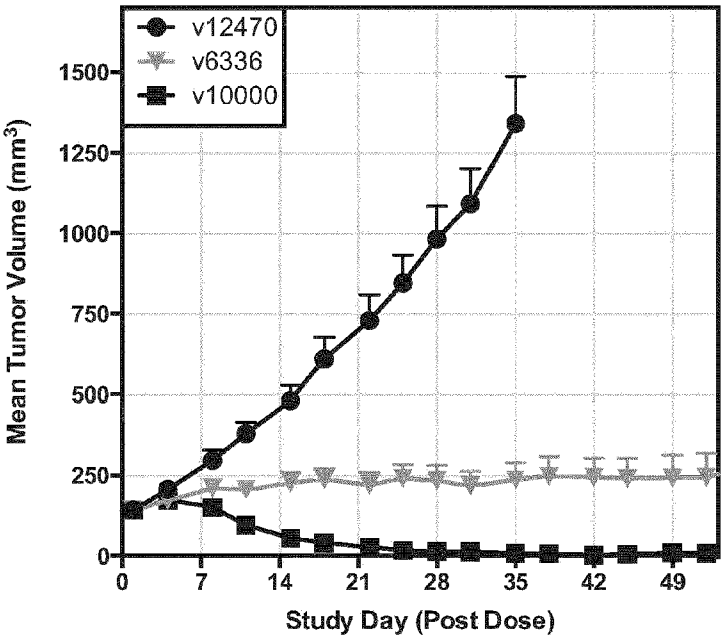

v12470
v6336
v10000
Mean Tumor Volume (mm3)
1500
1250
1000
750
500
250
0
0
7
14
21
28
35
42
49
Study Day (Post Dose)

FIG. 52

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.