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(54) **Title:** BISPECIFIC ANTIGEN-BINDING MOLECULES AND METHODS OF USE

(57) **Abstract:** The present invention provides bispecific antigen-binding molecules having a monovalent arm specific to a first target antigen (e.g., a T cell antigen, such as CD3) and a bivalent arm specific for a second target antigen (e.g., a tumor antigen, such as HER2). Bispecific antigen-binding molecules are useful in the treatment of disorders, such as cancer (e.g., HER2-positive cancer). The invention also features methods of producing bispecific antigen-binding molecules, methods of treating disorders using bispecific antigen-binding molecules, and compositions including bispecific antigen-binding molecules.

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BISPECIFIC ANTIGEN-BINDING MOLECULES AND METHODS OF USE

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 6, 2019, is named 50474-162WO2_Sequence_Listing_02.06.19_ST25 and is 293,645 bytes in size.

FIELD OF THE INVENTION

The present invention relates generally to bispecific antigen-binding molecules, compositions thereof, and methods for treating diseases, such as cancer.

BACKGROUND

Manipulating cell-to-cell contact between particular cell types in a patient represents a promising approach for treating various disease conditions. For example, bispecific antigen-binding molecules (e.g., bispecific antibodies) having two arms, each specific to a different target antigen, are under development for their ability to bring immune cells into contact with target cells. Such bispecific antibodies have shown promise in various disorders, such as cancer, in which potent immune-mediated killing of tumor target cells has been observed in clinical trials. To confer tumor-specificity, tumor-targeting arms of bispecific antibodies have been designed to target antigens that are overexpressed on tumor cells.

Existing bispecific antibodies can have several limitations, including short half-lives and toxicity to healthy tissues. Bispecific antibodies that rely on tumor cell overexpression of a target antigen often kill healthy, non-tumor cells that express normal levels of the antigen. Such on-target, off-tumor effects limit the therapeutic index of the bispecific antibody therapy by restraining the maximum dose tolerated by the subject. Thus, there is an unmet need in the field for the development of bispecific antigen-binding molecules (e.g., bispecific antibodies) with enhanced selectivity to a target cell or tissue.

SUMMARY OF THE INVENTION

The present invention relates to bispecific antigen-binding molecules having a monovalent arm and a bivalent arm (e.g., T cell-dependent bispecific (TDB) antibodies having a monovalent arm and a bivalent arm).

In one aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a first antigen-binding moiety, wherein the C-terminus of the first antigen-binding moiety is fused to the N-terminus of a first Fc subunit; (b) the bivalent arm comprises a second antigen-binding moiety and a third antigen-binding moiety, wherein the C-terminus of the third antigen-binding moiety is fused to the N-terminus of the second antigen-binding moiety, and the C-terminus of the second antigen-binding moiety is fused to an N-terminus of a second Fc subunit; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain, wherein the first antigen-binding moiety is capable of specific binding to a first target antigen, and the second antigen-binding moiety and the third antigen-binding moiety are each capable of specific binding to a second target cell antigen. In some embodiments, the first target antigen is an activating T cell antigen, such as CD3, and/or the second target cell antigen is a tumor antigen (e.g., HER2). In some embodiments, the tumor antigen is expressed on (a) a tumor cell in a subject and (b) at least one type of non-tumor cell in the subject.

In some embodiments, the ratio of tumor antigen copy number on the non-tumor cells to the tumor cells is from 1:10 to 1:1,000 (e.g., from 1:100 to 1:200). In some embodiments, the tumor antigen copy number is from 10^2 to 10^5 on a non-tumor cell and from 10^3 to 10^7 on a tumor cell.

In some embodiments, the tumor antigen copy number (e.g., average tumor antigen copy number, e.g., HER2 copy number, e.g., average HER2 copy number) on the tumor cells (e.g., HER2-positive tumor cells) is greater than 10^5 per cell (e.g., from 10^5 to 10^7 per cell, or from 10^5 to 10^6 per cell). In some embodiments, the tumor antigen copy number (e.g., average tumor antigen copy number, e.g., HER2 copy number, e.g., average HER2 copy number) is ≥ 200,000 per tumor cell (e.g., HER2-positive tumor cell). In some embodiments, the tumor antigen copy number (e.g., average tumor antigen copy number, e.g., HER2 copy number, e.g., average HER2 copy number) is ≤ 200,000 per non-tumor cell (e.g., non-cancerous cell, e.g., healthy cell).

In some embodiments, the monovalent binding affinity (K_D) of the second antigen-binding moiety and/or the third antigen-binding moiety is from 10 nM to 100 nM (e.g., from 20 nM to 90 nM, from 30 nM to 80 nM, from 40 nM to 60 nM, e.g., from 25 nM to 55 nM). In one embodiment, the monovalent binding affinity (K_D) of the second antigen-binding moiety and/or the third antigen-binding moiety is from 20 nM to 50 nM. In one embodiment, the monovalent binding affinity (K_D) of the second antigen-binding moiety and the third antigen-binding moiety is from 20 nM to 50 nM.

In some embodiments, the monovalent K_D of the second antigen-binding moiety is the K_D of the second antigen-binding moiety in Fab format measured using surface plasmon resonance (e.g., BIACORE® surface plasmon resonance) and wherein the monovalent K_D of the third antigen-binding moiety is the K_D of the third antigen-binding moiety in Fab format measured using surface plasmon resonance (e.g., BIACORE® surface plasmon resonance).

In some embodiments, the monovalent dissociation rate of the second antigen-binding moiety and/or the third antigen-binding moiety is from 10^{-3}/second to 10^{-1}/second (e.g., from 10^{-2}/second to 30^{-2}/second). In some embodiments, the monovalent K_D of the first antigen-binding moiety is from 10 nM to 100 nM (e.g., from 20 nM to 90 nM, from 20 nM to 80 nM, from 30 nM to 70 nM, or from 40 nM to 60 nM).

In any of the preceding embodiments, the first antigen-binding moiety may be a Fab molecule (Fab_A) comprising a variable heavy chain (VH_A) region and a variable light chain (VL_A) region; the second antigen-binding moiety is a Fab molecule (Fab_{B1}) comprising a variable heavy chain (VH_{B1}) region and a variable light chain (VL_{B1}) region; and/or the third antigen-binding moiety is a Fab molecule (Fab_{B2}) comprising a variable heavy chain (VH_{B2}) region and a variable light chain (VL_{B2}) region. Thus, in some embodiments, the first antigen-binding moiety is a Fab_A comprising a VH_A region and a VL_A region, the second antigen-binding moiety is a Fab_{B1} comprising a VH_{B1} region and a VL_{B1} region, and third antigen-binding moiety is a Fab_{B2} comprising a VH_{B2} region and a VL_{B2} region.

In some embodiments, the VH_{B1} and the VH_{B2} share at least 95% sequence identity. Additionally or alternatively, the VL_{B1} and the VL_{B2} share at least 95% sequence identity. Additionally or alternatively, the VH_{B1} and the VH_{B2} share at least 95% sequence identity and/or the VL_{B1} and the VL_{B2} share at least 95% sequence identity.

In some embodiments, the VH_{B1} region and/or the VH_{B2} region comprises an amino acid substitution at one, two, three, or all four residues of N54, D98, F100, and/or Y102, according to the Kabat numbering system. For example, the VH_{B1} region and/or the VH_{B2} region may feature an amino acid substitution at one, two, three, four, or all five of the following residues: N54E, D98A, D98T, F100A, and/or Y102V, according to the Kabat numbering system.

In some embodiments, the VL_{B1} region and/or the VL_{B2} region comprises an amino acid substitution at one, two, or all three residues of N30, Y55, and/or H91, according to the Kabat numbering system. For example, the VL_{B1} region and/or the VL_{B2} region may feature an amino acid substitution at one, two, or all three of the following residues: N30S, Y55E, and/or H91A.

In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17 (e.g., at least 96% sequence identity to SEQ ID NO: 17, at least 97% sequence identity to SEQ ID NO: 17, at least 98% sequence identity to SEQ ID NO: 17, at least 99% sequence identity to SEQ ID NO: 17, or 100% sequence identity to SEQ ID NO: 17). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 18, at least 98% sequence identity to SEQ ID NO: 18, at least 99% sequence identity to SEQ ID NO: 18, or 100% sequence identity to SEQ ID NO: 18). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17 (e.g., at least 96% sequence identity to SEQ ID NO: 17, at least 97% sequence identity to SEQ ID NO: 17, at least 98% sequence identity to SEQ ID NO: 17, at least 99% sequence identity to SEQ ID NO: 17, or 100% sequence identity to SEQ ID NO: 17); and the VL_{B1} region comprises an amino acid sequence having at least 95%

sequence identity to SEQ ID NO: 18 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 18, at least 98% sequence identity to SEQ ID NO: 18, at least 99% sequence identity to SEQ ID NO: 18, or 100% sequence identity to SEQ ID NO: 18). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 17; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17 (e.g., at least 96% sequence identity to SEQ ID NO: 17, at least 97% sequence identity to SEQ ID NO: 17, at least 98% sequence identity to SEQ ID NO: 17, at least 99% sequence identity to SEQ ID NO: 17, or 100% sequence identity to SEQ ID NO: 17). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 18, at least 98% sequence identity to SEQ ID NO: 18, at least 99% sequence identity to SEQ ID NO: 18, or 100% sequence identity to SEQ ID NO: 18). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13; and the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17 (e.g., at least 96% sequence identity to SEQ ID NO: 17, at least 97% sequence identity to SEQ ID NO: 17, at least 98% sequence identity to SEQ ID NO: 17, at least 99% sequence identity to SEQ ID NO: 17, or 100% sequence identity to SEQ ID NO: 17); and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 18, at least 98% sequence identity to SEQ ID NO: 18, at least 99% sequence identity to SEQ ID NO: 18, or 100% sequence identity to SEQ ID NO: 18). In some embodiments, the VH_{B2} region comprises the amino acid of SEQ ID NO: 17; and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the VL_{B1} region and/or the VL_{B2} region comprises an amino acid substitution at H91. For example, in some embodiments, the H91 residue is substituted with an amino acid having a nonpolar side chain. In some embodiments, the VL_{B1} region and/or the VL_{B2} region comprises the amino acid substitution of H91A. In some embodiments, the VL_{B1} region and/or the VL_{B2}

region comprises an amino acid substitution at Y55. For example, in some embodiments, the Y55 residue is substituted with an amino acid having an acidic side chain. In some embodiments, the VL_{B1} region and/or the VL_{B2} region comprises the amino acid substitution of Y55E. In some embodiments, the VH_{B1} region and/or the VH_{B2} region comprises an amino acid substitution at F100 and/or Y102. For example, in some embodiments, the F100 residue and/or the Y102 residue is substituted with an amino acid having a nonpolar side chain. In some embodiments, the VH_{B1} region and/or the VH_{B2} region comprises the amino acid substitution of F100A and/or Y102V.

In some embodiments, the VL_{B1} region and/or the VL_{B2} region comprises one or more liability fixed residues, e.g., one or more liability fixed residues comprising the amino acid substitution of N30S. Additionally or alternatively, the VH_{B1} region and/or the VH_{B2} region may feature one or more liability fixed residues, e.g., one or more liability fixed residues comprising one or more an amino acid substitutions selected from the group consisting of N54E, D98A, and D98T.

In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24 (e.g., at least 96% sequence identity to SEQ ID NO: 24, at least 97% sequence identity to SEQ ID NO: 24, at least 98% sequence identity to SEQ ID NO: 24, at least 99% sequence identity to SEQ ID NO: 24, or 100% sequence identity to SEQ ID NO: 24). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 24. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27 (e.g., at least 96% sequence identity to SEQ ID NO: 27, at least 97% sequence identity to SEQ ID NO: 27, at least 98% sequence identity to SEQ ID NO: 27, at least 99% sequence identity to SEQ ID NO: 27, or 100% sequence identity to SEQ ID NO: 27). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 27. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 H91A-1Fab-IgG TDB).

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24 (e.g., at least 96% sequence identity to SEQ ID NO: 24, at least 97% sequence identity to SEQ ID NO: 24, at least 98% sequence identity to SEQ ID NO: 24, at least 99% sequence identity to SEQ ID NO: 24, or 100% sequence identity to SEQ ID NO: 24); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27 (e.g., at least 96% sequence identity to SEQ ID NO: 27, at least 97% sequence identity to SEQ ID NO: 27, at least 98% sequence identity to SEQ ID NO: 27, at least 99% sequence identity to SEQ ID NO: 27, or 100% sequence identity to SEQ ID NO: 27). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 24; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 27. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 H91A-1Fab-IgG TDB).

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24 (e.g., at least 96% sequence identity to SEQ ID NO: 24, at least 97% sequence identity to SEQ ID NO: 24, at least 98% sequence identity to SEQ ID NO: 24, at least 99% sequence identity to SEQ ID NO: 24, or 100% sequence identity to SEQ ID NO: 24). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 24. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 27, at least 98% sequence identity to SEQ ID NO: 27, at least 99% sequence identity to SEQ ID NO: 27, or 100% sequence identity to SEQ ID NO: 27). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 27. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 H91A-1Fab-IgG TDB).

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20; and the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24 (e.g., at least 96% sequence identity to SEQ ID NO: 24, at least 97% sequence identity to SEQ ID NO: 24, at least 98% sequence identity to SEQ ID NO: 24, at least 99% sequence identity to SEQ ID NO: 24, or 100% sequence identity to SEQ ID NO: 24); and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27 (e.g., at least 96% sequence identity to SEQ ID NO: 27, at least 97% sequence identity to SEQ ID NO: 27, at least 98% sequence identity to SEQ ID NO: 27, at least 99% sequence identity to SEQ ID NO: 27, or 100% sequence identity to SEQ ID NO: 27). In some embodiments, the VH_{B2} region comprises the amino acid of SEQ ID NO: 24; and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 27. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 H91A-1Fab-IgG TDB).

In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33 (e.g., at least 96% sequence identity to SEQ ID NO: 33, at least 97% sequence identity to SEQ ID NO: 33, at least 98% sequence identity to SEQ ID NO: 33, at least 99% sequence identity to SEQ ID NO: 33, or 100% sequence identity to SEQ ID NO: 33). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 33. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25 (e.g., at least 96% sequence identity to SEQ ID NO: 25, at least 97% sequence identity to SEQ ID NO: 25, at least 98% sequence identity to SEQ ID NO: 25, at least 99% sequence identity to SEQ ID NO: 25, or 100% sequence identity to SEQ ID NO: 25). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 25. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 D98A.F100A.Y102V-1Fab-IgG TDB).

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33 (e.g., at least 96% sequence identity to SEQ ID NO: 33, at least 97% sequence identity to SEQ ID NO: 33, at least 98% sequence identity to SEQ ID NO: 33, at least 99% sequence identity to SEQ ID NO: 33, or 100% sequence identity to SEQ ID NO: 33); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25 (e.g., at least 96% sequence identity to SEQ ID NO: 25, at least 97% sequence identity to SEQ ID NO: 25, at least 98% sequence identity to SEQ ID NO: 25, at least 99% sequence identity to SEQ ID NO: 25, or 100% sequence identity to SEQ ID NO: 25). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 33; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 25. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 D98A.F100A.Y102V-1Fab-IgG TDB).

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33 (e.g., at least 96% sequence identity to SEQ ID NO: 33, at least 97% sequence identity to SEQ ID NO: 33, at least 98% sequence identity to SEQ ID NO: 33, at least 99% sequence identity to SEQ ID NO: 33, or 100% sequence identity to SEQ ID NO: 33). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 33. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25 (e.g., at least 96% sequence identity to SEQ ID NO: 25, at least 97% sequence identity to SEQ ID NO: 25, at least 98% sequence identity to SEQ ID NO: 25, at least 99% sequence identity to SEQ ID NO: 25, or 100% sequence identity to SEQ ID NO: 25). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 25. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 D98A.F100A.Y102V-1Fab-IgG TDB).

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32; and the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33 (e.g., at least 96% sequence identity to SEQ ID NO: 33, at least 97% sequence identity to SEQ ID NO: 33, at least 98% sequence identity to SEQ ID NO: 33, at least 99% sequence identity to SEQ ID NO: 33, or 100% sequence identity to SEQ ID NO: 33); and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25 (e.g., at least 96% sequence identity to SEQ ID NO: 25, at least 97% sequence identity to SEQ ID NO: 25, at least 98% sequence identity to SEQ ID NO: 25, at least 99% sequence identity to SEQ ID NO: 25, or 100% sequence identity to SEQ ID NO: 25). In some embodiments, the VH_{B2} region comprises the amino acid of SEQ ID NO: 33; and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 25. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 D98A.F100A.Y102V-1Fab-IgG TDB).

In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 41; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41); and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). In some embodiments, the VH_{B2} region comprises the amino acid of SEQ ID NO: 41; and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 44. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 41; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 44. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43; and the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44); and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). In some embodiments, the VH_{B2} region comprises the amino acid of SEQ ID NO: 44; and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,

(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 41; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41); and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). In some embodiments, the VH_{B2} region comprises the amino acid of SEQ ID NO: 41; and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 44. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). For example, in some embodiments, the VH$_{B1}$ region comprises the amino acid of SEQ ID NO: 44; and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In some embodiments, the VH$_{B2}$ region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44). In some embodiments, the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 44. In some embodiments, the VL$_{B2}$ region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). In some embodiments, the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In some embodiments, the VH$_{B2}$ comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43; and the VL$_{B2}$ comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44); and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). In some embodiments, the VH$_{B2}$ region comprises the amino acid of SEQ ID NO: 44; and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In some embodiments, the bispecific antigen-binding molecule of any of the preceding embodiments features a VH_A region comprising one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3. In some embodiments, the VH_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7 (e.g., at least 96% sequence identity to SEQ ID NO: 7, at least 97% sequence identity to SEQ ID NO: 7, at least 98% sequence identity to SEQ ID NO: 7, at least 99% sequence identity to SEQ ID NO: 7, or 100% sequence identity to SEQ ID NO: 7). For example, in some embodiments, the VH_A region comprises the amino acid sequence of SEQ ID NO: 7. In some embodiments, the VL_A region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the VL_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8 (e.g., at least 96% sequence identity to SEQ ID NO: 8, at least 97% sequence identity to SEQ ID NO: 8, at least 98% sequence identity to SEQ ID NO: 8, at least 99% sequence identity to SEQ ID NO: 8, or 100% sequence identity to SEQ ID NO: 8). For example, in some embodiments, the VL_A region comprises the amino acid sequence of SEQ ID NO: 8. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB, e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain).

In some embodiments, the VH_A comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3; and the VL_A comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the VH_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7 (e.g., at least 96% sequence identity to SEQ ID NO: 7, at least 97% sequence identity to SEQ ID NO: 7, at least 98% sequence identity to SEQ ID NO: 7, at least 99% sequence identity to SEQ ID NO: 7, or 100% sequence identity to SEQ ID NO: 7); and the VL_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8 (e.g., at least 96% sequence identity to SEQ ID NO: 8, at least 97% sequence identity to SEQ ID NO: 8, at least 98% sequence identity to SEQ ID NO: 8, at least 99% sequence identity to SEQ ID NO: 8, or 100% sequence identity to SEQ ID NO: 8). In some embodiments, the VH_A region comprises the amino acid of SEQ ID NO: 7; and the VL_A region comprises the amino acid sequence of SEQ ID NO: 8. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB, e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain).

In another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab_A, wherein the C-terminus of the Fab_A is fused to an N-terminus of a first Fc subunit, and wherein the Fab_A comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18. For example, in some embodiments, (a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 17, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 18; and (c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 17, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 18.

In another aspect, the invention provides a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 22, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27. For example, in some embodiments, (a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 24, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 27; and (c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 24, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 27.

In yet another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25. For example, in some embodiments, (a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 33, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 25; and (c) the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 33, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 25.

In another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab_A, wherein the C-terminus of the Fab_A is fused to an N-terminus of a first Fc subunit, and wherein the Fab_A comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab_{B1} and a Fab_{B2}, wherein the C-terminus of the Fab_{B2} is fused to the N-terminus of the Fab_{B1}, and the C-terminus of the Fab_{B1} is fused to an N-terminus of a second Fc subunit, wherein the Fab_{B1} and the Fab_{B2} each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab_A comprises a VH_A region and a VL_A region, wherein the VH_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab_{B1} comprises a VH_{B1} region and a VL_{B1} region, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48; and (c) the Fab_{B2} comprises a VH_{B2} region and a VL_{B2} region, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48. For example, in some embodiments, (a) the VH_A region comprises the amino acid sequence of SEQ ID NO: 7, and the VL_A region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 41, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48; and (c) the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 41, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48.

In another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab_A, wherein the C-terminus of the Fab_A is fused to an N-terminus of a first Fc subunit, and wherein the Fab_A comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab_{B1} and a Fab_{B2}, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_{A}$ comprises a VH$_{A}$ region and a VL$_{A}$ region, wherein the VH$_{A}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_{A}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48. For example, in some embodiments, (a) the VH$_{A}$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_{A}$ region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 44, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 48; and (c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 44, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3/HER2 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB, e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain and two 4D5 Y55E.H91A.N54E.D98T.Y102V HER2 binding domains).

In yet another aspect, the invention provides a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab$_{A}$, wherein the C-terminus of the Fab$_{A}$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_{A}$ comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_{A}$ comprises a VH$_{A}$ region and a VL$_{A}$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49. For example, in some embodiments, (a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 41, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 49; and (c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 41, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3/HER2 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB, e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain and two 4D5 N30S.Y55E.H91A.N54E.D98T HER2 binding domains).

In another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49. For example, in some embodiments, (a) the VH_A region comprises the amino acid sequence of SEQ ID NO: 7, and the VL_A region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 44, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49; and (c) the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 44, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3/HER2 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB, e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain and two 4D5 N30S.Y55E.H91A.N54E.D98T.Y102V HER2 binding domains).

In some embodiments of any of the preceding aspects, the Fc domain is an IgG Fc domain (e.g., an IgG_1 or IgG_4 Fc domain). The Fc domain can be a human Fc domain. In some embodiments, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. For example, in some embodiments, the one or more amino acid substitutions that reduces binding to an Fc receptor and/or effector function is at one or more position selected from the group of L234, L235, and P329 (e.g, wherein the first Fc subunit and the second Fc subunit each comprises the amino acid substitutions of L234A, L235A and P329G). In some embodiments, the one or more amino acid substitutions that reduces binding to an Fc receptor and/or effector function is at N297 (e.g., N297G). In some embodiments, the Fc receptor is an Fcγ receptor. In some embodiments, the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC).

In some embodiments of any of the preceding aspects, the Fc domain comprises a modification configured to promote the association of the first Fc subunit with the second Fc subunit. In some embodiments, an amino acid residue in the CH3 domain of the second Fc subunit is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the second Fc subunit which is positionable in a cavity within the CH3 domain of the first Fc subunit, and an amino acid residue in the CH3 domain of the first Fc subunit is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the first Fc subunit within which the protuberance within the CH3 domain of the second Fc subunit is positionable. In some embodiments, the CH3 domain of the second Fc subunit comprises the amino acid substitution of T366, and the CH3 domain of the first Fc subunit comprises amino acid substitutions at one, two, or all three of T366, L368, and/or Y407. In some embodiments, the CH3 domain of the second Fc subunit comprises the amino acid substitution of T366W, and the CH3 domain of the first Fc subunit comprises one, two, or all three amino acid substitutions of T366S, L368A, and/or Y407V.

In some embodiments of any of the preceding aspects, the C-terminus of the third antigen-binding moiety is fused to the N-terminus of the second antigen-binding moiety via a peptide linker. The peptide linker can be 5-20 amino acids in length (e.g., 5-10, 10-15, or 15-20, e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length).

In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 50. In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 55 (e.g., at least 96% sequence identity to SEQ ID NO: 55, at least 97% sequence identity to SEQ ID NO: 55, at least 98% sequence identity to SEQ ID NO: 55, at least 99% sequence identity to SEQ ID NO: 55, or 100% sequence identity to SEQ ID NO: 55). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 59 (e.g., at least 96% sequence identity to SEQ ID NO: 59, at least 97% sequence identity to SEQ ID NO: 59, at least 98% sequence identity to SEQ ID NO: 59, at least 99% sequence identity to SEQ ID NO: 59, or 100% sequence identity to SEQ ID NO: 59). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 63 (e.g., at least 96% sequence identity to SEQ ID NO: 63, at least 97% sequence identity to SEQ ID NO: 63, at least 98% sequence identity to SEQ ID NO: 63, at least 99% sequence identity to SEQ ID NO: 63, or 100% sequence identity to SEQ ID NO: 63). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 83 (e.g., at least 96% sequence identity to SEQ ID NO: 83, at least 97% sequence identity to SEQ ID NO: 83, at least 98% sequence identity to SEQ ID NO: 83, at least 99% sequence identity to SEQ ID NO: 83, or 100% sequence identity to SEQ ID NO: 83). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 85 (e.g., at least 96% sequence identity to SEQ ID NO: 85, at least 97% sequence identity to SEQ ID NO: 85, at least 98% sequence identity to SEQ ID NO: 85, at least 99% sequence identity to SEQ ID NO: 85, or 100% sequence identity to SEQ ID NO: 85). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 87 (e.g., at least 96% sequence identity to SEQ ID NO: 87, at least 97% sequence identity to SEQ ID NO: 87, at least 98% sequence identity to SEQ ID NO: 87, at least 99% sequence identity to SEQ ID NO: 87, or 100% sequence identity to SEQ ID NO: 87). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 89 (e.g., at least 96% sequence identity to SEQ ID NO: 89, at least 97% sequence identity to SEQ ID NO: 89, at least 98% sequence identity to SEQ ID NO: 89, at least 99% sequence identity to SEQ ID NO: 89, or 100% sequence identity to SEQ ID NO: 89).

In some embodiments, the peptide linker comprises the amino acid sequence of SEQ ID NO: 51. In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 56 (e.g., at least 96% sequence identity to SEQ ID NO: 56, at least 97% sequence identity to SEQ ID NO: 56, at least 98% sequence identity to SEQ ID NO: 56, at least 99% sequence identity to SEQ ID NO: 56, or 100% sequence identity to SEQ ID NO: 56). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 60 (e.g., at least 96% sequence identity to SEQ ID NO: 60, at least 97% sequence identity to SEQ ID NO: 60, at least 98% sequence identity to SEQ ID NO: 60, at least 99% sequence identity to SEQ ID NO: 60, or 100% sequence identity to SEQ ID NO: 60). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 64 (e.g., at least 96% sequence identity to SEQ ID NO: 64, at least 97% sequence identity to SEQ ID NO: 64, at least 98% sequence identity to SEQ ID NO: 64, at least 99% sequence identity to SEQ ID NO: 64, or 100% sequence identity to SEQ ID NO: 64). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 84 (e.g., at least 96% sequence identity to SEQ ID NO: 84, at least 97% sequence identity to SEQ ID NO: 84, at least 98% sequence identity to SEQ ID NO: 84, at least 99%

sequence identity to SEQ ID NO: 84, or 100% sequence identity to SEQ ID NO: 84). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 86 (e.g., at least 96% sequence identity to SEQ ID NO: 86, at least 97% sequence identity to SEQ ID NO: 86, at least 98% sequence identity to SEQ ID NO: 86, at least 99% sequence identity to SEQ ID NO: 86, or 100% sequence identity to SEQ ID NO: 86). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 88 (e.g., at least 96% sequence identity to SEQ ID NO: 88, at least 97% sequence identity to SEQ ID NO: 88, at least 98% sequence identity to SEQ ID NO: 88, at least 99% sequence identity to SEQ ID NO: 88, or 100% sequence identity to SEQ ID NO: 88). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 90 (e.g., at least 96% sequence identity to SEQ ID NO: 90, at least 97% sequence identity to SEQ ID NO: 90, at least 98% sequence identity to SEQ ID NO: 90, at least 99% sequence identity to SEQ ID NO: 90, or 100% sequence identity to SEQ ID NO: 90).

In another aspect, the invention features an isolated nucleic acid encoding the bispecific antigen-binding molecule of any of the preceding aspects.

In another aspect, the invention provides a vector comprising an isolated nucleic acid encoding the bispecific antigen-binding molecule of any of the preceding aspects.

In another aspect, the invention provides a host cell (e.g., an isolated host cell) comprising a vector comprising an isolated nucleic acid encoding the bispecific antigen-binding molecule of any of the preceding aspects. In some embodiments, the host cell is a mammalian cell (e.g., a Chinese hamster ovary (CHO) cell). In other embodiments, the host cell is a prokaryotic cell (e.g., an *E. coli* cell).

In another aspect, the invention features a method of producing the bispecific antigen-binding molecule of any of the preceding aspects. In some embodiments, the method comprises culturing any of the host cells described above (e.g., a host cell comprising a vector comprising an isolated nucleic acid encoding the bispecific antigen-binding molecule of any of the preceding aspects) in a culture medium. In some embodiments, the method further comprises recovering the bispecific antigen-binding molecule from the host cell or the culture medium.

In another aspect, the invention features a set of isolated nucleic acids encoding the bispecific antigen-binding molecule of any of the preceding aspects (e.g., a set comprising two, three, four, or more isolated nucleic acids encoding the bispecific antigen-binding molecule of any of the preceding aspects). In some embodiments, a set of isolated nucleic acids includes a first isolated nucleic acid and a second isolated nucleic acid, wherein the first isolated nucleic acid encodes one or more amino acid sequences of a first arm of the bispecific antigen-binding molecule and the second isolated nucleic acid encodes one or more amino acid sequences of a second arm of the bispecific antigen-binding molecule.

In another aspect, the invention provides a set of vectors, wherein each vector of the set comprises an isolated nucleic acid of a set of isolated nucleic acids, wherein the set of isolated nucleic acids encoding the bispecific antigen-binding molecule of any of the preceding aspects (e.g., a set comprising two, three, four, or more isolated nucleic acids encoding the bispecific antigen-binding molecule of any of the preceding aspects).

In another aspect, the invention provides a set of host cells (e.g., a set of isolated host cells). In some embodiments, each host cell of the set comprises an isolated nucleic acid of a set of isolated nucleic acids, wherein the set of isolated nucleic acids encoding the bispecific antigen-binding molecule of any of the preceding aspects (e.g., a set comprising two, three, four, or more isolated nucleic acids encoding the bispecific antigen-binding molecule of any of the preceding aspects). In some embodiments, each host cell of the set comprises a vector comprising an isolated nucleic acid of a set of isolated nucleic acids, wherein the set of isolated nucleic acids encoding the bispecific antigen-binding molecule of any of the preceding aspects (e.g., a set comprising two, three, four, or more isolated nucleic acids encoding the bispecific antigen-binding molecule of any of the preceding aspects). In some embodiments, the set of host cells comprises mammalian cells (e.g., CHO cells). In some embodiments, the set of host cells comprises prokaryotic cells (e.g., *E. coli* cells).

In another aspect, the invention provides a method of producing the bispecific antigen-binding molecule of any of the preceding aspects, wherein the method comprises culturing the set of host cells of the preceding aspect in a culture medium. In some embodiments, the method further comprises recovering the bispecific antigen-binding molecule from the set of host cells or the culture medium.

In another aspect, the invention features an immunoconjugate comprising the bispecific antigen-binding molecule of any of the preceding aspects and a cytotoxic agent.

In yet another aspect, the invention provides a composition comprising the bispecific antigen-binding molecule of any of the preceding aspects. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier, excipient, or diluent. For example, in some embodiments, the composition is a pharmaceutical composition. In some embodiments, the composition further comprises a PD-1 axis binding antagonist or an additional therapeutic agent.

In another aspect, the invention features a bispecific antigen-binding molecule of any one of the preceding aspects for use as a medicament. For example, in some embodiments, the bispecific antigen-binding molecules described herein are for use in treating or delaying progression of a cell proliferative disorder (e.g., cancer) or an autoimmune disorder in a subject in need thereof. In some embodiments, the bispecific antigen-binding molecule of any of the preceding aspects are for use in enhancing immune function in a subject having a cell proliferative disorder (e.g., cancer, e.g., a HER2-positive cancer) or an autoimmune disorder.

In another aspect, the invention features a use of the bispecific antigen-binding molecule of any of the preceding aspects in the manufacture of a medicament for treating or delaying progression of a disorder. In another aspect, the invention features a use of the bispecific antigen-binding molecule of any of the preceding aspects in the manufacture of a medicament for enhancing immune function in a subject having a disorder. In some embodiments, the disorder is a cell proliferative disorder (e.g., cancer, e.g., a HER2-positive cancer) or an autoimmune disorder.

In yet another aspect, the invention features a method of treating or delaying the progression of a disorder in a subject in need thereof, the method comprising administering to the subject the bispecific antigen-binding molecule of any of the preceding aspects. In another aspect, the invention provides a method of enhancing immune function in a subject having a disorder, the method comprising administering to the subject the bispecific antigen-binding molecule of any of the preceding aspects. In some embodiments, the disorder is a cell proliferative disorder (e.g., cancer, e.g., a HER2-positive cancer) or an autoimmune disorder.

In another aspect, the invention features a method of treating or delaying the progression of a disorder in a subject in need thereof, wherein the method comprises: (a) determining an expression of HER2 on a tumor cell, wherein the tumor cell expresses HER2 at an average copy number of 200,000 or more copies per cell; and (b) administering to the subject the bispecific antigen-binding molecule of any of the preceding aspects. In some embodiments, the disorder is a cell proliferative disorder (e.g., cancer, e.g., a HER2-positive cancer) or an autoimmune disorder.

In another aspect, the invention provides a method of enhancing immune function in a subject having a disorder, wherein the method comprises: (a) determining an expression of HER2 on a tumor cell, wherein the tumor cell expresses HER2 at an average copy number of 200,000 or more copies per cell; and (b) administering to the subject the bispecific antigen-binding molecule of any of the preceding aspects. In some embodiments, the disorder is a cell proliferative disorder (e.g., cancer, e.g., a HER2-positive cancer) or an autoimmune disorder.

In some embodiments of any of the preceding aspects, the cancer is selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, renal cancer, bladder cancer, pancreatic cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, glioblastoma, endometrial cancer, and osteosarcoma. In some embodiments, the cancer is a HER2-positive cancer (e.g., a HER2-positive breast cancer, a HER2-positive gastric cancer, a HER2-positive colorectal cancer, a HER2-positive non-small cell lung cancer, a HER2-positive renal cancer, a HER2-positive bladder cancer, a HER2-positive pancreatic cancer, a HER2-positive prostate cancer, a HER2-positive liver cancer, a HER2-positive head and neck cancer, a HER2-positive melanoma, a HER2-positive ovarian cancer, a HER2-positive mesothelioma, a HER2-positive glioblastoma, a HER2-positive endometrial cancer, or a HER2-positive osteosarcoma).

In some embodiments, the HER2-positive cancer (e.g., the HER2-positive breast cancer, the HER2-positive gastric cancer, the HER2-positive colorectal cancer, the HER2-positive non-small cell lung cancer, the HER2-positive renal cancer, the HER2-positive bladder cancer, the HER2-positive pancreatic cancer, the HER2-positive prostate cancer, the HER2-positive liver cancer, the HER2-positive head and neck cancer, the HER2-positive melanoma, the HER2-positive ovarian cancer, the HER2-positive mesothelioma, the HER2-positive glioblastoma, the HER2-positive endometrial cancer, or the HER2-positive osteosarcoma) is characterized by tumor cells that express HER2 at a copy number (e.g., an average copy number) of at least 200,000 per cell (e.g., at least 250,000 HER2 copies per cell, at least 300,000 HER2 copies per cell, at least 400,000 HER2 copies per cell, at least 500,000 HER2 copies per cell, at least 600,000 HER2 copies per cell, at least 700,000 HER2 copies per cell, at least 750,000 HER2 copies per cell, at least 800,000 HER2 copies per cell, at least 900,000 HER2 copies per cell, at least 1,000,000 HER2 copies per cell, at least 1,200,000 HER2 copies per cell, at least 1,500,000 HER2 copies per cell, at least 2,000,000 HER2 copies per cell, at least 2,500,000 HER2 copies per cell, at least 3,000,000 HER2 copies per cell, or more, e.g., from 200,000 to 3,000,000 HER2 copies per cell, from 250,000 to 2,500,000 HER2 copies per cell, from 300,000 to 2,000,000 HER2 copies per cell, from 400,000 to 1,500,000 HER2 copies per cell, or from 500,000 to 1,000,000 HER2 copies per cell, e.g., from 200,000 to 1,000,000 HER2 copies per cell (e.g., from 200,000 to 250,000 HER2 copies per cell, from 250,000 to 300,000 HER2 copies per cell, from 300,000 to 400,000 HER2 copies per cell, from 400,000 to 500,000 HER2 copies per cell, from 500,000 to 750,000 HER2 copies per cell, or from 750,000 to 1,000,000 HER2 copies per cell) or from 1,000,000 to 3,000,000 HER2 copies per cell (e.g., from 1,000,000 to 1,500,000 HER2 copies per cell, from 1,500,000 to 2,000,000 HER2 copies per cell, from 2,000,000 to 2,500,000 HER2 copies per cell, or from 2,500,000 to 3,000,000 HER2 copies per cell).

In some embodiments of any of the preceding aspects, the bispecific antigen-binding molecule is administered to the subject in a dosage of about 0.01 mg/kg to about 10 mg/kg (e.g., about 0.1 mg/kg to about 10 mg/kg, e.g., about 1 mg/kg).

In some embodiments, a PD-1 axis binding antagonist and/or an additional therapeutic agent is administered to the subject. In some embodiments, the PD-1 axis binding antagonist or additional therapeutic agent is administered prior to or subsequent to the administration of the bispecific antigen-binding molecule. In some embodiments, the PD-1 axis binding antagonist or additional therapeutic agent is administered concurrently with the bispecific antigen-binding molecule. In some embodiments, the PD-1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist (e.g., atezolizumab (MPDL3280A), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab)), a PD-1 binding antagonist (e.g., MDX-1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001 (spartalizumab), REGN2810 (cemiplimab), and BGB-108), and a PD-L2 binding antagonist (e.g., an antibody or an immunoadhesin).

In some embodiments, the bispecific antigen-binding molecule is administered subcutaneously, intravenously, intramuscularly, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. For example, in some embodiments, the bispecific antigen-binding molecule is administered subcutaneously. In other embodiments, the bispecific antigen-binding molecule is administered intravenously.

In some embodiments of any of the preceding aspects, the subject is a human.

In another aspect, the invention features a kit comprising: (a) a composition comprising the bispecific antigen-binding molecule of any of the preceding aspects; and (b) a package insert comprising instructions for administering the composition to a subject to treat or delay progression of a disorder (e.g., a cell proliferative disorder (e.g., cancer) or an autoimmune disorder). In some embodiments, the composition further comprises a pharmaceutically acceptable carrier, excipient, or diluent.

Brief Description of the Drawings

FIG. 1 is an immunoblot showing the relative expression of HER2 protein by SKBR3 cells and MCF7 cells.

FIGS. 2A-2F are graphs showing dose response curves of relative killing of SKBR3 cells (open squares) and MCF7 cells (solid dots) by various monovalent HER2 TDB (IgG TDB) molecules. Data are represented as mean ± standard deviation. FIG. 2A is a graph showing dose response curves of target cell killing by the wild-type 4D5 IgG TDB antibody (trastuzumab). FIG. 2B is a graph showing dose response curves of target cell killing by the 4D5 antibody variant, Y55E.Y102V-TDB. FIG. 2C is a graph showing dose response curves of target cell killing by the 4D5 antibody variant, Y55E.D98A.F100A.Y102V-IgG TDB. FIG. 2D is a graph showing dose response curves of target cell killing by the 4D5 antibody variant, D98A.F100A.Y102V-IgG TDB. FIG. 2E is a graph showing dose response curves of target cell killing by the 4D5 antibody variant, H91A-IgG TDB. FIG. 2F is a graph showing dose response curves of target cell killing by the 4D5 antibody variant, Y100A-IgG TDB.

FIG. 3 is a schematic drawing of a representative trivalent antibody of the 1Fab-IgG TDB format. The antibody features a bivalent arm with two anti-HER2 binding moieties and a monovalent arm with one anti-CD3 binding moiety.

FIG. 4A is a graph showing dose response curves quantifying binding of various 1Fab-IgG TDB antibodies, relative to the wild-type 4D5 IgG TDB antibody to SKBR3, as quantified by flow cytometry. Solid downward-pointing triangles represent the wildtype 4D5 IgG TDB antibody (trastuzumab); solid squares represent the 4D5 antibody variant, H91A-IgG TDB; solid upward-pointing triangles represent the 4D5 antibody variant, D98A.F100A.Y102V-IgG TDB; open downward-pointing triangles represent the 4D5 antibody variant, Y102V-IgG TDB; and open diamonds represent the 4D5 antibody variant, Y55E.Y102V-IgG TDB.

FIG. 4B is a graph showing dose response curves quantifying binding of various 1Fab-IgG TDB antibodies, relative to the wild-type 4D5 IgG TDB antibody, to MCF7, as quantified by flow cytometry. Solid downward-pointing triangles represent the wildtype 4D5 IgG TDB antibody (trastuzumab); solid squares represent the 4D5 antibody variant, H91A-IgG TDB; solid upward-pointing triangles represent the 4D5 antibody variant, D98A.F100A.Y102V-IgG TDB; open downward-pointing triangles represent the 4D5 antibody variant, Y102V-IgG TDB; and open diamonds represent the 4D5 antibody variant, Y55E.Y102V-IgG TDB.

FIG. 5A is a graph showing induction of apoptosis in SKBR3 cells as a result of various 1Fab-IgG TDB antibodies, relative to the wild-type 4D5 IgG TDB antibody, as quantified by caspase 3/7 activity over time. Solid dots represent the wildtype 4D5 IgG TDB antibody (trastuzumab); open squares represent the 4D5 antibody variant, H91A-IgG TDB; solid triangles represent the 4D5 antibody variant, D98A.F100A.Y102V-IgG TDB; open triangles represent the 4D5 antibody variant, Y102V-IgG TDB; and open diamonds represent the 4D5 antibody variant, Y55E.Y102V-IgG TDB. Data are represented as mean ± standard deviation.

FIG. 5B is a graph showing induction of apoptosis in MCF7 cells as a result of various 1Fab-IgG TDB antibodies, relative to the wild-type 4D5 IgG TDB antibody, as quantified by caspase 3/7 activity over time. Solid dots represent the wildtype 4D5 IgG TDB antibody (trastuzumab); open squares represent the 4D5 antibody variant, H91A-IgG TDB; solid triangles represent the 4D5 antibody variant, D98A.F100A.Y102V-IgG TDB; open triangles represent the 4D5 antibody variant, Y102V-IgG TDB; and open diamonds represent the 4D5 antibody variant, Y55E.Y102V-IgG TDB. Data are represented as mean ± standard deviation.

FIG. 6A is a graph showing cytotoxicity in SKBR3 cells in response to incubation with 50 ng/mL of various 1Fab-IgG TDB antibodies, relative to the wild-type 4D5 IgG TDB antibody, as measured by a CELLTITER-GLO® assay. The first bar (on the left) represents the wildtype 4D5 TDB antibody (trastuzumab); the second bar represents the 4D5 antibody variant, H91A-IgG TDB; the third bar represents the 4D5 antibody variant, D98A.F100A.Y102V-IgG TDB; the fourth bar represents the 4D5 antibody variant, Y102V-IgG TDB; and the fifth bar (on the right) represents the 4D5 antibody variant, Y55E.Y102V-IgG TDB. Data are represented as mean ± standard deviation.

FIG. 6B is a graph showing cytotoxicity in MCF7 cells in response to incubation with 50 ng/mL of various 1Fab-IgG TDB antibodies, relative to the wild-type 4D5 IgG TDB antibody, as measured by a CELLTITER-GLO® assay. The first bar (on the left) represents the wildtype 4D5 TDB antibody (trastuzumab); the second bar represents the 4D5 antibody variant, H91A-IgG TDB; the third bar represents the 4D5 antibody variant, D98A.F100A.Y102V-IgG TDB; the fourth bar represents the 4D5 antibody variant, Y102V-IgG TDB; and the fifth bar (on the right) represents the 4D5 antibody variant, Y55E.Y102V-IgG TDB. Data are represented as mean ± standard deviation.

FIG. 6C is a graph showing cytotoxicity in MCF7 cells in response to incubation with 50 µg/mL of various 1Fab-IgG TDB antibodies, relative to the wild-type 4D5 IgG TDB antibody, as measured by a CELLTITER-GLO® assay. The first bar (on the left) represents the wildtype 4D5 TDB antibody (trastuzumab); the second bar represents the 4D5 antibody variant, H91A-IgG TDB; the third bar represents the 4D5 antibody variant, D98A.F100A.Y102V-IgG TDB; the fourth bar represents the 4D5 antibody variant, Y102V-IgG TDB; and the fifth bar (on the right) represents the 4D5 antibody variant, Y55E.Y102V-IgG TDB. Data are represented as mean ± standard deviation.

FIG. 7A is a graph showing dose response curves quantifying the binding of various 1Fab-IgG TDB antibodies to SKBR3 cells, relative to the wild-type 4D5 IgG TDB antibody (trastuzumab; solid triangles), as quantified by flow cytometry. Open triangles represent the 4D5 antibody variant, Y55E.H91A-1Fab-IgG TDB; squares represent the 4D5 antibody variant, Y100Aa-1Fab-IgG TDB; and circles represent the 4D5 antibody variant, H91A.N30A-1Fab-IgG TDB.

FIG. 7B is a graph showing dose response curves quantifying the binding of various 1Fab-IgG TDB antibodies to MCF7 cells, relative to the wild-type 4D5 IgG TDB antibody (trastuzumab; solid triangles), as quantified by flow cytometry. Open triangles represent the 4D5 antibody variant, Y55E.H91A-1Fab-IgG TDB; squares represent the 4D5 antibody variant, Y100Aa-1Fab-IgG TDB; and circles represent the 4D5 antibody variant, H91A.N30A-1Fab-IgG TDB.

FIG. 8 is a graph showing dose response curves quantifying the cytotoxicity of various 1Fab-IgG TDB antibodies to SKBR3 cells, as measured by a CELLTITER-GLO® assay. Open squares represent the 4D5 antibody variant, H91A-1Fab-IgG TDB; solid squares represent the 4D5 antibody variant, Y100Aa-1Fab-IgG TDB; and circles represent the 4D5 antibody variant, H91A.N30A-1Fab-IgG TDB. Data are represented as mean ± standard deviation.

FIG. 9 is a graph showing dose response curves comparing the cytotoxicity of 4D5 IgG TDB antibodies relative to 4D5 H91A-1Fab-IgG TDB antibodies. Cytotoxicity induced by 4D5 IgG TDB antibodies in SKBR3 cells and MCF7 cells are represented by solid circles and solid squares, respectively. Cytotoxicity induced by 4D5 H91A-1Fab-IgG TDB antibodies in SKBR3 cells and MCF7 cells are represented by open triangles and open squares, respectively. Data are represented as mean ± standard deviation.

FIG. 10 is a graph showing results of an RNA-seq analysis of ErbB2 RNA expression in 90 breast cancer cell lines. Cell lines were classified as low ErbB2 expressing, medium ErbB2 expressing, and high ErbB2 expressing cell lines.

FIG. 11A is a graph showing cytotoxicity of 4D5 IgG TDB antibodies at a concentration of 50 ng/mL on low ErbB2 expressing cell lines (MDA-MB-436, PC3, MCF7/neo-cl3, MDA-MB-231, LS1034, and HT55), medium ErbB2 expressing cell lines (MDA-MB-453, MDA-MB-175-VII, JIMT-1, and MKN7), and high Erb2 expressing cell lines (MDA-MB-361, SKBR3, BT474-M1, SK-OV-3, and KPL4). Data are represented as mean ± standard deviation.

FIG. 11B is a graph showing cytotoxicity of 4D5 IgG TDB antibodies at a concentration of 50 µg/mL on low ErbB2 expressing cell lines (MDA-MB-436, PC3, MCF7/neo-cl3, MDA-MB-231, LS1034, and HT55), medium ErbB2 expressing cell lines (MDA-MB-453, MDA-MB-175-VII, JIMT-1, and MKN7), and high Erb2 expressing cell lines (MDA-MB-361, SKBR3, BT474-M1, SK-OV-3, and KPL4). Data are represented as mean ± standard deviation.

FIG. 11C is a graph showing the effect of 4D5 TDB antibodies at a concentration of 50 ng/mL on activation of human CD8$^+$ T cells cultured with low ErbB2 expressing cell lines (MDA-MB-436, PC3, MCF7/neo-cl3, MDA-MB-231, LS1034, and HT55), medium ErbB2 expressing cell lines (MDA-MB-453, MDA-MB-175-VII, JIMT-1, and MKN7), and high Erb2 expressing cell lines (MDA-MB-361, SKBR3, BT474-M1, SK-OV-3, and KPL4). T cell activation was measured by dual expression of CD69 and CD45. Data are represented as mean ± standard deviation.

FIG. 11D is an immunoblot showing HER2 protein expression by each of the cells lines represented in FIGS. 11A-11C.

FIG. 12A is a graph showing cytotoxicity of 4D5 H91A-1Fab-IgG TDB antibodies at a concentration of 50 ng/mL on low ErbB2 expressing cell lines (MDA-MB-436, PC3, MCF7/neo-cl3, MDA-MB-231, LS1034, and HT55), medium ErbB2 expressing cell lines (MDA-MB-453, MDA-MB-175-VII, JIMT-1, and MKN7), and high Erb2 expressing cell lines (MDA-MB-361, SKBR3, BT474-M1, SK-OV-3, and KPL4). Data are represented as mean ± standard deviation.

FIG. 12B is a graph showing cytotoxicity of 4D5 H91A-1Fab-IgG TDB1Fab-IgG TDB antibodies at a concentration of 50 µg/mL on low ErbB2 expressing cell lines (MDA-MB-436, PC3, MCF7/neo-cl3, MDA-MB-231, LS1034, and HT55), medium ErbB2 expressing cell lines (MDA-MB-453, MDA-MB-175-VII, JIMT-1, and MKN7), and high Erb2 expressing cell lines (MDA-MB-361, SKBR3, BT474-M1, SK-OV-3, and KPL4). Data are represented as mean ± standard deviation.

FIG. 12C is a graph showing the effect of 4D5 H91A-1Fab-IgG TDB antibodies at a concentration of 50 ng/mL on activation of human CD8$^+$ T cells cultured with low ErbB2 expressing cell lines (MDA-MB-436, PC3, MCF7/neo-cl3, MDA-MB-231, LS1034, and HT55), medium ErbB2 expressing cell lines (MDA-MB-453, MDA-MB-175-VII, JIMT-1, and MKN7), and high Erb2 expressing cell lines (MDA-MB-361, SKBR3, BT474-M1, SK-OV-3, and KPL4). T cell activation was measured by dual expression of CD69 and CD45. Data are represented as mean ± standard deviation.

FIG. 12D is an immunoblot showing HER2 protein expression by each of the cells lines represented in FIGS. 12A-12C.

FIG. 13 is a graph showing dose response curves quantifying the cytotoxicity of 4D5 H91A-IgG TDB antibodies (squares) and 4D5 H91A-1Fab-IgG TDB antibodies (circles) on HBL-100 cells.

FIG. 14A is a graph showing dose response curves quantifying the cytotoxicity of various concentrations of 4D5 H91A-IgG TDB antibodies on HBL-100 cells over time. Solid circles represent a dose of 5,000 ng/mL 4D5 H91A-IgG TDB, open squares represent a dose of 500 ng/mL 4D5 H91A-IgG TDB, solid upward-pointing triangles represent a dose of 50 ng/mL 4D5 H91A-IgG TDB, open downward-pointing triangles represent a dose of 5 ng/mL 4D5 H91A-IgG TDB, open diamonds represent a dose of 0.5 ng/mL 4D5 H91A-IgG TDB, and solid diamonds represent an untreated control.

FIG. 14B is a graph showing dose response curves quantifying the cytotoxicity of various concentrations of 4D5 H91A-1Fab-IgG TDB antibodies on HBL-100 cells over time. Solid circles represent a dose of 5,000 ng/mL 4D5 H91A-1Fab-IgG TDB, open squares represent a dose of 500 ng/mL 4D5 H91A- 1Fab-IgG TDB, solid upward-pointing triangles represent a dose of 50 ng/mL 4D5 H91A-1Fab-IgG TDB, open downward-pointing triangles represent a dose of 5 ng/mL 4D5 H91A-1Fab-IgG TDB, open diamonds represent a dose of 0.5 ng/mL 4D5 H91A- 1Fab-IgG TDB, and solid diamonds represent an untreated control.

FIG. 15A is a set of graphs showing the effect of killing of 4D5 H91A-IgG TDB and 4D5 H91A-1Fab-IgG TDB on cell lines expressing increasing amounts of HER2, from left to right, as shown by the underlying immunoblot and bar graph.

FIG. 15B is a series of photomicrographs showing the results of IHC and FISH detection assays of relative HER2 expression.

FIG. 15C is a graph showing killing of cells characterized in FIG. 15B by 4D5 H91A-1Fab-IgG TDB.

FIG. 16 is an immunoblot showing HER2 protein expression in MCF7 cells, HT55 cells, and HER2-amplified KPL4 cells.

FIG. 17 is a trellis plot showing KPL4 tumor volume over the course of treatment with various doses of 4D5 H91A-1Fab-IgG TDB antibodies or wildtype 4D5 TDB antibodies in mice supplemented with human PBMCs. Mice with established KPL4 tumors received a single intravenous dose at day 0 at indicated doses. Each panel in the trellis depicts one dose group, as indicated by the panel header. Bold, solid black lines represent the fitted tumor volume for each dose group. Dashed bold lines represent the fitted tumor volume for the control group, which received the histidine buffer vehicle. Dashed lines with open circles represent individual animals. Solid lines with solid dots represent animals that were removed from the study. Dashed horizontal gray lines mark a tumor volume of 500 mm^3.

FIG. 18 is a trellis plot showing HT55 tumor volume over the course of treatment with various doses of 4D5 H91A-1Fab-IgG TDB antibodies or wildtype 4D5 IgG TDB antibodies in mice supplemented with human PBMCs. Mice with established HT55 tumors received a single intravenous dose at day 0 at indicated doses. Each panel in the trellis depicts one dose group, as indicated by the panel header. Bold, solid black lines represent the fitted tumor volume for each dose group. Dashed bold lines represent the fitted tumor volume for the control group, which received the histidine buffer vehicle. Dashed lines with open circles represent individual animals. Solid lines with solid dots represent animals that were removed from the study. Dashed horizontal gray lines mark a tumor volume of 500 mm^3.

FIG. 19 is a trellis plot showing KPL4 tumor volume over the course of treatment with various doses of 4D5 D98A.F100A.Y102V-1Fab-IgG TDB antibodies or wildtype 4D5 IgG TDB antibodies in mice supplemented with human PBMCs. Mice with established KPL4 tumors received a single intravenous dose at day 0 at indicated doses. Each panel in the trellis depicts one dose group, as indicated by the panel header. Bold, solid black lines represent the fitted tumor volume for each dose group. Dashed bold lines represent the fitted tumor volume for the control group, which received the histidine buffer vehicle. Dashed lines with open circles represent individual animals. Solid lines with solid dots represent animals that were removed from the study. Dashed horizontal red lines mark a tumor volume of 500 mm^3.

FIG. 20 is a graph showing dose response curves quantifying the cytotoxicity of 4D5 H91A-1Fab-IgG TDB antibodies (solid circles) and 4D5 D98A.F100A.Y102V-1Fab-IgG TDB antibodies (open squares) on SKBR3 cells. Data are represented as mean ± standard deviation.

FIG. 21A is a graph showing killing by 4D5 H91A-1Fab-IgG TDB antibodies of high HER2-expressing cell lines (n = 20). High HER2-expressing cell lines are characterized by high expression of Erb2 RNA. Data are represented as mean ± standard deviation.

FIG. 21B is a graph showing killing by 4D5 D98A.F100A.Y102V-1Fab-IgG TDB antibodies of high HER2-expressing cell lines (n = 20). High HER2-expressing cell lines are characterized by high expression of Erb2 RNA. Data are represented as mean ± standard deviation.

FIG. 22 is a graph showing the relative potency of killing of high HER2-expressing cell lines by 4D5 D98A.F100.Y102V-1Fab-IgG TDB antibodies relative to 4D5 H91A-1Fab-IgG TDB antibodies. Data are derived from FIGS. 20A and 20B.

FIG. 23A is a graph showing killing by 4D5 H91A-1Fab-IgG TDB antibodies of medium HER2-expressing cell lines (n = 12). Killing of SKBR3 cells is provided as a positive control. Medium HER2-expressing cell lines are characterized by medium expression of Erb2 RNA. Data are represented as mean ± standard deviation.

FIG. 23B is a graph showing killing by 4D5 D98A.F100A.Y102V-1Fab-IgG TDB antibodies of medium HER2-expressing cell lines (n = 12). Killing of SKBR3 cells is provided as a positive control. Medium HER2-expressing cell lines are characterized by medium expression of Erb2 RNA. Data are represented as mean ± standard deviation.

FIG. 24 is a graph showing the relative potency of killing of medium HER2-expressing cell lines by 4D5 D98A.F100.Y102V-1Fab-IgG TDB antibodies relative to 4D5 H91A-1Fab-IgG TDB antibodies. Data are derived from FIGS. 22A and 22B.

FIG. 25A is a graph showing killing by 4D5 H91A-1Fab-IgG TDB antibodies of low HER2-expressing cell lines (n = 12). Killing of SKBR3 cells is provided as a positive control. Low HER2-expressing cell lines are characterized by low expression of Erb2 RNA. Data are represented as mean ± standard deviation.

FIG. 25B is a graph showing killing by 4D5 D98A.F100A.Y102V-1Fab-IgG TDB antibodies of low HER2-expressing cell lines (n = 12). Killing of SKBR3 cells is provided as a positive control. Low HER2-expressing cell lines are characterized by low expression of Erb2 RNA. Data are represented as mean ± standard deviation.

FIG. 26 is a graph showing the relative potency of killing of low HER2-expressing cell lines by 4D5 D98A.F100.Y102V-1Fab-IgG TDB antibodies relative to 4D5 H91A-1Fab-IgG TDB antibodies. Data are derived from FIGS. 24A and 24B.

FIG. 27A is a schematic drawing showing the position and sequences of the two peptide linkers tested in FIG. 27B. Each peptide linker fuses the C-terminus of the constant heavy chain (CH) region of the distal Fab to the N-terminus of the variable heavy chain (VH) region of the proximal Fab. DKTHTGGGGSGG (SEQ ID NO: 52) is represented by open squares, and DKTHT (SEQ ID NO: 50) is represented by solid circles, in FIG. 27B.

FIG. 27B is a graph showing dose response curves quantifying the binding to MCF7 cells of 4D5 H91A-1Fab-IgG TDB antibodies having the DKTHTGGGGSGG linker (open squares), relative to 4D5 H91A-1Fab-IgG TDB antibodies having the DKTHT linker (solid circles). Data are represented as mean ± standard deviation.

FIG. 27C is a graph showing dose response curves quantifying the killing of SKBR3 cells by 4D5 H91A-1Fab-IgG TDB antibodies having the DKTHTGGGGSGG linker (open squares), relative to 4D5 H91A-1Fab-IgG TDB antibodies having the DKTHT linker (solid circles). Data are represented as mean ± standard deviation.

FIG. 28A is a series of immunoblots showing the effect of anti-HER2-CD3 1Fab-IgG on the expression of pAKT^{S473} and pHER3 by SKBR3 cells over time.

FIG. 28B is a graph showing dose response curves quantifying the viability of SKBR3 cells in response to treatment with anti-HER2-CD3 1Fab-IgG, anti-HER2-CD3 IgG TDB, and Trastuzumab.

FIG. 29A is a sensorgram showing binding kinetics of the wildtype 4D5 Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE® surface plasmon resonance. The wildtype 4D5 Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIG. 29B is a sensorgram showing binding kinetics of the 4D5 Y55E.Y102V-Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE® surface plasmon resonance. The 4D5 Y55E.Y102V-Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIG. 29C is a sensorgram showing binding kinetics of the 4D5 D98A.F100A.Y102V-Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE® surface plasmon resonance. The 4D5 D98A.F100A.Y102V-Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIG. 29D is a sensorgram showing binding kinetics of the 4D5 H91A-Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE® surface plasmon resonance. The 4D5 H91A-Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIG. 30A is a sensorgram showing binding kinetics of the 4D5 N30S-Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE® surface plasmon resonance. The 4D5 N30S-Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIG. 30B is a sensorgram showing binding kinetics of the 4D5 N54E.D98T-Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE®

surface plasmon resonance. The 4D5 N54E.D98T-Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIG. 30C is a sensorgram showing binding kinetics of the 4D5 N30S.N54E.D98T-Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE® surface plasmon resonance. The 4D5 N30S.N54E.D98T-Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIG. 31A is a sensorgram showing binding kinetics of the 4D5 N30S.Y55E.N54E.D98T.Y102V-Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE® surface plasmon resonance. The 4D5 N30S.Y55E.N54E.D98T.Y102V-Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIG. 31B is a sensorgram showing binding kinetics of the 4D5 N30S.N54E.D98T.F100A.Y102V-Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE® surface plasmon resonance. The 4D5 N30S.N54E.D98T.F100A.Y102V-Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIG. 31C is a sensorgram showing binding kinetics of the 4D5 N30S.H91A.N54E.D98T-Fab to directly immobilized human HER2 (extracellular domain; Novus Biologicals), as measured using BIACORE® surface plasmon resonance. The 4D5 N30S.H91A.N54E.D98T-Fab samples ranged in concentrations of 0.27 nM to 200 nm, in 3-fold dilutions.

FIGS. 32A-32K is a series of sensorgrams showing binding kinetics of 4D5 IgG TDB antibodies to human HER2, as measured using BIACORE® surface plasmon resonance. FIG. 32A is a sensorgram showing binding kinetics of the wildtype 4D5 IgG TDB; FIG. 32B is a sensorgram showing binding kinetics of the 4D5 H91A-IgG TDB; FIG. 32C is a sensorgram showing binding kinetics of the 4D5 Y55E.H91A-IgG TDB; FIG. 32D is a sensorgram showing binding kinetics of the 4D5 Y55E.D98A.F100A.Y102V-IgG TDB; FIG. 32E is a sensorgram showing binding kinetics of the 4D5 D98A.F100A.Y102V-IgG TDB; FIG. 32F is a sensorgram showing binding kinetics of the 4D5 H91A.D98A.F100A.Y102V-IgG TDB; FIG. 32G is a sensorgram showing binding kinetics of the 4D5 Y55E.H91A.D98A.F100A.Y102V-IgG TDB; FIG. 32H is a sensorgram showing binding kinetics of the 4D5 Y55E.Y102V-IgG TDB; FIG. 32I is a sensorgram showing binding kinetics of the 4D5 Y102V-IgG TDB; FIG. 32J is a sensorgram showing binding kinetics of the 4D5 H91A.Y102V-IgG TDB; and FIG. 32K is a sensorgram showing binding kinetics of the 4D5 Y55E.H91A.Y102V-IgG TDB.

FIG. 33A is a graph showing dose response curves quantifying the killing of SKBR3 cells by 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB (lot 1) antibodies (open squares); 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB antibodies (solid squares); 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB (lot 2) antibodies (open downward-facing triangles); or 4D5 H91A-1Fab-IgG TDB antibodies (solid upward-facing triangles).

FIG. 33B is a graph showing dose response curves quantifying the killing of MCF7 cells by 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB (lot 1) antibodies (open squares); 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB antibodies (solid squares); 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB (lot 2) antibodies (open downward-facing triangles); or 4D5 H91A-1Fab-IgG TDB antibodies (solid upward-facing triangles).

FIG. 34A is a graph showing dose response curves quantifying binding of various 1Fab-IgG TDB variants to SKBR8 cells, as measured by flow cytometry. Open squares represent 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB (lot 1) antibodies; solid squares represent 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB antibodies; open downward-facing triangles represent 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB (lot 2) antibodies; and solid upward-facing triangles represent 4D5 H91A-1Fab-IgG TDB antibodies.

FIG. 34B is a graph showing dose response curves quantifying binding of various 1Fab-IgG TDB variants to MCF7 cells, as measured by flow cytometry. Open squares represent 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB (lot 1) antibodies; solid squares represent 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB antibodies; open downward-facing triangles represent 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB (lot 2) antibodies; and solid upward-facing triangles represent 4D5 H91A-1Fab-IgG TDB antibodies.

FIG. 35A is a schematic representation showing binding and activity of anti-HER2-CD3 IgG-TDBs with monovalent high affinity binding.

FIG. 35B is a schematic representation showing binding and activity of anti-HER2-CD3 IgG-TDBs with monovalent low affinity binding.

FIG. 35C is a schematic representation showing that bivalent HER2 binding at appropriate affinity (monovalent KD 20-50 nM) results in high binding to HER2-over-expressing cells due to avidity of the anti-HER2-CD3 1Fab-IgG TDB.

FIG. 36 provides the crystal structure of the HER2 extracellular domain (ECD) and highlights the regions to which the different HER2 antibodies bind.

FIG. 37 is a graph showing dose response curves quantifying killing of SKBR3 target cells by 38E4v1 4D5-H91A 1Fab-IgG TDB (open circles); SKBR3 target cells by 40G5c 4D5-H91A 1Fab-IgG TDB (solid circles); MCF7 target cells by 38E4v1 4D5-H91A 1Fab-IgG TDB (open squares); and MCF7 target cells by 40G5c 4D5-H91A 1Fab-IgG TDB (closed squares).

FIG. 38A is a graph showing level of 4D5 H91A 1Fab-IgG TDB and 4D5 IgG TDB-induced HER2 independent T cell activation as tested in the presence and absence of HER2 expressing cells.

FIG. 38B is a graph showing level of 4D5 H91A 1Fab-IgG TDB and 4D5 IgG TDB-induced HER2 independent T cell activation as tested in the presence and absence of bivalent anti-CD3 OKT3.

FIG. 38C is a series of graphs showing blood markers for inflammation (C-reactive protein; CRP), T cell activation (lymphocyte margination), and liver damage (alanine and aspartate aminotransferases; ALT and AST) as measured two days and eight days after dosing cynomolgus monkeys with H91A 1Fab-IgG TDB or vehicle control.

FIG. 38D is a graph and summary table showing PK parameters as detected by ELISA from cynomolgus monkeys dosed with H91A 1Fab-IgG TDB at 20 mg/kg and 3 mg/kg doses.

FIG. 38E is a graph showing H91A 1Fab-IgG TDB cynomolgus serum levels at 7 days and 14 days after dosing and subjected to healthy donor PBMC and SKBR3 cells for 24 hours using indicated dilutions. Parallel experiments were carried out using dilutions of fresh 4D5-H91A 1Fab-IgG TDB (Control).

FIG. 39 is a diagram showing the effect of various amino acid substitutions on the binding affinity of 4D5 anti-HER2 Fab variants.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

I. DEFINITIONS

The term “about” as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

“Affinity” refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the equilibrium dissociation constant (K_D). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

As used herein, the term “specifically binds to” or is “specific for,” as used herein, refers to measurable and reproducible interactions such as binding between a target and an antibody, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody that specifically binds to a target (which can be an epitope) is an antibody that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets. In one embodiment, the extent of binding of an antibody to an unrelated target is less than about 10% of the binding of the antibody to the target as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that specifically binds to a target has an equilibrium dissociation constant (K_D) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, or ≤ 0.1 nM. In certain embodiments, an antibody specifically binds to an epitope on a protein that is conserved among the protein from different species. In another embodiment, specific binding can include, but does not require exclusive binding.

The term “antibody” herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

An “antibody fragment” refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab’, Fab’-SH, F(ab’)$_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

By “antigen-binding moiety” is meant a part of a compound or a molecule that specifically binds to a target epitope, antigen, ligand, or receptor. Molecules featuring antigen-binding moieties include, but are not limited to, antibodies (e.g., monoclonal, polyclonal, recombinant, humanized, and chimeric antibodies), antibody fragments or portions thereof (e.g., Fab fragments, Fab’2, scFv antibodies, SMIP, domain antibodies, diabodies, minibodies, scFv-Fc, affibodies, nanobodies, and VH and/or VL domains of antibodies), receptors, ligands, aptamers, and other molecules having an identified binding partner. An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

As used herein, the term "monovalent," for example, in the context of a monovalent arm of a bispecific antigen-binding molecule, refers to a molecule or a portion thereof (e.g., a portion of an antigen-binding molecule, e.g., one of two arms of a bispecific antigen-binding molecule) that has a single antigen-binding moiety. Thus, a monovalent molecule or portion thereof is capable of specific binding to exactly one antigen. The "monovalent binding affinity" or "monovalent K_D" of one of the two antigen-binding moieties of a bivalent arm of a bispecific antibody (e.g., one of the HER2 antigen-binding moieties of a 1Fab-IgG TDB) refers to the binding affinity of the antigen-binding moiety in monovalent form, i.e., as a monovalent arm of a bispecific antibody capable of specific binding to two different antigens or as a Fab molecule.

As used herein, the term "bivalent," for example, in the context of a bivalent arm of a bispecific antigen-binding molecule, refers to a molecule or a portion thereof (e.g., a portion of an antigen-binding molecule, e.g., one of two arms of a bispecific antigen-binding molecule) that has exactly two antigen-binding moieties, each of which is capable of specific binding to an antigen. Thus, a bivalent molecule or portion thereof is capable of specific binding to two antigens or two different epitopes on the same antigen (e.g., two HER2 antigens expressed on the surface of a single tumor cell).

The term "cluster of differentiation 3" or "CD3," as used herein, refers to any native CD3 from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated, including, for example, CD3ε, CD3γ, CD3α, and CD3β chains. The term encompasses "full-length," unprocessed CD3 (e.g., unprocessed or unmodified CD3ε or CD3γ), as well as any form of CD3 that results from processing in the cell. The term also encompasses naturally occurring variants of CD3, including, for example, splice variants or allelic variants. CD3 includes, for example, human CD3ε protein (NCBI RefSeq No. NP_000724), which is 207 amino acids in length, and human CD3γ protein (NCBI RefSeq No. NP_000064), which is 182 amino acids in length.

The terms "anti-CD3 antibody" and "an antibody that binds to CD3" refer to an antibody that is capable of binding CD3 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD3. In one embodiment, the extent of binding of an anti-CD3 antibody to an unrelated, non-CD3 protein is less than about 10% of the binding of the antibody to CD3 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to CD3 has a dissociation constant (K_D) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g. 10^{-8} M or less, e.g. from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M). In certain embodiments, an anti-CD3 antibody binds to an epitope of CD3 that is conserved among CD3 from different species.

The terms "Fc region" or "Fc domain" are herein used interchangeably to refer to 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. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain.

However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. The term encompasses truncated Fc regions, such as those having a C-terminal truncation (e.g., a ΔGK truncation, e.g., as described in Hu, et al., *Biotechnol. Prog.* 2017, 33: 786–794 and Jiang, et al., *J. Pharm. Sci.* 2016, 105: 2066–2072. 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. A "subunit" of an Fc domain 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. In one embodiment, a subunit of an IgG Fc domain comprises an IgG CH2 and an IgG CH3 constant domain.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms "full-length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE^{TM} technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., *supra.* In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra.*

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 Daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

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 ("complementarity determining regions" or "CDRs") and/or form structurally defined loops ("hypervariable loops") and/or contain the antigen-contacting residues ("antigen contacts"). Generally, antibodies comprise six HVRs: three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). Exemplary HVRs herein include:

(a) hypervariable loops occurring at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3) (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987));

(b) CDRs occurring at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3), 31-35b (H1), 50-65 (H2), and 95-102 (H3) (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991));

(c) antigen contacts occurring at amino acid residues 27c-36 (L1), 46-55 (L2), 89-96 (L3), 30-35b (H1), 47-58 (H2), and 93-101 (H3) (MacCallum et al. *J. Mol. Biol.* 262: 732-745 (1996)); and

(d) combinations of (a), (b), and/or (c), including HVR amino acid residues 46-56 (L2), 47-56 (L2), 48-56 (L2), 49-56 (L2), 26-35 (H1), 26-35b (H1), 49-65 (H2), 93-102 (H3), and 94-102 (H3).

Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al.*, supra.*

The term “variable region” or “variable domain” refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al., *J. Immunol.* 150:880-887 (1993); Clarkson et al., *Nature* 352:624-628 (1991).

An “immunoconjugate” is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

An “isolated” antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B* 848:79-87 (2007).

“Percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

As used herein, the term "liability fix" (and any grammatical derivation thereof) refers to an amino acid substitution configured to enhance the chemical stability of the antibody (e.g., reduced rate of deamidation and/or isomerization), for example, by replacing an amino acid residue prone to deamidation or isomerization with a comparably inert amino acid residue or by replacing an amino acid flanking the residue prone to deamination or isomerization with a comparably inert amino acid residue. Examples of liability fixes to reduce deamidation include substitution of asparagine with serine or glutamic acid (e.g., N30S or N54E). Examples of liability fixes to reduce isomerization include substitution of aspartic acid with alanine or threonine (e.g., D98A or D98T).

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

"Isolated nucleic acid encoding an anti-CD3 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

As used herein, "administering" is meant a method of giving a dosage of a compound (e.g., an anti-CD3 antibody of the invention or a nucleic acid encoding an anti-CD3 antibody of the invention) or a composition (e.g., a pharmaceutical composition, e.g., a pharmaceutical composition including an anti-CD3 antibody of the invention) to a subject. The compositions utilized in the methods described herein can be administered, for example, intramuscularly, intravenously, intradermally, percutaneously, intraarterially, intraperitoneally, intralesionally, intracranially, intraarticularly, intraprostatically, intrapleurally, intratracheally, intranasally, intravitreally, intravaginally, intrarectally, topically, intratumorally, peritoneally, subcutaneously, subconjunctivally, intravesicularlly, mucosally, intrapericardially, intraumbilically, intraocularly, orally, topically, locally, by inhalation, by injection, by infusion, by continuous infusion, by localized perfusion bathing target cells directly, by catheter, by lavage, in cremes, or in lipid compositions. The method of administration can vary depending on various factors (e.g., the compound or composition being administered and the severity of the condition, disease, or disorder being treated).

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. 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, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

As used herein, "delaying progression" of a disorder or disease means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease or disorder (e.g., a cell proliferative disorder, e.g., cancer). This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. For example, a late stage cancer, such as development of metastasis, may be delayed.

By “reduce” or “inhibit” is meant the ability to cause an overall decrease, for example, of 20% or greater, of 50% or greater, or of 75%, 85%, 90%, 95%, or greater. In certain embodiments, reduce or inhibit can refer to the effector function of an antibody that is mediated by the antibody Fc region, such effector functions specifically including complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP).

A “subject” or an “individual” is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the subject or individual is a human.

A “disorder” is any condition that would benefit from treatment including, but not limited to, chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question.

The terms “cell proliferative disorder” and “proliferative disorder” refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer. In one embodiment, the cell proliferative disorder is a tumor.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Included in this definition are benign and malignant cancers. By “early stage cancer” or “early stage tumor” is meant a cancer that is not invasive or metastatic or is classified as a Stage 0, 1, or 2 cancer. Examples of a cancer include, but are not limited to, breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, non-Hodgkin’s lymphoma (NHL), B cell lymphoma, B cell leukemia, multiple myeloma, renal cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, glioblastoma, germinal-center B-cell-like (GCB) diffuse large B cell lymphoma (DLBCL), activated B cell-like (ABC) DLBCL, follicular lymphoma (FL), mantle cell lymphoma (MCL), acute myeloid leukemia (AML), chronic lymphoid leukemia (CLL), marginal zone lymphoma (MZL), small lymphocytic leukemia (SLL), lymphoplasmacytic lymphoma (LL), Waldenström macroglobulinemia (WM), central nervous system lymphoma (CNSL), Burkitt’s lymphoma (BL), B cell prolymphocytic leukemia, splenic marginal zone lymphoma, hairy cell leukemia, splenic lymphoma/leukemia, splenic diffuse red pulp small B cell lymphoma, hairy cell leukemia variant, α heavy chain disease, γ heavy chain disease, μ heavy chain disease, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, pediatric follicular lymphoma, primary cutaneous follicle centre lymphoma, T cell/histiocyte rich large B cell lymphoma, primary DLBCL of the central nervous system, primary cutaneous DLBCL (leg type), Epstein-Barr virus (EBV)-positive DLBCL of the elderly, DLBCL associated with chronic inflammation, lymphomatoid granulomatosis, primary mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, anaplastic lymphoma kinase (ALK)-positive large B cell lymphoma, plasmablastic lymphoma, large B cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, large B cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, or large B cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma)). In some embodiments, the cancer is a HER2-positive cancer (e.g., a HER2-positive breast cancer or a HER2-positive gastric cancer).

The term “HER2-positive” cancer comprises cancer cells which have higher than normal levels of HER2. Examples of HER2-positive cancer include HER2-positive breast cancer and HER2-positive gastric cancer. Optionally, HER2-positive cancer has an immunohistochemistry (IHC) score of 2+ or 3+ and/or an in situ hybridization (ISH) amplification ratio ≥2.0.

The term "tumor," as used herein, refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer", "cancerous", "cell proliferative disorder", "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

The term “tumor antigen,” as used herein, may be understood as those antigens that are presented on tumor cells. These antigens can be presented on the cell surface with an extracellular part, which is often combined with a transmembrane and cytoplasmic part of the molecule. These antigens can sometimes be presented only by tumor cells and never by the normal ones. Tumor antigens can be exclusively expressed on tumor cells or might represent a tumor specific mutation compared to normal cells. In this case, they are called tumor-specific antigens. More common are tumor antigens that are presented by tumor cells and normal cells, and they are called tumor-associated antigens. These tumor-associated antigens can be overexpressed compared to normal cells or are accessible for antibody binding in tumor cells due to the less compact structure of the tumor tissue compared to normal tissue.

An “effective amount” of a compound, for example, a bispecific antigen-binding molecule of the invention or a composition (e.g., pharmaceutical composition) thereof, is at least the minimum amount required to achieve the desired therapeutic or prophylactic result, such as a measurable improvement or prevention of a particular disorder (e.g., a cell proliferative disorder, e.g., cancer). An effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual. An effective amount is also one in which any toxic or detrimental effects of the treatment are outweighed by the therapeutically beneficial effects. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as decreasing one or more symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication such as via targeting, delaying the progression of the disease, and/or prolonging survival. In the case of cancer or tumor, an effective amount of the drug may have the effect in reducing the number of cancer cells; reducing the tumor size; inhibiting (i.e., slow to some extent or desirably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and desirably stop) tumor metastasis; inhibiting to some extent tumor growth; and/or relieving to some extent one or more of the symptoms associated with the disorder. An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

The term "PD-1 axis binding antagonist" refers to a molecule that inhibits the interaction of a PD-1 axis binding partner with one or more of its binding partners, so as to remove T cell dysfunction resulting from signaling on the PD-1 signaling axis, with a result being restored or enhanced T cell function. As used herein, a PD-1 axis binding antagonist includes a PD-1 binding antagonist and a PD-L1 binding antagonist, as well as molecules that interfere with the interaction between PD-L1 and PD-1 (e.g., a PD-L2-Fc fusion).

As used herein, a "PD-1 binding antagonist" is a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-1 with one or more of its binding partners, such as PD-L1 and/or PD-L2. In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its binding partners. In a specific aspect, the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1 and/or PD-L2. For example, PD-1 binding antagonists include anti-PD-1 antibodies and antigen-binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides, small molecule antagonists, polynucleotide antagonists, and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-1 with PD-L1 and/or PD-L2. In one embodiment, a PD-1 binding antagonist reduces the negative signal mediated by or through cell surface proteins expressed on T lymphocytes and other cells through PD-1 or PD-L1 so as to render a dysfunctional T-cell less dysfunctional. In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody. In a specific aspect, a PD-1 binding antagonist is MDX-1106 (nivolumab) described herein. In another specific aspect, a PD-1 binding antagonist is MK-3475 (pembrolizumab) described herein. In another specific aspect, a PD-1 binding antagonist is CT-011 (pidilizumab) described herein. In another specific aspect, a PD-1 binding antagonist is MEDI-0680 (AMP-514). In another specific aspect, a PD-1 binding antagonist is PDR001 (spartalizumab). In another specific aspect, a PD-1 binding antagonist is REGN2810 (cemiplimab). In another specific aspect, a PD-1 binding antagonist is BGB-108. In another specific aspect, a PD-1 binding antagonist is AMP-224 described herein.

As used herein, a "PD-L1 binding antagonist" is a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L1 with either one or more of its binding partners, such as PD-1 and/or B7-1. In some embodiments, a PD-L1 binding antagonist is a molecule that inhibits the binding of PD-L1 to its binding partners. In a specific aspect, the PD-L1 binding antagonist inhibits binding of PD-L1 to PD-1 and/or B7-1. In some embodiments, PD-L1 binding antagonists include anti-PD-L1 antibodies and antigen-binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides, small molecule antagonists, polynucleotide antagonists, and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L1 with one or more of its binding partners, such as PD-1 and/or B7-1. In one embodiment, a PD-L1 binding antagonist reduces the negative signal mediated by or through cell surface proteins expressed on T lymphocytes and other cells through PD-L1 or PD-1 so as to render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, a PD-L1 binding antagonist is an anti-PD-L1 antibody. In a specific aspect, an anti-PD-L1 antibody is YW243.55.S70 described herein. In another specific aspect, an anti-PD-L1 antibody is MDX-1105 described herein. In still another specific aspect, an anti-PD-L1 antibody is atezolizumab (CAS Registry Number: 1422185-06-5), also known as MPDL3280A, described herein. In still another specific aspect, an anti-PD-L1 antibody is MEDI4736 (druvalumab) described herein. In still another specific aspect, an anti-PD-L1 antibody is MSB0010718C (avelumab) described herein.

The terms “Programmed Death Ligand 1” and “PD-L1” refer herein to a native sequence PD-L1 polypeptide, polypeptide variants (i.e., PD-L1 polypeptide variants), and fragments of a native sequence polypeptide and polypeptide variants (which are further defined herein). The PD-L1 polypeptide described herein may be that which is isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant or synthetic methods.

A “native sequence PD-L1 polypeptide” comprises a polypeptide having the same amino acid sequence as the corresponding PD-L1 polypeptide derived from nature.

A “PD-L1 polypeptide variant,” or variations thereof, means a PD-L1 polypeptide, generally an active PD-L1 polypeptide, as defined herein having at least about 80% amino acid sequence identity with any of the native sequence PD-L1 polypeptide sequences as disclosed herein. Such PD-L1 polypeptide variants include, for instance, PD-L1 polypeptides wherein one or more amino acid residues are added, or deleted, at the N- or C-terminus of a native amino acid sequence. Ordinarily, a PD-L1 polypeptide variant will have at least about 80% amino acid sequence identity, alternatively at least about 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% amino acid sequence identity, to a native sequence PD-L1 polypeptide sequence as disclosed herein. Ordinarily, PD-L1 polypeptide variants are at least about 10 amino acids in length, alternatively at least about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 281, 282, 283, 284, 285, 286, 287, 288, or 289 amino acids in length, or more. Optionally, PD-L1 polypeptide variants will have no more than one conservative amino acid substitution as compared to a native PD-L1 polypeptide sequence, alternatively no more than 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions as compared to a native PD-L1 polypeptide sequence.

The term “PD-L2 binding antagonist” refers to a molecule that decreases, blocks, inhibits, abrogates or interferes with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In some embodiments, a PD-L2 binding antagonist is a molecule that inhibits the binding of PD-L2 to one or more of its binding partners. In a specific aspect, the PD-L2 binding antagonist inhibits binding of PD-L2 to PD-1. In some embodiments, the PD-L2 antagonists include anti-PD-L2 antibodies, antigen-binding fragments thereof, immunoadhesins, fusion proteins, oligopeptides and other molecules that decrease, block, inhibit, abrogate or interfere with signal transduction resulting from the interaction of PD-L2 with either one or more of its binding partners, such as PD-1. In one embodiment, a PD-L2 binding antagonist reduces the negative co-stimulatory signal mediated by or through cell surface proteins expressed on T lymphocytes mediated signaling through PD-L2 so as render a dysfunctional T-cell less dysfunctional (e.g., enhancing effector responses to antigen recognition). In some embodiments, a PD-L2 binding antagonist is an immunoadhesin.

II. COMPOSITIONS

In one aspect, the invention is based, in part, on bispecific antigen-binding molecules (e.g., bispecific antibodies). In certain embodiments, the bispecific antigen-binding molecules have a monovalent arm capable of specific binding to a first antigen (e.g., a T cell antigen, e.g., CD3) and a bivalent arm capable of specific binding to two additional antigens (e.g., tumor antigens, e.g., two HER2 antigens). For example, the bivalent arm may comprise two antigen-binding moieties, each capable of specific binding to a target antigen (e.g., HER2) to increase the avidity of the bispecific antigen-binding molecule to a cell expressing high levels of the target antigen. Bispecific antigen-binding molecules of the invention are useful, for example, for treating or delaying the progression of a cell proliferative disorder (e.g., cancer) or an autoimmune disorder, or for enhancing immune function in a subject having such a disorder.

The 1Fab-IgG TDBs of the present invention selectively kill tumor cells which overexpress the targeted tumor antigen with high potency, while sparing cells that express low amounts of the targeted tumor antigen, e.g., cells of normal or healthy human tissues. Selectivity is based on the avidity of two low affinity anti-tumor antigen Fab arms to high target density on cells that overexpress the tumor antigen. The increased selectivity to the tumor antigen overexpressing cells mitigates the on-target adverse effects of TDB. For example, using HER2 as the targeted tumor antigen, anti-HER2-IgG TDB with monovalent high affinity binding to HER2 bind to both HER2 over-expressing cells and cells that express low level of HER2. Therapeutic index of this TDB is based on higher activity on HER2 over-expressing cells due to high HER2 density. At high TDB doses, high affinity IgG1 HER2-TDB can affect cells that express low level of HER2 and has therefore risk of on-target off-tumor autoimmunity on normal tissues (FIG. 35A). Engineering the HER2 binding arm to have lower affinity results in lower binding and TDB activity in both HER2 over-expressing cells and cells that express low level of HER2, but does not improve selectivity (FIG. 35B). Bivalent HER2 binding at appropriate affinity (monovalent K_D ~20-50 nM) results in high binding to HER2-over-expressing cells due to avidity of the anti-HER2 1Fab-IgG TDB. The avidity effect is dependent on high HER2 density, and 1Fab-IgG TDB therefore does not bind to cells expressing low levels of HER2, e.g., cells of normal or healthy human tissues. As cell-binding correlates with the ability of TDBs to recruit T cell activity, the anti-HER2 1Fab-IgG TDB selectively kills only cells that over express HER2 and has reduced risk of inducing on-target off-tumor autoimmunity on normal tissues (FIG. 35C).

Exemplary Bispecific Antigen-Binding Molecules

In one aspect, the invention provides isolated bispecific antigen-binding molecules (e.g., bispecific antibodies) having a monovalent arm and a bivalent arm. For example, in one aspect, the invention provides a bispecific antigen-binding molecule having a monovalent arm and a bivalent arm, wherein (a) the monovalent arm comprises a first antigen-binding moiety, and (b) the bivalent arm comprises a second antigen-binding moiety and a third antigen-binding moiety. In some embodiments, the C-terminus of the first antigen-binding moiety is fused to the N-terminus of a first Fc subunit, the C-terminus of the third antigen-binding moiety is fused to the N-terminus of the second antigen-binding moiety, and the C-terminus of the second antigen-binding moiety is fused to an N-terminus of a second Fc subunit. In some embodiments, the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, the first antigen-binding moiety is capable of specific binding to a first target cell antigen, and the second antigen-binding moiety and the third antigen-binding moiety are each capable of specific binding to a second target cell antigen (e.g., at the same epitope or at different epitopes on the second target cell antigen).

The first antigen-binding moiety may be a Fab molecule (Fab_A) comprising a variable heavy chain (VH_A) region and a variable light chain (VL_A) region; the second antigen-binding moiety may be a Fab molecule (Fab_{B1}) comprising a variable heavy chain (VH_{B1}) region and a variable light chain (VL_{B1}) region; and/or the third antigen-binding moiety may be a Fab molecule (Fab_{B2}) comprising a variable heavy chain (VH_{B2}) region and a variable light chain (VL_{B2}) region. Thus, in some instances, the first antigen-binding moiety may be a Fab_A comprising a VH_A region and a VL_A region, the second antigen-binding moiety may be a Fab_{B1} comprising a VH_{B1} region and a VL_{B1} region, and third antigen-binding moiety may be a Fab_{B2} comprising a VH_{B2} region and a VL_{B2} region. The VH_{B1} and the VH_{B2} may share at least 95% sequence identity (e.g., 95%, 96%, 97%, 98%, 99%, or 100% sequence identity). Additionally or alternatively, the VL_{B1} and the VL_{B2} may share at least 95% sequence identity (e.g., 95%, 96%, 97%, 98%, 99%, or 100% sequence identity).

A 1Fab-IgG molecule refers to a bispecific antigen-binding molecule having a first antigen binding moiety, a second antigen-binding moiety, and a third antigen-binding moiety, wherein the first antigen-binding moiety is a Fab_A comprising a VH_A region and a VL_A region, the second antigen-binding moiety is a Fab_{B1} comprising a VH_{B1} region and a VL_{B1} region, and the third antigen-binding moiety is a Fab_{B2} comprising a VH_{B2} region and a VL_{B2} region, wherein the VH_{B1} and the VH_{B2} share at least 95% sequence identity (e.g., 95%, 96%, 97%, 98%, 99%, or 100% sequence identity), and the VL_{B1} and the VL_{B2} share at least 95% sequence identity (e.g., 95%, 96%, 97%, 98%, 99%, or 100% sequence identity). 1Fab-IgG molecules have a monovalent arm comprising the first antigen-binding moiety that binds a first target antigen and a bivalent arm comprising the second and third antigen-binding moieties, wherein each of the second and third antigen-binding molecules specifically bind a second target cell antigen. In some embodiments, a 1Fab-IgG molecule is a 1Fab-IgG T-cell dependent bispecific molecule (1Fab-IgG TDB) where the monovalent arm specifically binds an antigen on the surface of a T cell, and each antigen-binding moiety of the bivalent arm specifically binds an antigen on the surface of a second target cell (e.g., a tumor cell). As with a TDB with a standard bivalent IgG format (IgG TDB), 1Fab-IgG TDBs recruit cytolytic T cells to kill the cells expressing the targeted antigen.

For example, an anti-CD3/HER2 1Fab-IgG TDB has a monovalent arm that specifically binds CD3 (e.g., a CD3 molecule on the surface of a T cell) and a bivalent arm that has two antigen-binding moieties that each specifically bind HER2 (e.g., a HER2 molecule on the surface of a tumor cell). The two antigen-binding moieties on the bivalent arm may bind the same epitope, or each antigen-binding moiety on the bivalent arm may bind a different epitope on the same antigen. In one embodiment, the bivalent arm binds to the same epitope as 4D5. In one embodiment, the bivalent arm binds to domain IV of HER2. In one embodiment, the antigen-binding moiety on the monovalent arm, specific for CD3, is a 40G5c Fab, and each of the two antigen-binding moieties on the bivalent arm, specific for HER2, is a variant of a 4D5 Fab. Particular embodiments are exemplified herein.

The bispecific antigen-binding molecules of the invention can provide increased sensitivity to cells that preferentially express a high density of antigen (e.g., tumor antigen), which, in many cases, reduces damage to healthy tissues (e.g., by activating T cells to engage tumor cells rather than healthy cells expressing low levels of tumor antigen). Accordingly, in some embodiments, a tumor antigen is expressed on (a) a tumor cell in a subject and (b) at least one type of non-tumor cell in the subject (e.g., at a lower density than its expression on tumor cells). In some embodiments, the ratio of tumor antigen copy number on the non-tumor cells to the tumor cells is from 1:2 to 1:1,000,000 (e.g., from 1:3 to 1:500,000, from 1:4 to 1:100,000, from 1:5 to 1:50,000, from 1:6 to 1:40,000, from 1:7 to 1: 20,000, from 1:8 to 1:10,000, from 1:9 to 1:5,000, from 1:10 to 1:1,000, from 1:20 to 1:500, from 1:50 to 1:400, or from 1:100 to 1:200, e.g., from 1:2 to 1:10, from 1:10 to 1:20, from 1:20 to 1:50, from 1:50 to 1:100, from 1:100 to 1:200, from 1:200 to 1:300, from 1:300 to 1:400, from 1:400 to 1:500, from 1:500 to 1:600, from 1:600 to 1:700, from 1:700 to 1:800, from 1:800 to 1:900, from 1:900 to 1:1,000, from 1:1,000 to 1:5,000, from 1:5,000 to 1:10,000, from 1:10,000 to 1:50,000, from 1:50,000 to 1:100,000, from 1:100,000 to 1:500,000, or from 1:500,000 to 1:1,000,000, e.g., about 1:2, about 1:3, about 1:4, about 1:5, about 1:6, about 1:7, about 1:8, about 1:9, about 1:10, about 1:15, about 1:20, about 1:25, about 1:30, about 1:35, about 1:40, about 1:50, about 1:60, about 1:70, about 1:80, about 1:90, about 1:100, about 1:125, about 1:150, about 1:175, about 1:200, about 1:300, about 1:400, about 1:500, about 1:1,000, about 1:10,000, about 1:100,000, or about 1:1,000,000).

In some embodiments, the tumor antigen copy number on a non-tumor cell is from 10^1 to 10^6 (e.g., from 10^1 to 10^6, from 10^1 to 10^5, from 10^1 to 10^4, from 10^1 to 10^3, from 10^1 to 10^2, from 10^2 to 10^6, from 10^2 to 10^5, from 10^2 to 10^4, from 10^2 to 10^3, from 10^3 to 10^6, from 10^3 to 10^5, from 10^3 to 10^4, from 10^4 to 10^6, from 10^4 to 10^5, or from 10^5 to 10^6, e.g., about 10^1, 10^2, 10^3, 10^4, 10^5, or 10^6). In some embodiments, the tumor antigen copy number on a tumor cell is from 10^2 to 10^7 (e.g., from 10^2 to 10^7, from 10^2 to 10^6, from 10^2 to 10^5, from 10^2 to 10^4, from 10^2 to 10^3, from 10^3 to 10^7, from 10^3 to 10^6, from 10^3 to 10^5, from 10^3 to 10^4, from 10^4 to 10^7, from 10^4 to 10^6, from 10^4 to 10^5, from 10^5 to 10^7, from 10^5 to 10^6, or from 10^6 to 10^7, e.g., about 10^2, 10^3, 10^4, 10^5, 10^6, or 10^7).

In some embodiments, the tumor antigen copy number is from 10^1 to 10^6 on a non-tumor cell and from 10^2 to 10^8 on a tumor cell (e.g., from 10^2 to 10^5 on a non-tumor cell and from 10^3 to 10^7 on a tumor cell, from 10^2 to 10^5 on a non-tumor cell and from 10^4 to 10^7 on a tumor cell, from 10^3 to 10^5 on a non-tumor cell and from 10^5 to 10^7 on a tumor cell, from 10^3 to 10^4 on a non-tumor cell and from 10^5 to 10^6 on a tumor cell, or from 10^4 to 10^5 on a non-tumor cell and from 10^6 to 10^7 on a tumor cell).

In some embodiments, the tumor antigen copy number (e.g., average tumor antigen copy number, e.g., HER2 copy number, e.g., average HER2 copy number) is from 10^1 to 2.0×10^5 on a non-tumor cell and at least 2.0×10^5 on a tumor cell.

Tumor cells bound by an antigen-binding molecule of the present invention may express HER2 at a copy number (e.g., an average copy number) of at least 200,000 per cell (e.g., at least 250,000 HER2 copies per cell, at least 300,000 HER2 copies per cell, at least 400,000 HER2 copies per cell, at least

500,000 HER2 copies per cell, at least 600,000 HER2 copies per cell, at least 700,000 HER2 copies per cell, at least 750,000 HER2 copies per cell, at least 800,000 HER2 copies per cell, at least 900,000 HER2 copies per cell, at least 1,000,000 HER2 copies per cell, at least 1,200,000 HER2 copies per cell, at least 1,500,000 HER2 copies per cell, at least 2,000,000 HER2 copies per cell, at least 2,500,000 HER2 copies per cell, at least 3,000,000 HER2 copies per cell, or more, e.g., from 200,000 to 3,000,000 HER2 copies per cell, from 250,000 to 2,500,000 HER2 copies per cell, from 300,000 to 2,000,000 HER2 copies per cell, from 400,000 to 1,500,000 HER2 copies per cell, or from 500,000 to 1,000,000 HER2 copies per cell, e.g., from 200,000 to 1,000,000 HER2 copies per cell (e.g., from 200,000 to 250,000 HER2 copies per cell, from 250,000 to 300,000 HER2 copies per cell, from 300,000 to 400,000 HER2 copies per cell, from 400,000 to 500,000 HER2 copies per cell, from 500,000 to 750,000 HER2 copies per cell, or from 750,000 to 1,000,000 HER2 copies per cell) or from 1,000,000 to 3,000,000 HER2 copies per cell (e.g., from 1,000,000 to 1,500,000 HER2 copies per cell, from 1,500,000 to 2,000,000 HER2 copies per cell, from 2,000,000 to 2,500,000 HER2 copies per cell, or from 2,500,000 to 3,000,000 HER2 copies per cell). Thus, HER2-positive tumor cells having any of the aforementioned HER2 expression characteristics may be preferentially killed (e.g., selectively killed) by T cells upon binding of an antigen-binding molecule of the present invention, relative to non-tumor cells that have little or no HER2 expression (e.g., less than 200,000 HER2 copies per cell, e.g., from 0 to 200,000 HER2 copies per cell, from 0 to 150,000 HER2 copies per cell, from 0 to 100,000 HER2 copies per cell, from 0 to 50,000 HER2 copies per cell, from 0 to 20,000 HER2 copies per cell, from 0 to 10,000 HER2 copies per cell, from 0 to 5,000 HER2 copies per cell, or from 0 to 1,000 HER2 copies per cell).

Tumor antigen copy number (e.g., average tumor antigen copy number, e.g., HER2 copy number, e.g., average HER2 copy number) can be quantified using any suitable means known in the art or described herein. For example, HER2 copy number can be quantified using fluorescence quantitation by flow cytometry (e.g., using beads of known Molecules of Equivalent Soluble Fluorochrome (MESF)).

In some embodiments, the monovalent binding affinity (K_D) of the second antigen-binding moiety and/or the third antigen-binding moiety is from 10 nM to 100 nM (e.g., from 20 nM to 90 nM, from 30 nM to 80 nM, from 40 nM to 60 nM, e.g., from 25 nM to 55 nM). In one embodiment, the monovalent binding affinity (K_D) of the second antigen-binding moiety and/or the third antigen-binding moiety is from 20 nM to 50 nM. In one embodiment, the monovalent binding affinity (K_D) of the second antigen-binding moiety and the third antigen-binding moiety is from 20 nM to 50 nM.

In some embodiments, the monovalent dissociation rate of the second antigen-binding moiety and/or the third antigen-binding moiety is from 10^{-3}/second to 10^{-1}/second (e.g., from 10^{-2}/second to 30^{-2}/second).

In some embodiments, a first target antigen is an activating T cell antigen, such as CD3. Various T cell antigen-binding moieties are known in the art and suitable for use as part of the present invention. For example, in certain embodiments of the present invention, a suitable first antigen-binding moiety is the antibody clone 40G5c, or a fragment and/or variant thereof, e.g., as described in U.S. Publication No. 2015/0166661, which is incorporated herein by reference in its entirety. In particular embodiments, the first antigen-binding moiety is an anti-CD3 Fab (e.g., 40G5c) comprising an HVR-H1 of SEQ ID NO: 1, an HVR-H2 of SEQ ID NO: 2, an HVR-H3 of SEQ ID NO: 3, an HVR-L1 of SEQ ID NO: 4, an HVR-L2 of

SEQ ID NO: 5, and an HVR-L3 of SEQ ID NO: 6. In some embodiments, the first antigen-binding moiety is an anti-CD3 Fab (e.g., 40G5c) comprising a VH of SEQ ID NO: 7 and a VL of SEQ ID NO: 8. Amino acid sequences of 40G5c are also provided in Table 1.

Table 1. SEQ ID NOs corresponding to hypervariable region (HVR) and variable region heavy (VH) and light (VL) chain amino acid sequences for exemplary antibody clones.

	HVR						V	
	H1	H2	H3	L1	L2	L3	VH	VL
40G5c	1	2	3	4	5	6	7	8
4D5 Consensus	11	12	13	14	15	16	17	18
4D5 Wildtype (trastuzumab)	11	19	20	21	22	23	24	25
4D5-H91A	11	19	20	21	22	26	24	27
4D5-Y55E.Y102V	11	19	28	21	29	23	30	31
4D5-D98A.F100A.Y102V	11	19	32	21	22	23	33	25
4D5-D98T.F100A.Y102V	11	19	34	21	22	23	35	25
4D5-N30S.N54E.D98T	11	36	37	38	22	23	39	40
4D5-N30S.H91A.N54E.D98T	11	36	37	38	22	26	41	42
4D5-H91A.N54E.D98T	11	36	37	21	22	26	41	27
4D5-N30S.Y55E.N54E.D98T.Y102V	11	36	43	38	29	23	44	45
4D5-N30S	11	19	20	38	22	23	24	40
4D5-N54E.D98T	11	36	37	21	22	23	41	25
4D5-N30S.N54E.D98T.F100A.Y102V	11	36	34	38	22	23	46	40
4D5-N30S.N54E.D98A.F100A.Y102V	11	36	32	38	22	23	47	40
4D5-Y55E.H91A.N54E.D98T	11	36	37	21	29	26	41	48
4D5-Y55E.H91A.N54E.D98T.Y102V	11	36	43	21	29	26	44	48
4D5-N30S.Y55E.H91A.N54E.D98T	11	36	37	38	29	26	41	49
4D5-N30S.Y55E.H91A.N54E.D98T.Y102V	11	36	43	38	29	26	44	49
4D5-Y102V	11	19	28	21	22	23	30	25
4D5-N30A.H91A	11	19	20	53	22	26	24	54
4D5-Y55E.H91A	11	19	20	21	29	26	24	48
4D5-D98A.F100A.Y102V	11	19	32	21	22	23	33	25
4D5-Y55E.D98A.F100A.Y102V	11	19	32	21	29	23	33	31

In some embodiments, the first antigen-binding moiety binds to a human CD3 polypeptide or a cynomolgus monkey (cyno) CD3 polypeptide. In some embodiments, the human CD3 polypeptide or the cyno CD3 polypeptide is a human CD3ε polypeptide or a cyno CD3ε polypeptide, respectively. In some embodiments, the human CD3 polypeptide or the cyno CD3 polypeptide is a human CD3γ polypeptide or a cyno CD3γ polypeptide, respectively. Additional antigen-binding moieties (e.g., Fab molecules) that bind to CD3 are known in the art and described, for example, in U.S. Publication No. 2015/0166661, and can be adapted for use as part of the present invention.

In some embodiments, the monovalent K_D of the first antigen-binding moiety binds the human CD3ε polypeptide with a K_D of 250 nM or lower. In some embodiments, the anti-CD3 antibody binds the human CD3ε polypeptide with a K_D of 100 nM or lower. In some embodiments, the anti-CD3 antibody binds the human CD3ε polypeptide with a K_D of 15 nM or lower. In some embodiments, the anti-CD3 antibody binds the human CD3ε polypeptide with a K_D of 10 nM or lower. In some embodiments, the anti-CD3 antibody binds the human CD3ε polypeptide with a K_D of 5 nM or lower. In some embodiments, the monovalent K_D of the first antigen-binding moiety is from 10 nM to 100 nM (e.g., from 20 nM to 90 nM, from 20 nM to 80 nM, from 30 nM to 70 nM, or from 40 nM to 60 nM).

In some embodiments, the second target cell antigen is a tumor antigen.

The tumor antigen may be HER2. A suitable second and/or third antigen-binding moiety that binds HER2 is the antibody 4D5, or a fragment and/or variant thereof. Amino acid sequences of 4D5, and substitution variants thereof, are shown in Table 1 and described, for example, in U.S. Publication No. 2015/0166661, which is incorporated herein by reference in its entirety. In one embodiment, the second and/or third antigen-binding moiety binds to the same epitope as the antibody 4D5 (humanized version thereof known as trastuzumab (HERCEPTIN®; Genentech, Inc., South San Francisco, CA), Molina, et al., *Cancer Research* 2001, 61 (12): 4744-4749). In one embodiment, the second and the third antigen-binding moiety binds to the same epitope as the antibody 4D5.

In one embodiment, the second and/or third antigen-binding moiety binds to the same epitope as the antibody 2C4 (humanized version thereof known as pertuzumab (PERJETA®; Genentech, Inc., South San Francisco, CA), Franklin et al. *Cancer Cell* 2004, 5: 317-328). In one embodiment, the second and the third antigen-binding moiety binds to the same epitope as the antibody 2C4.

In one embodiment, the second and/or third antigen-binding moiety binds to the same epitope as the antibody 7C2 (U.S. Patent No. 9,518,118). In one embodiment, the second and the third antigen-binding moiety binds to the same epitope as the antibody 7C2.

Figure 36 provides the crystal structure of the HER2 ECD and highlights the regions to which the different HER2 antibodies bind. 4D5 binds to an epitope in domain IV of HER2 that is the protein region closest to the cellular membrane. 2C4 binds to an epitope in domain II of HER2 that is 50 Angstroms from the region to which hu4D5 binds. 7C2 binds to an epitope in domain I of HER2 that is 100 Angstroms from the HER2 region bound by hu4D5.

In one embodiment, the second and/or third antigen-binding moiety binds to domain IV of HER2. In one embodiment, the second and the third antigen-binding moiety binds to domain IV of HER2. In one embodiment, the second and/or third antigen-binding moiety binds to domain II of HER2. In one embodiment, the second and the third antigen-binding moiety binds to domain II of HER2.

In one embodiment, the second and/or third antigen-binding moiety binds to domain I of HER2. In one embodiment, the second and the third antigen-binding moiety binds to domain I of HER2.

In one embodiment, the second-binding moiety binds to domain IV of HER2 and the third antigen-binding moiety binds to domain I of HER2.

In one embodiment, the second-binding moiety binds to domain IV of HER2 and the third antigen-binding moiety binds to domain II of HER2.

In one embodiment, the second-binding moiety binds to domain II of HER2 and the third antigen-binding moiety binds to domain I of HER2.

In some embodiments, the bispecific antigen-binding molecule of any of the preceding embodiments features a VH_A region comprising one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3. In some embodiments, the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7 (e.g., at least 96% sequence identity to SEQ ID NO: 7, at least 97% sequence identity to SEQ ID NO: 7, at least 98% sequence identity to SEQ ID NO: 7, at least 99% sequence identity to SEQ ID NO: 7, or 100% sequence identity to SEQ ID NO: 7). For example, in some embodiments, the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7. In some embodiments, the VL$_A$ region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8 (e.g., at least 96% sequence identity to SEQ ID NO: 8, at least 97% sequence identity to SEQ ID NO: 8, at least 98% sequence identity to SEQ ID NO: 8, at least 99% sequence identity to SEQ ID NO: 8, or 100% sequence identity to SEQ ID NO: 8). For example, in some embodiments, the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB, e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain).

In some embodiments, the VH$_A$ comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3; and the VL$_A$ comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6. In some embodiments, the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7 (e.g., at least 96% sequence identity to SEQ ID NO: 7, at least 97% sequence identity to SEQ ID NO: 7, at least 98% sequence identity to SEQ ID NO: 7, at least 99% sequence identity to SEQ ID NO: 7, or 100% sequence identity to SEQ ID NO: 7); and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8 (e.g., at least 96% sequence identity to SEQ ID NO: 8, at least 97% sequence identity to SEQ ID NO: 8, at least 98% sequence identity to SEQ ID NO: 8, at least 99% sequence identity to SEQ ID NO: 8, or 100% sequence identity to SEQ ID NO: 8). In some embodiments, the VH$_A$ region comprises the amino acid of SEQ ID NO: 7; and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB , e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain).

In some embodiments, the VH$_{B1}$ region and/or the VH$_{B2}$ region comprises an amino acid substitution at one, two, three, or all four residues of N54, D98, F100, and/or Y102, according to the Kabat numbering system. For example, the VH$_{B1}$ region and/or the VH$_{B2}$ region may feature an amino acid substitution at one, two, three, four, or all five of the following residues: N54E, D98A, D98T, F100A, and/or Y102V, according to the Kabat numbering system.

In some embodiments, the VL$_{B1}$ region and/or the VL$_{B2}$ region comprises an amino acid substitution at one, two, or all three residues of N30, Y55, and/or H91, according to the Kabat numbering system. For example, the VL_{B1} region and/or the VL_{B2} region may feature an amino acid substitution at one, two, or all three of the following residues: N30S, Y55E, and/or H91A.

In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17 (e.g., at least 96% sequence identity to SEQ ID NO: 17, at least 97% sequence identity to SEQ ID NO: 17, at least 98% sequence identity to SEQ ID NO: 17, at least 99% sequence identity to SEQ ID NO: 17, or 100% sequence identity to SEQ ID NO: 17). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 18, at least 98% sequence identity to SEQ ID NO: 18, at least 99% sequence identity to SEQ ID NO: 18, or 100% sequence identity to SEQ ID NO: 18). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17 (e.g., at least 96% sequence identity to SEQ ID NO: 17, at least 97% sequence identity to SEQ ID NO: 17, at least 98% sequence identity to SEQ ID NO: 17, at least 99% sequence identity to SEQ ID NO: 17, or 100% sequence identity to SEQ ID NO: 17); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 18, at least 98% sequence identity to SEQ ID NO: 18, at least 99% sequence identity to SEQ ID NO: 18, or 100% sequence identity to SEQ ID NO: 18). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 17; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17 (e.g., at least 96% sequence identity to SEQ ID NO: 17, at least 97% sequence identity to SEQ ID NO: 17, at least 98% sequence identity to SEQ ID NO: 17, at least 99%

sequence identity to SEQ ID NO: 17, or 100% sequence identity to SEQ ID NO: 17). In some embodiments, the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL$_{B2}$ region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 18, at least 98% sequence identity to SEQ ID NO: 18, at least 99% sequence identity to SEQ ID NO: 18, or 100% sequence identity to SEQ ID NO: 18). In some embodiments, the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the VH$_{B2}$ comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13; and the VL$_{B2}$ comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17 (e.g., at least 96% sequence identity to SEQ ID NO: 17, at least 97% sequence identity to SEQ ID NO: 17, at least 98% sequence identity to SEQ ID NO: 17, at least 99% sequence identity to SEQ ID NO: 17, or 100% sequence identity to SEQ ID NO: 17); and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 18, at least 98% sequence identity to SEQ ID NO: 18, at least 99% sequence identity to SEQ ID NO: 18, or 100% sequence identity to SEQ ID NO: 18). In some embodiments, the VH$_{B2}$ region comprises the amino acid of SEQ ID NO: 17; and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 18.

In some embodiments, the VL$_{B1}$ region and/or the VL$_{B2}$ region comprises an amino acid substitution at H91. For example, in some embodiments, the H91 residue is substituted with an amino acid having a nonpolar side chain. In some embodiments, the VL$_{B1}$ region and/or the VL$_{B2}$ region comprises the amino acid substitution of H91A. In some embodiments, the VL$_{B1}$ region and/or the VL$_{B2}$ region comprises an amino acid substitution at Y55. For example, in some embodiments, the Y55 residue is substituted with an amino acid having an acidic side chain. In some embodiments, the VL$_{B1}$ region and/or the VL$_{B2}$ region comprises the amino acid substitution of Y55E. In some embodiments, the VH$_{B1}$ region and/or the VH$_{B2}$ region comprises an amino acid substitution at F100 and/or Y102. For example, in some embodiments, the F100 residue and/or the Y102 residue is substituted with an amino acid having a nonpolar side chain. In some embodiments, the VH$_{B1}$ region and/or the VH$_{B2}$ region comprises the amino acid substitution of F100A and/or Y102V.

In some embodiments of the bispecific antigen-binding moieties, the VL$_{B1}$ region and/or the VL$_{B2}$ region comprises one or more liability fixed residues, e.g., one or more liability fixed residues comprising the amino acid substitution of N30S. Additionally or alternatively, the VH$_{B1}$ region and/or the VH$_{B2}$ region may feature one or more liability fixed residues, e.g., one or more liability fixed residues comprising one or more an amino acid substitutions selected from the group consisting of N54E, D98A, and D98T.

The bispecific antigen-binding molecule may feature a mutation at residue H91 of the light chain of the second and/or third antigen-binding moiety (e.g., H91A). For example, in some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24 (e.g., at least 96% sequence identity to SEQ ID NO: 24, at least 97% sequence identity to SEQ ID NO: 24, at least 98% sequence identity to SEQ ID NO: 24, at least 99% sequence identity to SEQ ID NO: 24, or 100% sequence identity to SEQ ID NO: 24). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 24. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27 (e.g., at least 96% sequence identity to SEQ ID NO: 27, at least 97% sequence identity to SEQ ID NO: 27, at least 98% sequence identity to SEQ ID NO: 27, at least 99% sequence identity to SEQ ID NO: 27, or 100% sequence identity to SEQ ID NO: 27). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 27.

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24 (e.g., at least 96% sequence identity to SEQ ID NO: 24, at least 97% sequence identity to SEQ ID NO: 24, at least 98% sequence identity to SEQ ID NO: 24, at least 99% sequence identity to SEQ ID NO: 24, or 100% sequence identity to SEQ ID NO: 24); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27 (e.g., at least 96% sequence identity to SEQ ID NO: 27, at least 97% sequence identity to SEQ ID NO: 27, at least 98% sequence identity to SEQ ID NO: 27, at least 99% sequence identity to SEQ ID NO: 27, or 100% sequence identity to SEQ ID NO: 27). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 24; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 27.

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24 (e.g., at least 96% sequence identity to SEQ ID NO: 24, at least

97% sequence identity to SEQ ID NO: 24, at least 98% sequence identity to SEQ ID NO: 24, at least 99% sequence identity to SEQ ID NO: 24, or 100% sequence identity to SEQ ID NO: 24). In some embodiments, the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 24. In some embodiments, the VL$_{B2}$ region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27 (e.g., at least 96% sequence identity to SEQ ID NO: 18, at least 97% sequence identity to SEQ ID NO: 27, at least 98% sequence identity to SEQ ID NO: 27, at least 99% sequence identity to SEQ ID NO: 27, or 100% sequence identity to SEQ ID NO: 27). In some embodiments, the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 27.

In some instances, the VH$_{B2}$ comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20; and the VL$_{B2}$ comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24 (e.g., at least 96% sequence identity to SEQ ID NO: 24, at least 97% sequence identity to SEQ ID NO: 24, at least 98% sequence identity to SEQ ID NO: 24, at least 99% sequence identity to SEQ ID NO: 24, or 100% sequence identity to SEQ ID NO: 24); and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27 (e.g., at least 96% sequence identity to SEQ ID NO: 27, at least 97% sequence identity to SEQ ID NO: 27, at least 98% sequence identity to SEQ ID NO: 27, at least 99% sequence identity to SEQ ID NO: 27, or 100% sequence identity to SEQ ID NO: 27). In some embodiments, the VH$_{B2}$ region comprises the amino acid of SEQ ID NO: 24; and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 27. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 H91A-1Fab-IgG TDB).

In other instances, the bispecific antigen-binding molecule may feature mutations at residues D98, F100, and Y102 of the heavy chain of the second and/or third antigen-binding moiety (e.g., D98A, F100A, and Y102V). In some embodiments, the bispecific antigen-binding molecule features a VH$_{B1}$ region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32. In some embodiments, the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33 (e.g., at least 96% sequence identity to SEQ ID NO: 33, at least 97% sequence identity to SEQ ID NO: 33, at least 98% sequence identity to SEQ ID NO: 33, at least 99% sequence identity to SEQ ID NO: 33, or 100% sequence identity to SEQ ID NO: 33). For example, in some embodiments, the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 33. In some embodiments, the VL$_{B1}$ region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or

(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25 (e.g., at least 96% sequence identity to SEQ ID NO: 25, at least 97% sequence identity to SEQ ID NO: 25, at least 98% sequence identity to SEQ ID NO: 25, at least 99% sequence identity to SEQ ID NO: 25, or 100% sequence identity to SEQ ID NO: 25). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33 (e.g., at least 96% sequence identity to SEQ ID NO: 33, at least 97% sequence identity to SEQ ID NO: 33, at least 98% sequence identity to SEQ ID NO: 33, at least 99% sequence identity to SEQ ID NO: 33, or 100% sequence identity to SEQ ID NO: 33); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25 (e.g., at least 96% sequence identity to SEQ ID NO: 25, at least 97% sequence identity to SEQ ID NO: 25, at least 98% sequence identity to SEQ ID NO: 25, at least 99% sequence identity to SEQ ID NO: 25, or 100% sequence identity to SEQ ID NO: 25). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 33; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33 (e.g., at least 96% sequence identity to SEQ ID NO: 33, at least 97% sequence identity to SEQ ID NO: 33, at least 98% sequence identity to SEQ ID NO: 33, at least 99% sequence identity to SEQ ID NO: 33, or 100% sequence identity to SEQ ID NO: 33). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 33. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25 (e.g., at least 96% sequence identity to SEQ ID NO: 25, at least 97% sequence identity to SEQ ID NO: 25, at least 98% sequence identity to SEQ ID NO: 25, at least 99% sequence identity to SEQ ID NO: 25, or 100% sequence identity to SEQ ID NO: 25). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 25.

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32; and the VL_{B2}

comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33 (e.g., at least 96% sequence identity to SEQ ID NO: 33, at least 97% sequence identity to SEQ ID NO: 33, at least 98% sequence identity to SEQ ID NO: 33, at least 99% sequence identity to SEQ ID NO: 33, or 100% sequence identity to SEQ ID NO: 33); and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25 (e.g., at least 96% sequence identity to SEQ ID NO: 25, at least 97% sequence identity to SEQ ID NO: 25, at least 98% sequence identity to SEQ ID NO: 25, at least 99% sequence identity to SEQ ID NO: 25, or 100% sequence identity to SEQ ID NO: 25). In some embodiments, the VH$_{B2}$ region comprises the amino acid of SEQ ID NO: 33; and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 25. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 D98A.F100A.Y102V-1Fab-IgG TDB).

In other embodiments, the bispecific antigen-binding molecule may feature mutations at residues Y55 and H91 of the light chain and at residues N54 and D98 of the heavy chain of the second and/or third antigen-binding moiety (e.g., Y55E, H91A, N54E, and D98T). In some embodiments, the bispecific antigen-binding molecule features a VH$_{B1}$ region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41). For example, in some embodiments, the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the VL$_{B1}$ region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). For example, in some embodiments, the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 48.

In some embodiments, the VH$_{B1}$ comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL$_{B1}$ comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41); and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). For example, in some embodiments, the VH$_{B1}$ region comprises the amino acid of SEQ ID NO: 41; and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 48.

In some embodiments, the VH$_{B2}$ region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41). In some embodiments, the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the VL$_{B2}$ region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). In some embodiments, the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 48.

In some embodiments, the VH$_{B2}$ comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL$_{B2}$ comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41); and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). In some embodiments, the VH$_{B2}$ region comprises the amino acid of SEQ ID NO: 41; and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In other embodiments, the bispecific antigen-binding molecule may feature mutations at residues Y55 and H91 of the light chain and at residues N54, D98, and Y102 of the heavy chain of the second and/or third antigen-binding moiety (e.g., Y55E, H91A, N54E, D98T, and Y102). In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 44. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48.

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 41; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48.

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least

95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 44. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48.

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43; and the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44); and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48 (e.g., at least 96% sequence identity to SEQ ID NO: 48, at least 97% sequence identity to SEQ ID NO: 48, at least 98% sequence identity to SEQ ID NO: 48, at least 99% sequence identity to SEQ ID NO: 48, or 100% sequence identity to SEQ ID NO: 48). In some embodiments, the VH_{B2} region comprises the amino acid of SEQ ID NO: 44; and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In other embodiments, the bispecific antigen-binding molecule may feature mutations at residues N30, Y55, and H91 of the light chain and at residues N54 and D98 of the heavy chain of the second and/or third antigen-binding moiety (e.g., N30S, Y55E, H91A, N54E, and D98T). In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 41.

In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49.

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 41; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49.

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 41. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 49.

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41 (e.g., at least 96% sequence identity to SEQ ID NO: 41, at least 97% sequence identity to SEQ ID NO: 41, at least 98% sequence identity to SEQ ID NO: 41, at least 99% sequence identity to SEQ ID NO: 41, or 100% sequence identity to SEQ ID NO: 41); and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). In some embodiments, the VH_{B2} region comprises the amino acid of SEQ ID NO: 41; and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T-1Fab-IgG TDB).

In other embodiments, the bispecific antigen-binding molecule may feature mutations at residues N30, Y55, and H91 of the light chain and at residues N54, D98, and Y102 of the heavy chain of the second and/or third antigen-binding moiety (e.g., N30S, Y55E, H91A, N54E, D98T, and Y102V). In some embodiments, the bispecific antigen-binding molecule features a VH_{B1} region which comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44). For example, in some embodiments, the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 44. In some embodiments, the VL_{B1} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). For example, in some embodiments, the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49.

In some embodiments, the VH_{B1} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43; and the VL_{B1} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,

(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44); and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). For example, in some embodiments, the VH_{B1} region comprises the amino acid of SEQ ID NO: 44; and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49.

In some embodiments, the VH_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44). In some embodiments, the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 44. In some embodiments, the VL_{B2} region comprises one, two, or all three of the following HVRs: (a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or (c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). In some embodiments, the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 49.

In some embodiments, the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43; and the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44 (e.g., at least 96% sequence identity to SEQ ID NO: 44, at least 97% sequence identity to SEQ ID NO: 44, at least 98% sequence identity to SEQ ID NO: 44, at least 99% sequence identity to SEQ ID NO: 44, or 100% sequence identity to SEQ ID NO: 44); and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49 (e.g., at least 96% sequence identity to SEQ ID NO: 49, at least 97% sequence identity to SEQ ID NO: 49, at least 98% sequence identity to SEQ ID NO: 49, at least 99% sequence identity to SEQ ID NO: 49, or 100% sequence identity to SEQ ID NO: 49). In some embodiments, the VH_{B2} region comprises the amino acid of SEQ ID NO: 44; and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-HER2 bispecific antigen-binding molecule (e.g., 4D5 N30S.Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB).

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 28; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 30. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 30 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 31.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 34; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 35 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 25.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 39 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 40.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37;

and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 41 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 42.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 41 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 27.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 44 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 45.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 24 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 40.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 41 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 25.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 34; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 46 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 40.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 47 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 40.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 28; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and

(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 30 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 25.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 24 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 54.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 24 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 48.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 33 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 25.

In some embodiments, the VH_{B1} and/or the VH_{B2} comprises the following HVRs: (a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and (c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32; and the VL_{B1} and/or the VL_{B2} comprises the following HVRs: (d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the VH_{B1} and/or the VH_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 33 and the VL_{B1} and/or the VL_{B2} comprises an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, at least 97%, at least 98%, at least 99% sequence identity) to, or the sequence of, SEQ ID NO: 31.

In another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab_A, wherein the C-terminus of the Fab_A is fused to an N-terminus of a first Fc subunit, and wherein the Fab_A comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab_{B1} and a Fab_{B2}, wherein the C-terminus of the Fab_{B2} is fused to the N-terminus of the Fab_{B1}, and the C-terminus of the Fab_{B1} is fused to an N-terminus of a second Fc subunit, wherein the Fab_{B1} and the Fab_{B2} each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 12, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 13, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 14, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 15, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 16; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab_A comprises a VH_A region and a VL_A region, wherein the VH_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab_{B1} comprises a VH_{B1} region and a VL_{B1} region, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17, and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18; and (c) the Fab_{B2} comprises a VH_{B2} region and a VL_{B2} region, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 17, and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 18. For example, in some embodiments, (a) the VH_A region comprises the amino acid sequence of SEQ ID NO: 7, and the VL_A region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 17, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 18; and (c) the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 17, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 18.

In another aspect, the invention provides a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 22, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27. For example, in some embodiments, (a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 24, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 27; and (c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 24, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 27.

In yet another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25. For example, in some embodiments, (a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 33, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 25; and (c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 33, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 25.

In another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48. For example, in some embodiments, (a) the VH_A region comprises the amino acid sequence of SEQ ID NO: 7, and the VL_A region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 41, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48; and (c) the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 41, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48.

In another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab_A, wherein the C-terminus of the Fab_A is fused to an N-terminus of a first Fc subunit, and wherein the Fab_A comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab_{B1} and a Fab_{B2}, wherein the C-terminus of the Fab_{B2} is fused to the N-terminus of the Fab_{B1}, and the C-terminus of the Fab_{B1} is fused to an N-terminus of a second Fc subunit, wherein the Fab_{B1} and the Fab_{B2} each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab_A comprises a VH_A region and a VL_A region, wherein the VH_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab_{B1} comprises a VH_{B1} region and a VL_{B1} region, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48; and (c) the Fab_{B2} comprises a VH_{B2} region and a VL_{B2} region, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48. For example, in some embodiments, (a) the VH_A region comprises the amino acid sequence of SEQ ID NO: 7, and the VL_A region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 44, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48; and (c) the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 44, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3/HER2 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB, e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain and two 4D5 Y55E.H91A.N54E.D98T.Y102V HER2 binding domains).

In yet another aspect, the invention provides a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab$_{A}$, wherein the C-terminus of the Fab$_{A}$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_{A}$ comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_{A}$ comprises a VH$_{A}$ region and a VL$_{A}$ region, wherein the VH$_{A}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_{A}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49. For example, in some embodiments, (a) the VH$_{A}$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_{A}$ region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 41, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 49; and (c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 41, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3/HER2 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB, e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain and two 4D5 N30S.Y55E.H91A.N54E.D98T HER2 binding domains).

In another aspect, the invention features a bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein: (a) the monovalent arm comprises a Fab$_{A}$, wherein the C-terminus of the Fab$_{A}$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_{A}$ comprises the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6; (b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs: (i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11, (ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, (iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43, (iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38, (v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and (vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and (c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain. In some embodiments, (a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8; (b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49; and (c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49. For example, in some embodiments, (a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8; (b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 44, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 49; and (c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 44, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 49. For example, in some embodiments, the bispecific antigen-binding molecule is an anti-CD3/HER2 bispecific antigen-binding molecule (e.g., an anti-CD3/HER2 1Fab-IgG TDB, e.g., an anti-CD3/HER2 1Fab-IgG TDB having a 40G5c CD3 binding domain and two 4D5 N30S.Y55E.H91A.N54E.D98T.Y102V HER2 binding domains).

Peptide linkers fusing the second and third antigen-binding moieties

In some embodiments, a bispecific antigen-binding molecule of the invention features a structure wherein the C-terminus of the third antigen-binding moiety is fused to the N-terminus of the second antigen-binding moiety via a peptide linker. The peptide linker can be 5-20 amino acids in length (e.g., 5-10, 10-15, or 15-20, e.g., 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length). In some embodiments, the peptide linker is the natural amino acid sequence of the variable heavy chain hinge region (e.g., DKTHT; SEQ ID NO: 50). Alternatively, in some embodiments, the peptide linker includes the G$_4$SG$_2$ linker (SEQ ID NO: 51). In some embodiments, the peptide linker comprises the G$_4$SG$_2$ linker and the hinge region (e.g., SEQ ID NO: 52).

The amino acid sequences of exemplary heavy chain polypeptides comprising the second and third antigen-binding moiety heavy chains, with and without a G$_4$SG$_2$ linker, are provided in Table 2.

Table 2. SEQ ID NOs corresponding to polypeptides comprising the Fc domain, the second antigen-binding moiety heavy chain region, and the third antigen-binding moiety heavy chain region of exemplary 1Fab-IgG TDB molecules, including and without a G_4SG_2 linker. The sequences shown below include a hinge sequence as part of the linker peptide fusing the second and third antigen-binding moiety heavy chains.

	Without G_4SG_2	**Including G_4SG_2**
4D5 Consensus	55	56
4D5 Wildtype (trastuzumab)	57	58
4D5 H91A	59	60
4D5 Y55E.Y102V	61	62
4D5 D98A.F100A.Y102V	63	64
4D5 D98T.F100A.Y102V	65	66
4D5 N30S.N54E.D98T	67	68
4D5 N30S.H91A.N54E.D98T	69	70
4D5 H91A.N54E.D98T	71	72
4D5 N30S.Y55E.N54E.D98T.Y102V	73	74
4D5 N30S	75	76
4D5 N54E.D98T	77	78
4D5 N30S.N54E.D98T.F100A.Y102V	79	80
4D5 N30S.N54E.D98A.F100A.Y102V	81	82
4D5 Y55E.H91A.N54E.D98T	83	84
4D5 Y55E.H91A.N54E.D98T.Y102V	85	86
4D5 N30S.Y55E.H91A.N54E.D98T	87	88
4D5 N30S.Y55E.H91A.N54E.D98T.Y102V	89	90

In particular, a bispecific antigen-binding molecule of the invention may feature a peptide linker comprising the amino acid sequence of SEQ ID NO: 50. In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 55 (e.g., at least 96% sequence identity to SEQ ID NO: 55, at least 97% sequence identity to SEQ ID NO: 55, at least 98% sequence identity to SEQ ID NO: 55, at least 99% sequence identity to SEQ ID NO: 55, or 100% sequence identity to SEQ ID NO: 55). In other instances, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 59 (e.g., at least 96% sequence identity to SEQ ID NO: 59, at least 97% sequence identity to SEQ ID NO: 59, at least 98% sequence identity to SEQ ID NO: 59, at least 99% sequence identity to SEQ ID NO: 59, or 100% sequence identity to SEQ ID NO: 59). Alternatively, the antigen-binding molecule may comprise an amino acid sequence having at least 95% sequence identity SEQ ID NO: 63 (e.g., at least 96% sequence identity to SEQ ID NO: 63, at least 97% sequence identity to SEQ ID NO: 63, at least 98% sequence identity to SEQ ID NO: 63, at least 99% sequence identity to SEQ ID NO: 63, or 100% sequence identity to SEQ ID NO: 63). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 83 (e.g., at least 96% sequence identity to SEQ ID NO: 83, at least 97% sequence identity to SEQ ID NO: 83, at least 98% sequence identity to SEQ ID NO: 83, at least 99% sequence identity to SEQ ID NO: 83, or 100% sequence identity to SEQ ID NO: 83). In other instances, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 85 (e.g., at least 96% sequence identity to SEQ ID NO: 85, at least 97% sequence identity to SEQ ID NO: 85, at least 98% sequence identity to SEQ ID NO: 85, at least 99% sequence identity to SEQ ID NO: 85, or 100% sequence identity to SEQ ID NO: 85). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 87 (e.g., at least 96% sequence identity to SEQ ID NO: 87, at least 97% sequence identity to SEQ ID NO: 87, at least 98% sequence identity to SEQ ID NO: 87, at least 99% sequence identity to SEQ ID NO: 87, or 100% sequence identity to SEQ ID NO: 87). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 89 (e.g., at least 96% sequence identity to SEQ ID NO: 89, at least 97% sequence identity to SEQ ID NO: 89, at least 98% sequence identity to SEQ ID NO: 89, at least 99% sequence identity to SEQ ID NO: 89, or 100% sequence identity to SEQ ID NO: 89).

In other instances, the peptide linker comprises the amino acid sequence of SEQ ID NO: 51. The antigen-binding molecule may comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 56 (e.g., at least 96% sequence identity to SEQ ID NO: 56, at least 97% sequence identity to SEQ ID NO: 56, at least 98% sequence identity to SEQ ID NO: 56, at least 99% sequence identity to SEQ ID NO: 56, or 100% sequence identity to SEQ ID NO: 56). In some embodiments, the antigen-binding molecule may comprise an amino acid sequence having at least 95% sequence identity SEQ ID NO: 60 (e.g., at least 96% sequence identity to SEQ ID NO: 60, at least 97% sequence identity to SEQ ID NO: 60, at least 98% sequence identity to SEQ ID NO: 60, at least 99% sequence identity to SEQ ID NO: 60, or 100% sequence identity to SEQ ID NO: 60). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 64 (e.g., at least 96% sequence identity to SEQ ID NO: 64, at least 97% sequence identity to SEQ ID NO: 64, at least 98% sequence identity to SEQ ID NO: 64, at least 99% sequence identity to SEQ ID NO: 64, or 100% sequence identity to SEQ ID NO: 64). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 84 (e.g., at least 96% sequence identity to SEQ ID NO: 84, at least 97% sequence identity to SEQ ID NO: 84, at least 98% sequence identity to SEQ ID NO: 84, at least 99% sequence identity to SEQ ID NO: 84, or 100% sequence identity to SEQ ID NO: 84). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 86 (e.g., at least 96% sequence identity to SEQ ID NO: 86, at least 97% sequence identity to SEQ ID NO: 86, at least 98% sequence identity to SEQ ID NO: 86, at least 99% sequence identity to SEQ ID NO: 86, or 100% sequence identity to SEQ ID NO: 86). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 88 (e.g., at least 96% sequence identity to SEQ ID NO: 88, at least 97% sequence identity to SEQ ID NO: 88, at least 98% sequence identity to SEQ ID NO: 88, at least 99% sequence identity to SEQ ID NO: 88, or 100% sequence identity to SEQ ID NO: 88). In some embodiments, the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity SEQ ID NO: 90 (e.g., at least 96% sequence identity to SEQ ID NO: 90, at least 97% sequence identity to SEQ ID NO: 90, at least 98% sequence identity to SEQ ID NO: 90, at least 99% sequence identity to SEQ ID NO: 90, or 100% sequence identity to SEQ ID NO: 90).

Fc domains

Bispecific antigen-binding molecules of the invention may feature an Fc domain. The Fc domain may be an IgG Fc domain (e.g., an IgG_1 or IgG_4 Fc domain). For example, the Fc domain can be a human Fc domain. In some embodiments, the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function. For example, in some embodiments, the one or more amino acid substitutions that reduces binding to an Fc receptor and/or effector function is at one or more position selected from the group of L234, L235, and P329 (e.g., wherein the first Fc subunit and the second Fc subunit each comprises the amino acid substitutions of L234A, L235A and P329G). The Fc receptor may be, for example, an Fcγ receptor. Thus, the bispecific antigen-binding molecules of the invention can be configured to reduce antibody-dependent cell-mediated cytotoxicity (ADCC).

In some instances, the Fc domain comprises a modification configured to promote the association of the first Fc subunit with the second Fc subunit. “Knob-in-hole” engineering of bispecific antibodies may be utilized to generate a first arm containing a knob and a second arm containing the hole into which the knob of the first arm may bind. The knob of the multispecific antibodies of the invention may be a monovalent arm (e.g., anti-CD3 arm) in one embodiment. Alternatively, the knob of the multispecific antibodies of the invention may be a bivalent arm (e.g., anti-tumor arm). The hole of the multispecific antibodies of the invention may be a monovalent arm (e.g., anti-CD3 arm) in one embodiment. Alternatively, the hole of the multispecific antibodies of the invention may be a bivalent arm (e.g., anti-tumor arm). Bispecific antibodies may also be engineered using immunoglobulin crossover (also known as Fab domain exchange or CrossMab format) technology (see e.g., WO 2009/080253; Schaefer et al., *Proc. Natl. Acad. Sci. USA*, 108:11187-11192 (2011)). Bispecific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., US Patent No. 4,676,980, and Brennan et al., *Science*, 229: 81 (1985)); or by using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992)).

An amino acid residue in the CH3 domain of the second Fc subunit may be replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance (e.g., a knob) within the CH3 domain of the second Fc subunit which is positionable in a cavity (e.g., a hole) within the CH3 domain of the first Fc subunit, and an amino acid residue in the CH3 domain of the first Fc subunit may be replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity (e.g., a hole) within the CH3 domain of the first Fc subunit within which the protuberance (e.g., a knob) within the CH3 domain of the second Fc subunit may be positionable. In some embodiments, the

CH3 domain of the second Fc subunit comprises the amino acid substitution of T366, and the CH3 domain of the first Fc subunit comprises amino acid substitutions at one, two, or all three of T366, L368, and/or Y407. In some embodiments, the CH3 domain of the second Fc subunit comprises the amino acid substitution of T366W, and the CH3 domain of the first Fc subunit comprises one, two, or all three amino acid substitutions of T366S, L368A, and/or Y407V.

It is expressly contemplated that such bispecific antigen-binding molecules or other antibodies described herein for use in any of the instances enumerated herein may have any of the features, singly or in combination, described in Sections 1-6 below.

1. Antibody Affinity

In certain instances, a bispecific antigen-binding molecule has an equilibrium dissociation constant (K_D) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM (e.g., 10^{-8} M or less, e.g., from 10^{-8} M to 10^{-13} M, e.g., from 10^{-9} M to 10^{-13} M).

In one instance, K_D is measured by a radiolabeled antigen binding assay (RIA). In one instance, an RIA is performed with the Fab version of an antibody of interest and its antigen. For example, solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (about 23℃). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

According to another instance, K_D is measured using a BIACORE® surface plasmon resonance assay. For example, an assay using a BIACORE®-2000 or a BIACORE®-3000 (BIACORE®, Inc., Piscataway, NJ) is performed at 25℃ with immobilized antigen CM5 chips at ~10 response units (RU). In one instance, carboxymethylated dextran biosensor chips (CM5, BIACORE®, Inc.) are activated with *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve about 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with

0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25℃ at a flow rate of about 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off}/k_{on}. See, for example, Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds $10^6\ M^{-1}s^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25℃ of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO ™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. *Antibody Fragments*

In certain instances, a bispecific antigen-binding molecule provided herein is includes one or more antibody fragments. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased *in vivo* half-life, see U.S. Patent No. 5,869,046.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al. *Nat. Med.* 9:129-134 (2003); and Hollinger et al. *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al. *Nat. Med.* 9:129-134 (2003).

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain instances, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g., *E. coli* or phage), as described herein.

3. *Chimeric and Humanized Antibodies*

In certain instances, a bispecific antigen-binding molecule provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al. *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

In certain instances, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some instances, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.

Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008), and are further described, e.g., in Riechmann et al., *Nature* 332:323-329 (1988); Queen et al., *Proc. Natl. Acad. Sci. USA* 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, *Methods* 36:25-34 (2005) (describing specificity determining region (SDR) grafting); Padlan, *Mol. Immunol.* 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua et al., *Methods* 36:43-60 (2005) (describing "FR shuffling"); and Osbourn et al., *Methods* 36:61-68 (2005) and Klimka et al., *Br. J. Cancer*, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.* 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); and Presta et al. *J. Immunol.*, 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.* 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.* 272:10678-10684 (1997) and Rosok et al., *J. Biol. Chem.* 271:22611-22618 (1996)).

4. Human Antibodies

In certain instances, a bispecific antigen-binding molecule provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5: 368-74 (2001) and Lonberg, *Curr. Opin. Immunol.* 20:450-459 (2008).

Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:1117-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology. Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.

Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, e.g., Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., *J. Immunol.*, 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also described in Li et al., *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, *Xiandai Mianyixue*, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, *Histology and Histopathology*, 20(3):927-937 (2005) and Vollmers and Brandlein, *Methods and Findings in Experimental and Clinical Pharmacology*, 27(3):185-91 (2005).

Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. *Library-Derived Antibodies*

Bispecific antigen-binding molecules of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, 2001) and further described, e.g., in the McCafferty et al., *Nature* 348:552-554; Clackson et al., *Nature* 352: 624-628 (1991); Marks et al., *J. Mol. Biol.* 222: 581-597 (1992); Marks and Bradbury, in *Methods in Molecular Biology* 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2): 119-132(2004).

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter et al., *Ann. Rev. Immunol.*, 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths et al., *EMBO J*, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, *J. Mol. Biol.*, 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Antigen-Binding Molecule Variants

In certain instances, amino acid sequence variants of the bispecific antigen-binding molecules of the invention are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, for example, antigen-binding.

In certain embodiments, a bispecific antigen-binding molecule of the invention comprises one or more modifications in the VH/VL region and/or CH1/CL region to facilitate correct heavy/light chain pairing. In some embodiments, a bispecific antigen-binding molecule of the invention comprises one or more modifications in the Fc region to facilitate heterodimerization of the two arms of the bispecific antigen-binding molecule. Such modifications in the VH/VL region, CH1/CL region, and/or FC region are described in International Patent Publication No. WO 2016/172485, which is herein incorporated by reference in its entirety.

A. Substitution, Insertion, and Deletion Variants

In certain instances, antigen-binding moiety variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 3 under the heading of “preferred substitutions.” More substantial changes are provided in Table 3 under the heading of “exemplary substitutions,” and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, for example, retained/improved antigen binding, decreased immunogenicity, or improved Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) or Complement Dependent Cytotoxicity (CDC).

Table 3. Exemplary and Preferred Amino Acid Substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;
(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;
(3) acidic: Asp, Glu;
(4) basic: His, Lys, Arg;
(5) residues that influence chain orientation: Gly, Pro; or
(6) aromatic: Trp, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity and/or reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, for example, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g., binding affinity).

Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or residues that contact antigen, with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001)). In some instances of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain instances, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may, for example, be outside of antigen-contacting residues in the HVRs. In certain instances of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

B. Glycosylation variants

In certain instances, antigen-binding molecules of the invention can be altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody of the invention may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antigen-binding molecule comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH* 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some instances, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

In one instance, antigen-binding molecule variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g., complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, for example, U.S. Publication Nos. US 2003/0157108 and US 2004/0093621. Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.* 336:1239-1249 (2004); Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.* 249:533-545 (1986); U.S. Pat. Appl. No. US 2003/0157108 A1; and WO 2004/056312 A1, Adams et al., especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, *FUT8,* knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.* 87: 614 (2004); Kanda, Y. et al., *Biotechnol. Bioeng.*, 94(4):680-688 (2006); and WO2003/085107).

Antigen-binding molecule variants are further provided with bisected oligosaccharides, for example, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by

GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878; US Patent No. 6,602,684; and US 2005/0123546. Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

C. Fc region variants

In certain instances, one or more amino acid modifications may be introduced into the Fc region of an antibody of the invention, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g., a substitution) at one or more amino acid positions.

In certain instances, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest are described in U.S. Patent No. 5,500,362 (see, e.g., Hellstrom, I. et al. *Proc. Natl. Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al., *Proc. Natl. Acad. Sci. USA* 82:1499-1502 (1985); U.S. Patent No. 5,821,337 (see Bruggemann, M. et al., *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CYTOTOX 96® non-radioactive cytotoxicity assay (Promega, Madison, WI))). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al. *Proc. Natl. Acad. Sci. USA* 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, e.g., Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg et al., *Blood.* 101:1045-1052 (2003); and Cragg et al., *Blood.* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art (see, e.g., Petkova et al. *Int'l. Immunol.* 18(12):1759-1769 (2006)).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent Nos. 6,737,056 and 8,219,149). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent Nos. 7,332,581 and 8,219,149).

Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

In certain instances, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

In some instances, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.* 164: 4178-4184 (2000).

Antibodies with increased half-lives and improved binding to the neonatal Fc 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)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).

See also Duncan & Winter, *Nature* 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

D. Cysteine engineered antibody variants

In certain instances, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular instances, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain instances, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

E. Antibody derivatives

In certain instances, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

In another instance, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one instance, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA* 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

F. Immunoconjugates

The invention also provides immunoconjugates comprising bispecific antigen-binding molecule conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one instance, an immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Patent Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Patent Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Patent Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.* 53:3336-3342 (1993); and Lode et al., *Cancer Res.* 58:2925-2928 (1998)); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.* 13:477-523 (2006); Jeffrey et al., *Bioorganic & Med. Chem. Letters* 16:358-362 (2006); Torgov et al., *Bioconj. Chem.* 16:717-721 (2005); Nagy et al., *Proc. Natl. Acad. Sci. USA* 97:829-834 (2000); Dubowchik et al., *Bioorg. & Med. Chem. Letters* 12:1529-1532 (2002); King et al., *J. Med. Chem.* 45:4336-4343 (2002); and U.S. Patent No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

In another instance, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

In another instance, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science* 238:1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a “cleavable linker” facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.* 52:127-131 (1992); U.S. Patent No. 5,208,020) may be used.

The immunuoconjugates or ADCs herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., U.S.A).

III. Recombinant Methods and Compositions

Bispecific antigen-binding molecules of the invention may be produced using recombinant methods and compositions, for example, as described in U.S. Patent No. 4,816,567 and in U.S. Publication No. 2013/0078249, which is incorporated herein by reference in its entirety. In one embodiment, isolated nucleic acid (e.g., a polynucleotide) encoding bispecific antigen-binding molecule described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising a VH of the bispecific antigen-binding molecule (e.g., the light and/or heavy chains of the either arm of the bispecific antigen-binding molecule). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided.

Polynucleotides encoding bispecific antigen-binding molecules of the invention may be expressed as a single polynucleotide molecule or as multiple (e.g., two or more) polynucleotides that are co-expressed. Polypeptides encoded by polynucleotides that are co-expressed may associate through, e.g., disulfide bonds or other means to form a functional bispecific antigen-binding molecule. For example, a light chain portion of an antigen binding moiety may be encoded by a separate polynucleotide from the portion of the bispecific antigen-binding molecule comprising the heavy chain portion of the antigen binding moiety, an Fc domain subunit and optionally another antigen binding moiety. When co-expressed, the heavy chain polypeptides will associate with the light chain polypeptides to form the antigen binding moiety. In another example, the portion of the bispecific antigen-binding molecule comprising one of the two Fc domain subunits and optionally one or more antigen-binding moieties could be encoded by a separate polynucleotide from the portion of the T cell activating bispecific antigen binding molecule comprising the other of the two Fc domain subunits and optionally an antigen binding moiety. When co-expressed, the Fc domain subunits will associate to form the Fc domain.

In certain embodiments, an isolated polynucleotide of the invention encodes a fragment of a bispecific antigen-binding molecule comprising a first and a second antigen-binding moiety, and an Fc domain consisting of two subunits. In one embodiment, an isolated polynucleotide of the invention encodes the first antigen binding moiety and a subunit of the Fc domain. In another embodiment, an isolated polynucleotide of the invention encodes the heavy chain of the second antigen binding moiety and a subunit of the Fc domain. In a more specific embodiment, the isolated polynucleotide encodes a polypeptide, wherein a Fab heavy chain shares a C-terminal peptide bond with an Fc domain subunit. In yet another embodiment, an isolated polynucleotide of the invention encodes the heavy chain of the third antigen-binding moiety, the heavy chain of the second antigen binding moiety, and a subunit of the Fc domain. In some embodiments, the light chains of the second and third antigen-binding moieties are co-expressed and associate with the heavy chain regions to form Fab domains.

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 at least one VL of the bispecific antigen-binding molecule and an amino acid sequence comprising at least one VH of the bispecific antigen-binding molecule, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising a VL of the bispecific antigen-binding molecule and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising a VH of the bispecific antigen-binding molecule. In one embodiment, the host cell is eukaryotic, e.g., a Chinese Hamster Ovary (CHO) cell or lymphoid cell. In one embodiment, a method of making a bispecific antigen-binding molecule is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the bispecific antigen-binding molecule, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of a bispecific antigen-binding molecule, a polynucleotide encoding a bispecific antigen-binding molecule, 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 bispecific antigen-binding molecule).

Suitable host cells for cloning or expression of bispecific antigen-binding molecule-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent 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, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli.*) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

IV. Pharmaceutical Formulations

Therapeutic formulations of the bispecific antigen-binding molecules of the present invention are prepared for storage by mixing the bispecific antigen-binding molecule having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients, or stabilizers in the form of lyophilized formulations or aqueous solutions. For general information concerning formulations, see, e.g., Gilman et al. (eds.) *The Pharmacological Bases of Therapeutics*, 8th Ed., Pergamon Press, 1990; A. Gennaro (ed.), *Remington's Pharmaceutical Sciences,* 18th Edition, Mack Publishing Co., Pennsylvania, 1990; Avis et al. (eds.) *Pharmaceutical Dosage Forms: Parenteral Medications* Dekker, New York, 1993; Lieberman et al. (eds.) *Pharmaceutical Dosage Forms: Tablets* Dekker, New York, 1990; Lieberman et al. (eds.), *Pharmaceutical Dosage Forms: Disperse Systems* Dekker, New York, 1990; and Walters (ed.) *Dermatological and Transdermal Formulations* (Drugs and the Pharmaceutical Sciences), Vol 119, Marcel Dekker, 2002.

Acceptable carriers, excipients, or stabilizers are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™, or polyethylene glycol (PEG).

The formulation herein may also contain more than one active compound, preferably those with complementary activities that do not adversely affect each other. The type and effective amounts of such medicaments depend, for example, on the amount and type of antagonist present in the formulation, and clinical parameters of the subjects.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

V. Therapeutic Methods

Any of the bispecific antigen-binding molecules of the invention (e.g., bispecific antigen-binding molecules having a monovalent arm capable of specific binding to a first antigen and a bivalent arm capable of specific binding to two second antigens) or compositions thereof may be used in any of the therapeutic methods described herein.

In one aspect, a bispecific antigen-binding molecule for use as a medicament is provided. For example, in some embodiments, the bispecific antigen-binding molecules described herein are for use in treating or delaying progression of a cell proliferative disorder (e.g., cancer) or an autoimmune disorder in a subject in need thereof. In some embodiments, the bispecific antigen-binding molecule of any of the preceding aspects are for use in enhancing immune function in a subject having a cell proliferative disorder (e.g., cancer) or an autoimmune disorder. In certain embodiments, the invention provides bispecific antigen-binding molecule for use in a method of enhancing immune function in an individual having a cell proliferative disorder or an autoimmune disorder comprising administering to the individual an effective of the bispecific antigen-binding molecule to activate effector cells (e.g., T cells, e.g., CD8+ and/or CD4+ T cells), expand (increase) an effector cell population, reduce a population of target cells (e.g., a population of cells expressing a second target cell antigen recognized by a bispecific antigen-binding molecule of the invention), and/or kill a target cell (e.g., target tumor cell).

The invention features use of the bispecific antigen-binding molecule of the invention in the manufacture of a medicament for treating or delaying progression of a disorder, such as a cell proliferative disorder (e.g., a cancer) or an autoimmune disorder. In a further embodiment, the medicament is for use a method of treating a cell proliferative disorder or an autoimmune disorder comprising administering to an individual having a cell proliferative disorder or an autoimmune disorder an effective amount of the medicament. In one such embodiment, the method further comprises administering to the subject an effective amount of at least one additional therapeutic agent, for example, as described below. In a further embodiment, the medicament is for activating effector cells (e.g., T cells, e.g., CD8+ and/or CD4+ T cells), expanding (increasing) an effector cell population, reducing a population of target cells (e.g., a population of cells expressing a second target cell antigen recognized by a bispecific antigen-binding molecule of the invention), and/or killing a target cell (e.g., target tumor cell).

In a further aspect, the invention provides a method of treating or delaying the progression of a disorder in a subject in need thereof, the method comprising administering to the subject the bispecific antigen-binding molecule of any of the preceding aspects. In one embodiment, the method comprises administering to a subject having such a cell proliferative disorder or an autoimmune disorder an effective amount of a bispecific antigen-binding molecule (e.g., a bispecific antigen-binding molecule having an anti-CD3 monovalent arm and an anti-tumor cell antigen bivalent arm). In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, for example, as described below.

In another aspect, the invention provides a method of enhancing immune function in a subject having a disorder, the method comprising administering to the subject the bispecific antigen-binding molecule of any of the preceding aspects (e.g., a bispecific antigen-binding molecule having an anti-CD3 monovalent arm and an anti-tumor cell antigen bivalent arm). In some embodiments, the disorder is a cell proliferative disorder (e.g., cancer) or an autoimmune disorder. In certain embodiments, the method includes administering an effective amount of the bispecific antigen-binding molecule to activate effector cells (e.g., T cells, e.g., CD8+ and/or CD4+ T cells), expand (increase) an effector cell population, reduce a population of target cells (e.g., a population of cells expressing a second target cell antigen recognized by a bispecific antigen-binding molecule of the invention), and/or kill a target cell (e.g., target tumor cell).

In some embodiments, the bispecific antigen-binding molecules provided herein, compositions, and methods of use thereof, are used for the treatment of cancer, such as breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, non-Hodgkin's lymphoma (NHL), B cell lymphoma, B cell leukemia, multiple myeloma, renal cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, glioblastoma, germinal-center B-cell-like (GCB) diffuse large B cell lymphoma (DLBCL), activated B cell-like (ABC) DLBCL, follicular lymphoma (FL), mantle cell lymphoma (MCL), acute myeloid leukemia (AML), chronic lymphoid leukemia (CLL), marginal zone lymphoma (MZL), small lymphocytic leukemia (SLL), lymphoplasmacytic lymphoma (LL), Waldenström macroglobulinemia (WM), central nervous system lymphoma (CNSL), Burkitt's lymphoma (BL), B cell prolymphocytic leukemia, splenic marginal zone lymphoma, hairy cell leukemia, splenic lymphoma/leukemia, splenic diffuse red pulp small B cell lymphoma, hairy cell leukemia variant, α heavy chain disease, γ heavy chain disease, μ heavy chain disease, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, pediatric follicular lymphoma, primary cutaneous follicle centre lymphoma, T cell/histiocyte rich large B cell lymphoma, primary DLBCL of the central nervous system, primary cutaneous DLBCL (leg type), Epstein-Barr virus (EBV)-positive DLBCL of the elderly, DLBCL associated with chronic inflammation, lymphomatoid granulomatosis, primary mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, anaplastic lymphoma kinase (ALK)-positive large B cell lymphoma, plasmablastic lymphoma, large B cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, large B cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, or large B cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma)). In some embodiments, the bispecific antigen-binding molecules provided herein, compositions, and methods of use thereof, are used for the treatment of a HER2-positive cancer (e.g., a HER2-positive breast cancer or a HER2-positive gastric cancer).

The invention further provides combination therapies including administration of a bispecific antigen-binding molecule with one or more additional therapeutic agents (e.g., one, two, three, four, five, or more additional therapeutic agents) administered in parallel or as part of a complex regimen. In one embodiment, a bispecific antigen-binding molecule is co-administered with one or more biological modifiers selected from a BCL-2 inhibitor (e.g., GDC-0199/ABT-199), lenalidomide (REVLIMID®), a PI3K-delta inhibitor (such as idelalisib (ZYDELIG®)), an agonist, e.g., agonist antibody directed against an activating co-stimulatory molecule, e.g., CD40, CD226, CD28, OX40 (e.g., AgonOX), GITR, CD137 (also known as TNFRSF9, 4-1BB, or ILA), CD27 (e.g., CDX-1127), HVEM, or CD127, an antagonist, e.g., antagonist antibody, directed against an inhibitory co-stimulatory molecule, e.g., CTLA-4 (also known as CD152), PD-1, TIM-3, BTLA, VISTA, LAG-3, B7-H3, B7-H4, IDO (e.g., 1-methyl-D-tryptophan (also known as 1-D-MT)), TIGIT, MICA/B, GITR (e.g., TRX518) or arginase, ipilimumab (also known as MDX-010, MDX-101, or YERVOY®), tremelimumab (also known as ticilimumab or CP-675,206, urelumab (also known as BMS-663513), MGA271, an antagonist directed against a TGF-β, e.g., metelimumab (also known as CAT-192), fresolimumab (also known as GC1008), LY2157299k, and an adoptive transfer of a T cell (e.g., a cytotoxic T cell) expressing a chimeric antigen receptor (CAR), e.g., adoptive transfer of a T cell comprising a dominant-negative TGF-β receptor, e.g., a dominant-negative TGF-β type II receptor.

In a particular embodiment, the biological modifier is a PD-1 axis binding antagonist. In some embodiments, the PD-1 axis binding antagonist or additional therapeutic agent is administered prior to or subsequent to the administration of the bispecific antigen-binding molecule. In some embodiments, the PD-1 axis binding antagonist or additional therapeutic agent is administered concurrently with the bispecific antigen-binding molecule. In some embodiments, the PD-1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist (e.g., atezolizumab (MPDL3280A), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab)), a PD-1 binding antagonist (e.g., MDX-1106 (nivolumab), MK-3475 (pembrolizumab), CT-011 (pidilizumab), MEDI-0680 (AMP-514), PDR001 (spartalizumab), REGN2810 (cemiplimab), and BGB-108), and a PD-L2 binding antagonist (e.g., an antibody or an immunoadhesin).

Additionally or alternatively, a bispecific antigen-binding molecule of the invention may be co-administered with one or more chemotherapeutic agents (e.g., as part of a method of treating a subject having a cell proliferative disorder, e.g., a cancer).

In a further aspect, the invention provides a method for treating a HER2-positive cancer. In one embodiment, the method comprises administering to an individual having a HER2-positive cancer an effective amount of a bispecific antigen-binding molecule of the invention, such as a 1Fab-IgG TDB molecule having a bivalent anti-HER2 arm and a monovalent anti-CD3 arm. In a particular embodiment, the anti-HER2 arm possesses an acceptable toxicity profile when administered in an effective dose in a patient. In one embodiment, the anti-CD3 arm of 1Fab-IgG TDB molecule with an acceptable toxicity profile is a low affinity anti-CD3 antigen-binding moiety. In one embodiment, the anti-CD3 antigen-binding moiety of the 1Fab-IgG TDB with an acceptable toxicity profile is 40G5c.

In a particular embodiment, the HER2-positive cancer is a HER2-positive breast cancer or HER2-positive gastric cancer. The HER2-positive cancer (e.g., the HER2-positive breast cancer or the HER2-positive gastric cancer) may be characterized by tumor cells that express HER2 at a copy number (e.g., an average copy number) of at least 200,000 per cell (e.g., at least 250,000 HER2 copies per cell, at least 300,000 HER2 copies per cell, at least 400,000 HER2 copies per cell, at least 500,000 HER2 copies per cell, at least 600,000 HER2 copies per cell, at least 700,000 HER2 copies per cell, at least 750,000 HER2 copies per cell, at least 800,000 HER2 copies per cell, at least 900,000 HER2 copies per cell, at least 1,000,000 HER2 copies per cell, at least 1,200,000 HER2 copies per cell, at least 1,500,000 HER2 copies per cell, at least 2,000,000 HER2 copies per cell, at least 2,500,000 HER2 copies per cell, at least 3,000,000 HER2 copies per cell, or more, e.g., from 200,000 to 2,000,000 HER2 copies per cell, from 300,000 to 1,500,000 HER2 copies per cell, from 400,000 to 1,200,000 HER2 copies per cell, or from 500,000 to 1,000,000 HER2 copies per cell, e.g., from 200,000 to 1,000,000 HER2 copies per cell (e.g., from 200,000 to 250,000 HER2 copies per cell, from 250,000 to 300,000 HER2 copies per cell, from 300,000 to 400,000 HER2 copies per cell, from 400,000 to 500,000 HER2 copies per cell, from 500,000 to 750,000 HER2 copies per cell, or from 750,000 to 1,000,000 HER2 copies per cell) or from 1,000,000 to 3,000,000 HER2 copies per cell (e.g., from 1,000,000 to 1,500,000 HER2 copies per cell, from 1,500,000 to 2,000,000 HER2 copies per cell, from 2,000,000 to 2,500,000 HER2 copies per cell, or from 2,500,000 to 3,000,000 HER2 copies per cell).

In one embodiment, an anti-HER2 bispecific antigen-binding molecule is co-administered with one or more additional therapeutic agents that target the HER pathway. In one embodiment, the therapeutic agent that targets the HER pathway is selected from an EGFR inhibitor, a HER2 inhibitor, a HER3 inhibitor, and/or a HER4 inhibitor. In one embodiment, a HER2 TDB (e.g., a 1Fab-IgG TDB) is co-administered with one or more additional therapeutic agents selected from trastuzumab (HERCEPTIN®), T-DM1 (KADCYLA®) and pertuzumab (PERJETA®). In one embodiment, an anti-HER2/CD3 bispecific antigen-binding molecule is co-administered with trastuzumab. In one embodiment, an anti-HER2/CD3 bispecific antigen-binding molecule is co-administered with T-DM1. In one embodiment, an anti-HER2/CD3 bispecific antigen-binding molecule is co-administered with pertuzumab. In one embodiment, an anti-HER2/CD3 bispecific antigen-binding molecule is co-administered with trastuzumab and pertuzumab. In one embodiment, an anti-HER2/CD3 bispecific antigen-binding molecule is co-administered with T-DM1 and pertuzumab.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the bispecific antigen-binding molecule of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent or agents. In one embodiment, administration of the anti-CD3 antibody and administration of an additional therapeutic agent occur within about one month, or within about one, two or three weeks, or within about one, two, three, four, five, or six days, of each other.

A bispecific antigen-binding molecule of the invention (and/or any additional therapeutic agent) can be administered by any suitable means, including subcutaneously, intravenously, intramuscularly, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally. For example, in some embodiments, the bispecific antigen-binding molecule is administered subcutaneously. In other embodiments, the bispecific antigen-binding molecule is administered intravenously. In some embodiments, a bispecific antigen-binding molecule administered by subcutaneous injection exhibits a less toxic response in a patient than the same bispecific antigen-binding molecule administered by intravenous injection. Dosing can be by any suitable route, for example, by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Bispecific antigen-binding molecules of the invention can be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual subject, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The bispecific antigen-binding molecule need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of a bispecific antigen-binding molecule of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the severity and course of the disease, whether the bispecific antigen-binding molecule is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the bispecific antigen-binding molecule, and the discretion of the attending physician. The bispecific antigen-binding molecule is suitably administered to the patient at one time or over a series of treatments.

As a general proposition, the therapeutically effective amount of the bispecific antigen-binding molecule administered to a human will be in the range of about 0.01 to about 100 mg/kg of patient body weight whether by one or more administrations. In some embodiments, the bispecific antigen-binding molecule used is about 0.01 to about 45 mg/kg, about 0.01 to about 40 mg/kg, about 0.01 to about 35 mg/kg, about 0.01 to about 30 mg/kg, about 0.01 to about 25 mg/kg, about 0.01 to about 20 mg/kg, about 0.01 to about 15 mg/kg, about 0.01 to about 10 mg/kg, about 0.01 to about 5 mg/kg, or about 0.01 to about 1 mg/kg administered daily, for example. In one embodiment, an bispecific antigen-binding molecule described herein is administered to a human at a dose of about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg or about 1400 mg on day 1 of 21-day cycles.

The dose may be administered as a single dose or as multiple doses (e.g., 2 or 3 doses), such as infusions. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg, or 10 mg/kg (or any combination thereof) may be administered to the subject. Such doses may be administered intermittently, for example, every week or every three weeks (e.g., such that the patient receives from about two to about twenty, or, for example, about six doses of the anti-CD3 antibody). An initial higher loading dose, followed by one or more lower doses may be administered. The progress of this therapy is easily monitored by conventional techniques and assays. In some embodiments of any of the preceding aspects of the invention, the subject, patient, or individual is a human.

VI. Kits

Provided herein are kits including one or more compositions described herein (e.g., a composition including any one or more of the bispecific antigen-binding molecules described herein and a pharmaceutically acceptable carrier) and a package insert for administering the composition to a subject to treat or delay progression of a disorder (e.g., a cell proliferative disorder (e.g., a cancer, such as breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, non-Hodgkin's lymphoma (NHL), B cell lymphoma, B cell leukemia, multiple myeloma, renal cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, glioblastoma, germinal-center B-cell-like (GCB) diffuse large B cell lymphoma (DLBCL), activated B cell-like (ABC) DLBCL, follicular lymphoma (FL), mantle cell lymphoma (MCL), acute myeloid leukemia (AML), chronic lymphoid leukemia (CLL), marginal zone lymphoma (MZL), small lymphocytic leukemia (SLL), lymphoplasmacytic lymphoma (LL), Waldenström macroglobulinemia (WM), central nervous system lymphoma (CNSL), Burkitt's lymphoma (BL), B cell prolymphocytic leukemia, splenic marginal zone lymphoma, hairy cell leukemia, splenic lymphoma/leukemia, splenic diffuse red pulp small B cell lymphoma, hairy cell leukemia variant, α heavy chain disease, γ heavy chain disease, μ heavy chain disease, plasma cell myeloma, solitary plasmacytoma of bone, extraosseous plasmacytoma, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma), nodal marginal zone lymphoma, pediatric nodal marginal zone lymphoma, pediatric follicular lymphoma, primary cutaneous follicle centre lymphoma, T cell/histiocyte rich large B cell lymphoma, primary DLBCL of the central nervous system, primary cutaneous DLBCL (leg type), Epstein-Barr virus (EBV)-positive DLBCL of the elderly, DLBCL associated with chronic inflammation, lymphomatoid granulomatosis, primary mediastinal (thymic) large B cell lymphoma, intravascular large B cell lymphoma, anaplastic lymphoma kinase (ALK)-positive large B cell lymphoma, plasmablastic lymphoma, large B cell lymphoma arising in HHV8-associated multicentric Castleman disease, primary effusion lymphoma, large B cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, or large B cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma)). In some embodiments, the cancer is a HER2-positive cancer (e.g., a HER2-positive breast cancer or a HER2-positive gastric cancer). Additionally or alternatively, the kit may include a package insert for administering the composition to a subject to treat or delay progression of an autoimmune disorder.

EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1. Materials and Methods

Gene synthesis

Fab

Genes encoding variants of anti-HER2 (4D5-8; Carter et al., *Proc. Natl. Acad. Sci. USA*. 1992, 89: 4285-4289) Fabs were obtained by gene synthesis of light chain and heavy chain variable domains. The anti-HER2.4D5 Fab and variants were produced in 30 mL scale in Expi293TTM cells and purified in batch using anti-Flag affinity resin. After a 3x PBS wash step, Fabs were eluted with 50 mM phosphoric acid (pH 3.0), neutralized with 20x PBS (pH 11.0), and filtered. All Fabs were ≥95% pure and verified by mass spectrometry.

IgG TDB & 1Fab-IgG TDB

Knob (anti-HER2.N297G.knob (4D5-8 and variants);Kelley and O'Connell, *Biochemistry* 1993, 32: 6828-6835) and hole (anti-CD3.N297G.hole (40G5c, unless otherwise specified)) half-antibodies were generated as previously described (Dillon et al., *MAbs* 2017, 9: 213-230). For 1Fab-IgG TDB constructs, the coding region for anti-HER2 Fab (E1-T225) directly preceded the coding region of the anti-HER2.knob sequence and was synthesized with or without a G4SGG linker between them.

Luciferase fusions of 1Fab-IgG TDB

The mature protein sequence for luciferase (AAG54095.1) K18-D185 was fused C-terminally to the anti-HER2.hu4D5 light chain. Anti-HER2.hu4D5 Fab with and without the luciferase light chain fusion produced similar binding kinetics to HER2 using a BIACORE® T200 (GE Healthcare) and method described below.

Antibody expression and purification

IgG TDB, 1Fab-IgG TDB, and Luciferase fusions of 1Fab-IgG TDB were produced as follows. Half-antibodies anti-HER2.hu4D5.knob and variants and anti-CD3.40G5c.hole were produced in CHO cells at ≥1L scale and purified by MAbSelect SuRe (GE Healthcare). Bispecific IgG TDB, 1Fab-IgG TDB, or 1Fab-IgG TDB Luciferase fusion assembly from half-antibodies was performed as described in Junttila et al., *Cancer Res.* 2014, 74: 5561-5571; Spiess et al., *Nat. Biotechnol.* 2013, 31: 753-758.

HER2 Western blot analysis

Cell lysates were generated using Cell Signaling Technology lysis buffer. Protein was quantitated using BCA assay reagents (Pierce Cat. Nos. 23228 and 23224). 40 µg protein was loaded onto SDS-PAGE gels and a HER2 antibody (Dako Cat. No. A0485) was used for detection.

Immunohistochemistry (IHC)

Immunohistochemistry for HER2 was performed on five-µm formalin-fixed, paraffin-embedded tissue samples and tissue microarray (TMA) samples with anti-Her-2/neu (Ventana Medical Systems, clone 4B5, Tuscan, AZ) rabbit monoclonal primary antibody using the Ventana Ultraview-Benchmark system with Ventana DAB detection. Staining results were scored as 0, 1+, 2+, or 3+ using ASCO-CAP recommended clinical HER2 scoring guidelines.

Fluorescence in-situ hybridization (FISH)

Probes

A commercially available, FDA approved HER2 FISH probe (PathVysion, Abbott Molecular) was used by Core Diagnostics for the analysis of the cell line TMA. The PathVysion HER-2 DNA Probe cocktail consists of two labeled DNA probes. The LSI HER2 probe that spans the entire HER2 gene is labeled in SpectrumOrange. The CEP17 probe is labeled in SpectrumGreen and hybridizes to the alpha satellite DNA located at the centromere of chromosome 17 (17p11.1-q11.1). Inclusion of the CEP17 probe allows for the relative copy number of the HER2 gene to be determined.

Assay

Five-µm thick FFPE sections from the TMA block were subjected to FISH analysis as described previously with modifications. Following overnight incubation at 56ºC, the slides were de-paraffinized in three washes of CitroSolv for five minutes each, dehydrated by two alcohol washes and air-dried. Subsequently, the FFPE sections were incubated in a Pretreatment reagent (Abbott Molecular, IL) for 30 minutes at 80ºC and were then treated with Protease II (Abbott Molecular, IL) prior to additional washes in water and a series of ethanol. Dried slides were then co-denatured at 76°C for six minutes with the probes and were hybridized overnight at 37°C (ThermoBrite; Abbott Molecular). Post-hybridization washes and counter-staining were done in a manner similar to those previously described.

The sections were viewed using fluorescence microscopes equipped with computer-driven imaging systems (BioView Duet, BioView Ltd.). A minimum of 50 non-overlapping tumor cells from each sample were enumerated for HER2 and CEP17 hybridization signals. The current HER2 scoring criteria and guidelines were used for the analysis of the slides. A cell line was considered FISH-positive if it had a HER2-to-CEP17 ratio of two or more or had an average HER2 copy number of more than six signals per nuclei in cells with a HER2-to-CEP17 ratio of less than two. Samples with a HER2-to-CEP17 ratio of less than two and an average HER2 copy number of less than four signals per nuclei were considered as negative and samples with a HER2-to-CEP17 ratio of less than two and an average HER2 copy number between four to six signals per nuclei were considered as equivocal.

Cellular HER2 binding assays

Cellular binding of Luciferase fusions of 1Fab-IgGs - Direct binding assay:

Cells (SKBR3: 40,000 per well, MCF7: 200,000 per well) were plated into 96 flat-bottom white sterile tissue culture treated plates (Thermo Scientific; Cat #136101) and allowed to adhere overnight. Cells were then washed with binding buffer (PBS + 3% BSA + 0.05% sodium azide; pH 7.2), and varying concentrations (0.002 nM – 100 nM) of TDB luciferase fusion proteins in binding buffer were added to each well. Plates were then incubated at 4℃ for four hours and subsequently washed three times with binding buffer. After the final wash, 20 µl binding buffer containing 50 µl of the luciferase substrate (New England Biolab, Cat #E3300L) was added to each well. Luminescence values were read on Perkin Elmer Envision Plate Reader. The binding data was fit using the GraphPad Prism model, “One Site-Specific Binding” to determine the binding affinity of the antibody.

Cellular binding of Luciferase fusions of 1Fab-IgGs - Competition binding assay:

Cells were plated and washed as described above. To measure competitive binding, a fixed concentration of a TDB luciferase fusion protein (15 nM 4D5-WT or 50 nM 4D5-H91A) was mixed with increasing concentrations of TDB (0.007 nM-400 nM) in binding buffer and added to the cells. Plates were incubated for four hours at 4℃, washed, and read as described above. The binding data was fit using the GraphPad Prism model, “One Site-Fit Ki” to determine the binding affinity of the antibody.

Cellular binding by flow cytometry

MCF7 and SKBR3 cells were plated at 100,000 cells per well in 96-well U bottom plates in 50 µL of FACS Buffer (1% BSA, 2 mM EDTA in PBS). Test articles were serially diluted 1:5 in FACS buffer starting at either 50 µg/mL or 10 µg/mL and incubated with cells on ice for 30 minutes. Cells were washed twice with FACS buffer and resuspended in 100 µL Alexa 647 Goat Anti-Human IgG, Fcγ Fragment (Jackson ImmunoResearch No. 109-606-098), at a 1:200 dilution, in FACS buffer and incubated on ice for 30 minutes. Cells were resuspended in 100 µL FACS Buffer containing propidium iodide (PI; eBioscience #00-6990-50) at a 1:100 dilution and analyzed on BD FACSCaliber.

Blood cell fractionation

Peripheral blood mononuclear cells (PBMC) were separated from the blood of healthy volunteers using lymphocyte separation medium (MP Biomedicals). $CD8^+$ cells were extracted from PBMC using human $CD8^+$ Isolation Kit (Miltenyi Biotec No. 130-094-156) by negative selection.

T cell activation

All antibodies for flow cytometry cell staining were obtained from BD Biosciences (San Jose, CA). Human PBMC and target cells (10:1 ratio) were incubated in the presence of test article for 24 hours in flat-bottom 96 well plate (BD). After incubation, cells were transferred to a new V-bottom 96 well plate. Cells were stained with anti-CD8-FITC, anti-CD69-PE, and anti-CD25-APC. CD69 and CD25 surface expression was detected on CD8+ T cells by flow cytometry. The percentage of CD8+CD69+CD25+ was reported as T cell activation. To determine HER2-independent T cell activation, Jurkat-Dual NF-κB/IRF reporter cells (Invivogen Cat# jktd-isnf) were incubated at 37° C with TDB or OKT CD3 antibody for 24 hours. 10 μL of supernatant was removed from each well and added to a white, clear bottom 96 well plate. 100 μL of Quantiluc solution (Invivogen) was added to the harvested supernatants and plates were read on Perkin Elmer Envision plate reader.

In vitro apoptosis

Target cells were plated at 10,000 cells per well in culture media into a clear-bottom 96 black well plate (Corning No. 3904) and placed in 37ºC, 5% CO_2 incubator overnight. The test article, 50,000 purified $CD8^+$ cells, and a 1:1000 dilution of Caspase 3/7 reagent (Essen No. 4440) were added to pre-plated cells and placed into the Incucyte zoom. Images were collected every four hours over 40 hours and analyzed for number of apoptotic events per mm^2.

Cell viability

Cells were grown to confluence in RPMI supplemented with 10% FBS, penicillin (100 U/mL), streptavidin (100 μg/mL), and L-Glutamate (0.2 mM). Cells were dissociated with PBS based cell dissociation media and plated in black, clear-bottomed 96-well plates at density of 10,000 cells per well. On day four, media was discarded and plates were washed twice with PBS. 100 μL CELLTITER-GLO® Luminescent Cell Viability reagent (Promega Cat. No. G7570) was added and plates were read on luminometer as described in the instructions.

Breast cancer cell proliferation

$CD8^+$ cells were used as effectors in a 3:1 effector:target ratio. Breast cancer cell proliferation/viability was detected using the CELLTITER-GLO® Luminescent Cell Viability Assay (Promega). For the assay, 15×10^3 cells per well were plated in 96-well plates and incubated overnight for cell attachment before treatments.

In vivo efficacy

Female NOD scid gamma (NSG) mice were obtained from The Jackson Laboratory. Human PBMC were used as effector cells in the mice. PBMC were purified from the blood of healthy donors using the Lymphocyte Separation Medium (MP Biomedical, LLC, Salon, Ohio) and cryopreserved at -80ºC. Prior to transferring into mice PBMCs were thawed and cultured overnight in 10% FBS containing RPMI medium at 37ºC, 5% CO2. PBMCs were inoculated into mice intraperitoneally, one day after tumor cell inoculation at a concentration of 10×10^6 cells per mouse, in a volume of 100 μL of Hank's balanced salt solution (HBSS) buffer. To evaluate efficacy in treatment of HER2 amplified breast cancer, animals were inoculated with 3×10^6 KPL-4 tumor cells in HBSS/MATRIGEL® via subcutaneous administration in the right thoracic mammary fat pad. To evaluate activity in treatment of low HER2-expressing tumors, animals were subcutaneously inoculated with 5×10^6 HT55 tumor cells in HBSS. Single dose treatments were administered intravenously at day 0 as indicated in the figure legends.

Cynomolgus monkey safety and pharmacokinetic (PK) study

Eleven female cynomolgus monkeys (Macaca fascicularis; ages and weights at the initiation of dosing ranging from 24-60 months and 2-5 kg) were randomly assigned to three groups. Animals received vehicle control (N=3) or anti-HER2-CD3 4D4-H91A 1Fab-IgG TDB at 3 mg/kg (N=3), or TDB at 20 mg/kg (N=5) via one-hour intravenous infusion at a volume of two mL/kg/hour. Animals were monitored twice daily for any abnormalities and signs of pain or distress. Additional endpoints included food consumption, body weights, physical examinations, body temperature, respiration rate, and heart rate.

PK analysis

Cynomolgus monkey blood was collected at various times throughout the study to assess the pharmacokinetics. Blood was allowed to clot at room temperature for at least 30 minutes. Samples were centrifuged at about 1500-2000 x g at 2-8 ° C within one hour of collection. Serum was collected as stored at -60℃ to -80℃ until shipped for analysis. Serum PK samples were analyzed by GRIP ELISA (Yang et al., *Journal of Immunological Methods* 2008, 335: 8-20). Serum concentration-time profiles were used to estimate the following PK parameters using non-compartmental analysis (WinNonlin, version 5.2.1; Pharsight Corporation, Mountain View, CA): total drug exposure defined as area under the serum concentration-time curve (AUC), antibody clearance (CL), and observed maximum serum concentration (Cmax). Each animal was analyzed separately, and results for each dose group were summarized as mean ± standard deviation (SD).

Clinical Pathology

At least twice during the predose phase and on study days 2 and 8 blood was collected into tubescontaining the anticoagulant sodium citrate for coagulation tests or potassium EDTA for hematology tests.

BIACORE®

Binding kinetics of the anti-HER2 Fabs or anti-HER2/CD3 T cell dependent bispecific (TDB) antibodies were measured using surface plasmon resonance on a BIACORE® T200 or 8K instrument (GE Healthcare). All kinetics experiments were performed at a flow rate of 30 µL/minutes at 37˚C, and with a running buffer of 10 mM HEPES, pH 7.2, 150 mM NaCl, 0.05% Tween 20, and 1 mM EDTA (HBS-EP Buffer). Human HER2 extracellular domain (ECD; Sino Biologicals 10004-H08H or Novus Biologicals NBP1-94702) or Cynomolgus monkey HER2 ECD was immobilized on a CM5 sensor chip via amine-based coupling. For human HER2 ECD, a 3-fold concentration series of anti-HER2 Fabs or TDBs ranging from 0.27 to 200 nM was used to analyze binding. For cynomolgus monkey HER2 ECD, a 1.5-fold concentration series of anti-HER2 Fabs ranging from 87.8 to 1000 nM was used to analyze binding. Sensorgrams for binding of HER2 were recorded using an injection time of 180 seconds followed by 300 seconds of dissociation time and regeneration of the surface with 50 mM HCl. All sensorgrams observed for antigen binding to antibodies were analyzed using a 1:1 Langmuir binding, with the exception of 4D5

Fab variants binding to cynomolgus monkey HER2, which used a steady state binding model to calculate the kinetics and binding constants. Due to the requirement of high Fab concentrations to measure weak affinities to cynomolgus monkey HER2, all Fab variants were buffer exchanged into HBS-EP buffer prior to the experiment in order to prevent buffer effects and ensure accurate measurements. The mass of all 4D5 variants was verified by mass spectrometry.

Example 2. HER2 affinity of the anti-HER2/CD3 IgG TDBhas a direct effect on killing activity, but not on selectivity to HER2-overexpressing cells

Anti-HER2/CD3 bispecific antibodies that are monovalent for each binding arm have been shown to have robust activity in HER2-amplified in vitro and in vivo breast cancer models. However, this IgG TDB format also retains low level activity against cell lines expressing low levels of HER2, similar to normal HER2-positive human epithelial cells. It is desirable to increase the selectivity of anti-HER2/CD3 TDB to HER2 amplified cells in order to minimize the risk of on-target off-tumor activity and thereby increase the therapeutic index of the HER2 targeting TDB. Initially, the impact of HER2 affinity on selectivity of the IgG TDB to HER2 amplified cells was investigated. Multiple variants of anti-HER2 4D5 with monovalent binding affinities as Fabs ranging from 0.3 to 213 nM (Table 4) were generated. For all variants, the main driver for reduced affinity was the increase in (i.e., faster) dissociation rate (k_d), whereas the association rate (k_a) remained unaltered.

Table 4. Monovalent binding affinities of 4D5 variants.

Anti-HER2 (4D5) Fab	**k_a (1/Ms, 1E+5)**	**k_d (1/s, 1E-3)**	**K_D (nM)**	**K_D Ratio (Variant/WT)**
WT (trastuzumab)	8.74 ± 1.74	0.25 ± 0.03	0.30 ± 0.01	1.0
Y102V	9.28 ± 0.34	0.30 ± 0.02	0.32 ± 0.03	1.1
Y55E.Y102V	5.83 ± 1.04	1.43 ± 0.10	2.60 ± 0.34	8.7
Y55E.D98A.F100A.Y102V	11.5 ± 3.34	15.6 ± 2.58	14.0 ± 2.08	47
D98A.F100A.Y102V	10.1 ± 1.47	22.8 ± 2.12	22.7 ± 2.07	76
H91A	7.07 ± 2.43	33.6 ± 6.54	49.3 ± 8.57	164
Y55E.H91A	5.10 ± 0.20	87.5 ± 3.60	172 ± 13.2	573
Y100aA	4.61 ± 1.27	91.9 ± 11.2	213 ± 81.8	710
N30A.H91A	5.75 ± 0.56	201 ± 19.1	353 ± 62.7	1177

For the primary selectivity test, SKBR3 and MCF7 were used as model cell lines. SKBR3 is a HER2-amplified breast cancer cell line, whereas MCF7 is breast cancer cell line expressing low levels of HER2 (FIG. 1), similar to levels detected in non-cancerous cells, such as cultured cardiomyocytes (Junttila et al. *Cancer Res.* 74(19): 5561-5571, 2014). The cell membrane copy number of HER2 in SKBR3 and MCF7 cells has been reported as 2×10^6 per cell and 1.5×10^4 per cell, respectively (Aguilar et al. *Oncogene* 18(44): 6050-6062, 1999). Decreasing HER2 binding affinity gradually reduced the killing activity of the anti-HER2 IgG TDB on both SKBR3 and MCF7 cell lines (FIGS. 2A-2F). Killing activity was practically abolished with IgG TDBs having an affinity to HER2 of 49 nM or higher. Similar levels of activity decrease were observed in SKBR3 and MCF7 cells. In summary, monovalent HER2 affinity plays a key role in the activity of the anti-HER2/CD3 IgG TDB but did not affect selectivity to HER2-overexpressing cells.

Example 3. Characterizing the effect of bivalent low affinity HER2 binding on selectivity for HER2 overexpressing cells

Antibodies having bivalent HER2 binding and monovalent CD3 binding (1Fab-IgG TDB format) were constructed based on monovalent 4D5 affinity variants (FIG. 3). 1Fab-IgG TDBs were tested for cellular binding to SKBR3 cells (FIGS. 4A and 7A) and to MCF7 cells (FIGS. 4B and 7B) and for ability to mediate apoptosis (FIGS. 5A and 5B) and killing (FIGS. 6A-6C and 8) of SKBR3 and MCF7 cells. Multiple 4D5 1Fab-IgG TDB variants demonstrated similar binding to SKBR3 and MCF7 cells compared to a control IgG TDB with a monovalent high affinity HER2 arm based on wild-type 4D5 (trastuzumab; FIGS. 4A and 4B). Y55E.H91A, Y100aA, or N30A.H91A substitutions (monovalent K_Ds of 172, 213, and 353 nM, respectively) resulted in substantially reduced cellular binding in the 1Fab-IgG TDB format compared to the monovalent high-affinity HER2 IgG TDB format (FIGS. 7A and 7B). Lower binding was reflected in substantially reduced killing of SKBR3 cells (FIG. 8). Y102V and Y55E.Y102V substitutions (monovalent K_D 0.3 and 2.6 nM, respectively) in the 1Fab-IgG TDB format resulted in similar binding to both SKBR3 and MCF7 cell lines compared to control IgG TDB (FIGS. 4A and 4B). Consistent with cellular binding, Y102V and Y55E.Y102V 1Fab-IgG TDB variants demonstrated substantial potency in killing of low HER2-expressing MCF7 cells, suggesting that these modifications did not improve selectivity to HER2 overexpressing cells (FIGS. 5A, 5B, 6B, and 6C).

Two 4D5 variants, D98A.F100A.Y102V and H91A (monovalent K_D 23 and 49 nM, respectively), retained high cellular binding to SKBR3 cells, while demonstrating only minimal binding to MCF7 cells (FIGS. 4A, 4B, 5A, and 5B). This cellular binding was reflected in killing activity. In particular, the D98A.F100A.Y102V and H91A 1Fab-IgG TDB variants induced potent apoptosis and killing of SKBR3 cells but were unable to mediate killing of low HER2-expressing MCF7 cells despite very high concentrations (50 ng/mL to 50 µg/mL). In vitro activity of monovalent high HER2 affinity HER2-IgG TDB has been reported to saturate at ~10 ng/mL concentration (Junttila et al. *Cancer Res.* 74(19): 5561-5571, 2014). These results suggest that the enhanced avidity of the 1Fab-IgG TDB, relative to conventional antibodies, can enable enhanced selectivity to HER2-overexpressing cells. The window for monovalent affinity (K_D) resulting in increased selectivity was in the 20-50 nM range, measured as Fabs. Smaller reduction in affinity does not improve selectivity and larger reduction in affinity significantly compromises the potency of the TDB.

Example 4. Characterizing enhanced selectivity of the 1Fab-IgG TDB format for HER2 overexpressing cells

The H91A-1Fab-IgG TDB variant of 4D5 was selected to further analyze in vitro activity. A dose response study demonstrated that 4D5 H91A-1Fab-IgG TDB activity on SKBR3 was nearly identical compared to monovalent high HER2-affinity IgG TDB (FIG. 9; EC_{50} 6.9 and 6.3 pM, respectively). In contrast to IgG TDB, no activity was seen on any dose level of 1Fab-IgG TDB against MCF7 cells (FIG.

9). 90 breast cancer cell lines were arbitrarily split to high (>400 normalized reads per kilobase of transcript length per million mapped read (nRPKM)), medium (50-400 nRPKM) , and low (<50 nRPKM). HER2-expressing cells based on HER2-RNA expression reported in Klijn 2015 (FIG. 10; Klijn et al. *Nat. Biotechnol.* 33(3): 306-312, 2015). Selectivity studies were extended to 15 cell lines with variable HER2 expression levels. At a high dose level (50 ng/mL), no clear correlation was seen between T cell activation and HER2 expression for the monovalent high HER2 affinity IgG TDB (FIGS. 11A-11D). In striking contrast, T cell activation was induced only in the cell lines with the highest HER2 expression level when H91A affinity variant 1Fab-IgG TDB was tested (FIGS. 12A-12D). Similarly, target cell killing was limited to high HER2-expressing cell lines with the 1Fab-IgG TDB molecule, whereas the high HER2 affinity IgG TDB was broadly active also in inducing killing of the low HER2-expressing cells when used at high dose levels (FIGS. 11A-11D and 12A-12D). A 50 ng/mL dose of control IgG TDB induced T cell activation and killing of low HER2-expressing cells, while a 50 µg/ml 1Fab-IgG TDB did not impact viability of low HER2-expressing cells. Therefore, the improvement in selectivity of 1Fab-IgG TDB molecules relative to control IgG TDB to HER2 was calculated to be ≥1000-fold.

The activity was further tested in human non-cancer cell lines derived from lung, skin, and breast tissues. Low level HER2 expression was confirmed in all tested non-cancerous cells by flow cytometry. Table 5 shows a characterization of these cells and binding of IgG TDBs and 1Fab-IgG TDB.

Table 5. Binding to low HER2-expressing human non-cancer cells.

Cell Line	**Tissue Source**	**HER2 MFI***	IgG TDB **EC_{50}**	IgG TDB **MED****	**1Fab-IgG TDB MED****
MRC-5	Normal lung	74	No activity	No activity	No activity
HBL-100	Epithelial	60	2 ng/mL	5 ng/mL	No activity
BEAS-2B	Normal lung	33	No activity	No activity	No activity
Hs 888.Sk	Normal skin	35	7 ng/mL	50 ng/mL	No activity
hTERT-HME1	Normal breast + hTERT	9	32 ng/mL	50 ng/mL	No activity
Kd	Normal fibroblast lip	46	No activity	No activity	No activity
HMEC-1	Microvascular endothelial foreskins	15	No activity	50 ng/mL	No activity

*MFI = Mean fluorescence intensity

**MED = Minimal efficacious dose level

H91A affinity variant 1Fab-IgG TDB demonstrated no apoptotic activity against any of the 8 tested cells at concentrations ≤ 5 µg/mL. A representative comparison of dose-response curves between 1Fab-IgG TDB and IgG TDB cultured with HBL-100 cells shows that H91A-IgG TDB potently kills low HER2-expressing HBL-100 cells, in contrast to H91A-1Fab-IgG TDB (FIG. 13). Kinetic assays confirm the dose-dependence of H91A-IgG TDB on HBL-100 killing over time and further demonstrate that H91A-1Fab-IgG TDB-induced killing is minimal over the course of 70 hours (FIGS. 14A and 14B). These results demonstrate that in vitro potency of the 1Fab-IgG TDB is similar to monovalent high HER2-affinity IgG TDB in killing of high HER2-expressing cell lines. However, 1Fab-IgG TDB had no detectable activity on any tested low HER2-expressing cell lines, suggesting that it is selective to HER2-overexpressing cells.

The threshold of HER2 expression level that is required for activity of H91A affinity variant 1Fab-IgG TDB was then investigated. Upon incubation of 1Fab-IgG TDB with 13 target cell lines having various HER2 expression profiles, killing was observed in target cells expressing HER2 at a copy number of greater than 200,000 HER2 molecules per cell (FIG. 15A). In particular, robust killing activity was detected in all cell lines expressing HER2 RNA ≥ 489 nRPKM (i.e., cell lines expressing HER2 at a copy number ≥ 290,579). Minimal activity was detected in all cell lines that expressed HER2 RNA ≤ 260 nRPKM (i.e., cell lines expressing HER2 at a copy number ≤ 146,208). HER2 copy number for each cell line was quantified by FACS using Quantum-Alexa647 MESF beads, and results are summarized in Table 6, below.

Table 6. HER2 copy number of various cell lines tested for 1Fab-IgG TDB killing

Cell Line	Mean HER2 Copy Number	Standard Deviation	% Standard Deviation
HCC1187	38,436	3,238	8%
NCI-H522	26,954	198	1%
VP267	70,761	7,515	11%
EFM-19	38,479	51	0%
ZFR-75-1	58,337	18,222	31%
NUGC-4	59,298	3,020	5%
YMB-1	69,655	964	1%
SNU-216	175,357	1,819	1%
OE-33	146,208	68,969	47%
EFM-192C	290,579	34,503	12%
HCC202	368,513	82,560	22%
SKBR3	774,501	109,335	14%
Calu-3	525,657	42,062	8%
HT55	26,337	1,157	4%
KPL4	636,978	31,743	5%
CHO-K1	0	0	0%

The activity was further benchmarked to HER2 diagnostic tests that are clinically validated and used for patient selection. Out of 20 cell tested cell lines, substantial killing activity was seen only in IHC 3+, FISH+ cell lines using the H91A affinity variant 1Fab-IgG TDB (FIGS. 15B and 15C).

Example 5. Assessing in vivo selectivity of 1Fab-IgG TDB for HER2 overexpressing tumors

In vivo activity of the H91A affinity variant 1Fab-IgG TDB was evaluated. NSG mice supplemented with human PBMC were grafted with HER2-amplified KPL4 tumors or HT55 tumors, which expressed a similar level of HER2 as MCF7 cells (FIG. 16). The H91A-1Fab-IgG TDB induced dose regressions in response to a single 0.5-5 mg/kg dose (FIG. 17), whereas the monovalent high HER2 affinity IgG TDB induced regressions at 0.05 mg/kg dose, indicating that the 1Fab-IgG TDB is about 10-fold less active in vivo. As expected based on the in vitro results, less activity was detected in treatment of HT55 tumors (FIG. 18). Monovalent high HER2 affinity IgG TDB induced HT55 tumor regressions at single 0.1 mg/kg dose. The H91A-1Fab-IgG TDB did not have any anti-tumor activity in the treatment of

HT55 tumors at doses ≤ 50 mg/kg. These results demonstrated that both molecules were highly potent in treatment of HER2 amplified tumors and provide support for a positive therapeutic index based on HER2 expression level in HER2 amplified cancer. The H91A-1Fab-IgG TDB HER2 TDB demonstrated ≥ 100-fold in vivo selectivity to HER2 amplified tumors compared to tumors that express normal, low levels of HER2 (e.g., similar to normal human tissues).

Example 6. Adjusting HER2 affinity for enhanced selectivity

Two 4D5 variants, D98A.F100A.Y102V and H91A (monovalent K_D 23 and 49 nM, respectively), which demonstrated improved selectivity in 1Fab-IgG TDB format, were selected for extended characterization to identify optimal affinity to achieve maximal selectivity to HER2-overexpressing cells. In an in vitro dose response assay against SKBR3 cells, D98A.F100A.Y102V and H91A variants demonstrated near identical activity (FIG. 20). Both molecules demonstrated robust anti-tumor activity on 0.5 mg/kg doses in treatment of HER2 amplified KPL4 tumor xenografts (FIGS. 17 and 19). In vitro activity assays were extended by treating multiple cell lines expressing high, medium, or low levels of HER2 with a high concentration (50 ng/mL) of 1Fab-IgG TDB variants, H91A-1FabIg (FIGS. 21A, 23A, and 25A) and D98A.F100A.Y102V (FIGS. 21B, 23B, and 25B). Responses of the cell lines were generally similar to both molecules. However, a clear trend was observed in which potency of the D98A.F100A.Y102V variant was higher compared to H91A variant (FIGS. 22, 24, and 26). As this trend was detectable in low HER2-expressing cells, it was determined that the H91A substitution in the 4D5 HVR that binds HER2 with monovalent K_D of 49 nM resulted in maximal selectivity to HER2-overexpressing cells.

Example 7. Characterizing the effect of linker sequence length

To quantify the effect of the length of the linker sequence fusing the anti-HER2 Fabs in the heavy chain bivalent HER2 binding and activity, two linker sequences were tested (FIG. 27A). All 1Fab-IgG TDB molecules in previous experiments included the extended linker sequence (DKTHTGGGGSGG, SEQ ID NO: 52) including therewithin the natural DKTHT sequence (SEQ ID NO: 50) derived from the upper IgG1 hinge of the distal Fab, followed by a GGGGSGG extension (SEQ ID NO: 51). The extended linker sequence was compared to a 1Fab-IgG TDB molecule having a linker of the natural DKTHT sequence (SEQ ID NO: 50). The impact of the linkers was tested using H91A-1Fab-IgG TDB. No difference in binding to SKBR3 or MCF7 cells was observed (FIGS. 27B and 27C). In vitro dose response in SKBR3 killing, as well as broader cell line activity screen, demonstrated near identical activity between the variants. The natural linker variant performed similarly in treatment of KPL4 and HT55 tumor xenografts, relative to the extended linker. These data suggest that anti-HER2 1Fab-IgG TDB having monovalent affinities for HER2 ranging from K_D 23 nM to 49 nM exhibit favorable activity profiles. In these studies, maximal selectivity to HER2-overexpressing cells was achieved using the H91A 4D5 variant (K_D 49 nM).

Example 8. T cell-independent anti-signaling effect of trastuzumab is attenuated in 1Fab-IgG TDB molecule with bivalent low affinity binding to HER2

Binding of trastuzumab to overexpressed HER2 in HER2 amplified cancer cells results in robust interference of constitutive ligand independent PI3K signaling initiated by the deregulated HER2-HER3 complex (Junttila et al. *Cancer Res.* 74(19): 5561-5571, 2014). The 1Fab-IgG TDB molecule binds to HER2 in bivalent form. In vitro treatment of SKBR3 cells with incubation with 1Fab-IgG TDB resulted in transient non-durable reduction of pAKT, without detectable effect on HER3 phosphorylation (FIG. 28A). In addition, neither monovalent high HER2 affinity IgG TDB, nor bivalent low HER2 affinity 1Fab-IgG TDB substantially affected the proliferation/viability of SKBR3 cells (FIG. 28B). In summary, bivalent low affinity binding to HER2 does not inhibit proliferation of HER2-amplified cells.

Example 9. Combining 4D5 antibody variants with liability fix variants

To enhance stability of 4D5 antibody affinity variants, liability fixes were introduced. To reduce deamidation, 4D5 light chain residue N30 was substituted with a serine residue (N30S), and 4D5 heavy chain residue N54 was substituted with a glutamic acid residue (N54E). To reduce isomerization, 4D5 heavy chain residue D98 was substituted with an alanine residue (D98A) and/or a threonine residue (D98T). Binding affinities of the wildtype 4D5 Fab and affinity variants thereof (4D5 Y55E.Y102V-Fab, 4D5 D98A.F100A.Y102V-Fab, and 4D5 H91A-Fab; FIGS. 29A-29D); liability fix variants (4D5 N30S-Fab, 4D5 N54E.D98T-Fab, and N30S.N54E.D98T-Fab; FIGS. 30A-30C); and affinity variants with liability fixes (4D5 N30S.Y55E.N54E.D98T.Y102V-Fab, 4D5 N30S.N54E.D98A.F100A.Y102V-Fab, and 4D5 H91A.N30S.N54E.D98T-Fab; FIGS. 31A-31C) were quantified by BIACORE®.

4D5 affinity variants having liability fixes were generated and characterized. The molecular weights (MW; Daltons) of each 4D5 Fab variant was measured by mass spectrometry and the degree of aggregation was quantified by size exclusion chromatography (SEC). Results are shown in Table 7, below. The observed difference in MW between observed and expected values was linked to C-terminal lysine clipping.

Table 7. Results of mass spectrometry and SEC experiments.

4D5 Fab	Observed MW	Expected MW	Delta MW	% Monomer
WT	48,605	48,773	-128	99.11
H91A	48,539	48,667	-128	97.89
Y55E.Y102V	48,507	48,635	-128	96.28
D98A.F100A.Y102V	48,421	48,549	-128	99.28
D98T.F100A.Y102V	48,451	48,579	-128	98.71
N30S.N54E.D98T	48,579	48,707	-128	98.69
N30S.H91A.N54E.D98T	48,513	48,641	-128	98.86
H91A.N54E.D98T	48,540	48,668	-128	99.03
N30S.Y55E.N54E.D98T.Y102V	48,442	48,570	-128	99.65
N30S	48,578	48,706	-128	95.13
N54E.D98T	48,606	48,734	-128	97.16
N30S.N54E.D98A.F100A.Y102V	48,409	48,537	-128	97.24
N30S.N54E.D98T.F100A.Y102V	48,439	48,567	-128	98.67

Variants of 4D5 affinity variants were formatted into IgG TDB antibodies by associating a monovalent 4D5 arm with an anti-CD3 (40G5c N297G) arm (see, e.g., U.S. Publication No. 2015/095392, which is incorporated herein by reference in its entirety). The antibodies were tested for binding to human HER2 by BIACORE®. Results are shown in FIGS. 32A-32K and summarized in Table 8, below.

Table 8. Binding characteristics of 4D5 IgG TDB antibodies to human HER2.

Anti-HER2 Variant (IgG TDB)	**k_a (1/Ms)**	**k_d (1/s)**	**K_D (nM) Hu HER2**	**K_D Ratio (Variant/WT)**	**Rmax (RU)**	**Chi^2 (RU^2)**
H91A.D98A.F100A.Y102V	—	—	—	—	—	—
Y55E.H91A.D98A.F100A.Y102V	—	—	—	—	—	—
Y55E.H91A.Y102V	—	—	—	—	—	—
WT	3.20E+05	2.75E-04	0.86	1	42.4	6.02
Y102V	2.59E+05	3.27E-04	1.26	1.5	41.2	4.58
Y55E.Y102V	1.82E+05	1.30E-03	7.15	8.3	36.7	2.93
Y55E.D98A.F100A.Y102V	2.65E+05	1.12E-02	42.1	48.8	33.1	4.11
D98A.F100A.Y102V	3.12E+05	1.51E-02	48.5	56.3	32.8	6.24
H91A	2.37E+05	2.17E-02	91.6	106.3	28.6	2.28
H91A.Y102V	2.61E+05	3.17E-02	121	140.4	25.4	2.07
Y55E.H91A	1.55E+05	4.67E-02	301	349.2	12.2	2.26

No binding was detected in H91A.D98T.F100A.Y102V-IgG TDB, Y55E.H91A.D98A.F100A.Y102V-IgG TDB, or Y55E.H91A.Y102V-IgG TDB variants. In general, lower K_D values (stronger binding affinities) were largely associated with slower off-rates. In the IgG TDB format, about two-fold weaker affinity was observed compared to the Fab format (Tables 9 and 10). However, when a K_D ratio is used, wherein the K_D of a 4D5 variant is divided by the K_D of a 4D5 wildtype, the result is a relatively consistent value of 100-200 for the H91A variant in either the IgG TDB or Fab format.

Table 9. Binding characteristics of liability fixed 4D5 Fab molecules to human HER2.

4D5 Fab	**k_a (1/Ms)**	**k_d (1/s)**	**K_D (nM)**	**K_D ratio (Variant/WT)**	**R_{max} (RU)**	**χ^2 (RU^2)**
N30S.N54E.D98T	1.07E+6	6.18E-5	0.06	0.23	42.1	0.681
N54E.D98T	9.62E+5	6.89E-5	0.07	0.29	40.9	0.722
WT	1.02E+6	2.54E-4	0.25	1	43	0.544
N30S	1.05E+6	2.82E-4	0.27	1.07	41.2	0.656
Y55E.Y102V	6.60E+5	1.46E-3	2.21	8.84	42.3	0.555
H91A.N54E.D98T	7.82E+5	5.02E-3	6.42	25.7	39.6	0.185
N30S.H91A.N54E.D98T	8.22E+5	6.55E-3	7.97	31.9	39.7	0.15
N30S.N54E.D98T.F100A.Y102V	7.39E+5	6.76E-3	9.15	36.6	38.3	0.185
N30S.N54E.D98A.F100A.Y102V	7.58E+5	9.58E-3	12.6	50.4	37.2	0.235
D98T.F100A.Y102V	1.14E+6	1.47E-2	12.9	51.6	38.9	0.212
D98A.F100A.Y102V	1.14E+6	2.37E-2	20.7	82.8	37.7	0.319
H91A	6.63E+5	2.82E-2	42.6	170	36	0.281
Y55E.H91A.N54E.D98T.Y102V	6.42E+5	3.18E-2	49.5	198	35.8	0.113

A panel of 4D5 variants in Fab format was generated to obtain a range of affinities for screening in either IgG TDB (Table 9) or 1Fab-IgG TDB format (Table 10). Because the affinity and kinetics were most desirable in the H91A variant, elimination of possible molecular liabilities in this variant was undertaken. Liabilities included light chain position N(30)T in HVR-L1 and heavy chain positions N(54)G in HVR-H2 and D(98)G in HVR-H3. The objective was to prevent possible D(98)G isomerization and possible N(30)T and N(54)G deamidation. Initially, rational design was used to screen the variants. However, combining weak affinity variants with high affinity variants did not yield predictive affinity, and even combinations of two or three variants yielded unpredictable results, as illustrated in FIG. 38. For example, Y55E typically weakened affinity 3.5- to 20-fold, but in one case yielded a small increase in affinity. H91A weakened affinity from 91 to 163-fold and Y102V either increased affinity or did not change affinity. Therefore, a small matrix was tested using N30S.N54E.D98A/T to fix the molecular liabilities. N30S was used to prevent possible deamidation in the HVR-L1 and had little-to-no effect on affinity. N54E.D98T increased affinity to 0.07 nM, so affinity weakening mutations were required to achieve a K_D of about 50 nM. Through the use of the affinity mutations Y55E, H91A, and/or Y102V, in addition to the liability fixes, two variants having similar kinetics to H91A were identified (Table 10). 4D5 N30S.Y55E.H91A.HC-N54E.D98T-Fab features fixes in all three liabilities, while 4D5 Y55E.H91A.HC-N54E.D98T.Y102V-Fab features two liability fixed residues, and both have K_Ds of about 50 nM. Another variant, 4D5 N30S.Y55E.H91A.N54E.D98T.Y102V-Fab exhibited a consistently slightly faster off-rate and had a K_D of 70 nM.

Table 10. Binding characteristics of liability fixed 4D5 Fab molecules to human HER2.

4D5 Fab	**k_a (1/Ms, E+5)**	**k_d (1/s, E-3)**	**K_D (nM)**	**K_D ratio (Variant/WT)**
WT	8.74 ± 1.74	0.25 ± 0.03	0.30 ± 0.01	1.0
N30S.Y55E.H91A.N54E.D98T	6.76 ± 1.41	32.8 ± 4.07	49.2 ± 5.06	164
H91A	7.07 ± 2.43	33.6 ± 6.54	49.3 ± 8.57	164
Y55E.H91A.N54E.D98T.Y102V	5.98 ± 0.18	32.2 ± 1.45	54.0 ± 2.51	180
N30S.Y55E.H91A.N54E.D98T.Y102V	6.34 ± 0.33	44.0 ± 1.97	69.8 ± 6.95	233
Y55E.H91A.N54E.D98T	1.85 ± 0.49	22.7 ± 3.25	126 ± 18.0	420

As shown in Table 11, monovalent affinity of wildtype 4D5 Fab to cynomolgus monkey HER2 (3.3 nM) was roughly 10-fold weaker than to human HER2 (0.3 nM). The 4D5 H91A-Fab affinity variant and the liability fixed variants exhibit monovalent affinities greater than 300 nM to cynomolgus monkey HER2 (Table 12). However, the ratio of K_D to the cynomolgus HER2 protein of the cynomolgus monkey variant 4D5 H91A-Fab and 4D5 wildtype was roughly 100-200, within a similar range of that observed in the human format.

Table 11. Binding characteristics of liability fixed 4D5 Fab molecules to cynomolgus monkey HER2.

4D5 Fab	K_D (nM)	K_D ratio (Variant/WT)	Cyno K_D (nM)	Human K_D (nM)	Cyno/ Human K_D
WT	3.35	1	3.35	0.30	11.2
H91A	334	100	325	49.3	6.77
Y55E.H91A.N54E.D98T	≥ 770	230	≥728	126	≥6.11
Y55E.H91A.N54E.D98T.Y102V	≥ 710	212	≥692	54.0	≥13.1
N30S.Y55E.H91A.N54E.D98T	420	125	400	49.2	8.54
N30S.Y55E.H91A.N54E.D98T.Y102V	452	134	429	69.8	6.48

Example 10. Characterizing 4D5 liability fixed affinity variants

Several 4D5 liability fixed affinity variants were selected for further characterization. Y55E.H91A.N54E.D98T and Y55E.H91A.N54E.D98T.Y102V variants were formatted into a 1Fab-IgG TDB antibody having a natural (short) linker (hinge; DKTHT) and compared to H91A-1Fab-IgG TDB in a dose response assay to quantify killing of SKBR3 (FIG. 33A) or MCF7 (FIG. 33B) target cells. Each well was seeded with 1.5 × 10^4 SKBR3 or MCF7 target cells and co-cultured with PMBC-derived $CD8^+$ effector cells at a 1:3 effector:target ratio. Results are summarized in Table 12, below.

Table 12. SKBR3 target cell killing by selected 4D5 1Fab-IgG TDB variants

4D5 1Fab-IgG TDB	SKBR3 IC_{50} (ng/mL)	MCF7 IC_{50} (ng/mL)
Y55E.H91A.N54E.D98T.Y102V	2.61	>1,000
Y55E.H91A.N54E.D98T	1.63	>1,000
Y55E.H91A.N54E.D98T.Y102V	1.83	>1,000
H91A	2.01	>1,000

The binding of 4D5 Y55E.H91A.N54E.D98T-1Fab-IgG TDB and 4D5 Y55E.H91A.N54E.D98T.Y102V antibodies to SKBR3 cells (FIG. 34A) and MCF7 cells (FIG. 34B) was also characterized by flow cytometry. In general, 4D5 Y55E.H91A.N54E.D98T.Y102V-1Fab-IgG TDB were similar to 4D5 H91A-1Fab-IgG TDB in terms of cytotoxicity and binding to SKBR3 cells and MCF7 cells.

Example 11. Cellular Binding Affinity

To understand the contribution of bivalent HER2 engagement, or avidity, to cell-based affinity in greater detail, the apparent affinities of the 4D5-WT 1Fab-IgG TDB and 4D5-H91A 1Fab-IgG TDB molecules to SKBR3, MCF7, and cells transfected to express cynomolgus monkey HER2 at levels comparable to MCF7 was determined (Table 13). Despite the 164-fold difference between 4D5 and the affinity attenuated 4D5-H91A variant as monovalent Fab, the cell-based binding affinities of the 1Fab-IgG molecules to SKBR3 cells were comparable, as determined by direct cell binding or competition binding assays. The apparent affinity of 4D5-H91A 1Fab-IgG TDB for HER2 expressed on SKBR3 cells was in the range of 1.5-5 nM, which is ~10-fold higher compared to its monovalent Fab affinity measured by BIACORE®. This result suggests that the low monovalent Fab affinity can be compensated by avidity that is mediated by bivalent binding to HER2 in a cellular context. In contrast, 4D5-H91A 1Fab-IgG TDB did not demonstrate substantial binding to MCF7 or cyno HER2-expressing cells (K_D >100 nM for both in direct cell binding and no binding detected in competition binding), suggesting that the lower HER2 copy number on these cells is not sufficient for bivalent HER2 engagement. Results are summarized in Table 13.

Table 13. Binding affinity of the 1Fab-IgG TDBs for cynomolgus monkey HER2.

1Fab IgG	**K_D (nM)**		
	SKBR3	**MCF7**	**CHO-cynoHER2 High**
WT	3.26±1.22	11.57±5.91	1.51±0.15
H91A	5.02±1.96	>100	>100
N30S.Y55E.H91A.N54E.D98T	5.08±1.22	>100	>100

The effect of CD3 binding affinity on TDB-mediated target cell killing was also assessed. 4D5-H91A 1Fab-IgG TDB having the low-affinity 40G5c anti-CD3 arm (“40G5c 4D5-H91A 1Fab-IgG TDB”; CD3 K_D ~50 nM) was compared to an analogous 4D5-H91A 1Fab-IgG TDB having a high-affinity 38E4v1 anti-CD3 arm (“38E4v1 4D5-H91A 1Fab-IgG TDB”; CD3 K_D ~1 nM). In an in vitro cell killing assay using the high-HER2-expressing target cell line SKBR3, 40G5c 4D5-H91A 1Fab-IgG TDB exhibited similar potency in target cell killing as 38E4v1 4D5-H91A 1Fab-IgG TDB (FIG. 37). In contrast, in an analogous assay using the low-HER2-expressing target cell line MCF7, 38E4v1 4D5-H91A 1Fab-IgG TDB was substantially more potent in target cell killing than 40G5c 4D5-H91A 1Fab-IgG TDB, which exhibited very little cell-killing ability. These results, summarized in Table 14, below, show that low anti-CD3 binding dramatically enhances the ability of 4D5-H91A 1Fab-IgG TDB to selectively kill cells that express high levels of HER2.

Table 14. Effect of CD3 binding affinity on 4D5-H91A 1Fab-IgG TDB-mediated target cell killing

Anti-CD3 clone	**Cell Line**	**IC_{50} (ng/mL)**	**IC_{50} (pM)**
40G5c (low-affinity)	MCF7	>1,000	>1,000
40G5c (low-affinity)	SKBR3	4.2	28
38E4v1 (high-affinity)	MCF7	150	970
38E4v1 (high-affinity)	SKBR3	11	1.8

Example 12. High dose of 4D5-H91A 1Fab-IgG TDB does not result in HER2-independent T cell activation

FcγR binding has been attenuated in TDBs by N297G substitution in the Fc-region. To confirm that TDBs do not induce target-independent T cell activation at high concentration, a Jurkat reporter cell line was used (FIG. 38A). A robust signal was detected when reporter cells were stimulated with TDB in the presence of HER2 expressing cells. Neither 4D5-WT IgG TDB nor 4D5 H91A 1Fab-IgG TDB activated T cells in the absence of HER2. High levels of TDB were spiked into human PBMC (FIG. 38B). Bivalent anti-CD3 (OKT-3) induced T cell activation at ≥ 10 ng/ml concentration, whereas no T cell activation was detected at 100 µg/ml concentration, indicating that neither TDB format induces HER2-independent T cell activation at high concentrations.

Example 13. 4D5-H91A 1Fab-IgG TDB has limited pharmacological activity and is well-tolerated in cynomolgus monkey

Cynomolgus monkey (cyno) tissues express low levels of HER2, similar to human normal tissues. 4D5-H91A 1Fab-IgG TDB does not substantially bind or induce apoptosis of cells that express low-to-moderate levels of cyno-HER2 (Table 13). To test the tolerability of 4D5-H91A 1Fab-IgG TDB, a single dose tolerability study was designed to study the pharmacodynamic activity (PD) and pharmacokinetics (PK) in cynomolgus monkey. Female monkeys were dosed with slow intravenous infusion at 3 mg/kg (N=3) and 20 mg/kg (N=5) dose level. Tolerability/PK/PD readouts included clinical observations, clinical chemistry, hematology, cytokines, and PK. Histopathology was not included in the analysis. No or minimal test article-related changes were detected in clinical observations (body weight, heart rate, temperature, respiration rate). Typical TDB-associated pharmacological and clinical pathology responses (Bargou et al., *Science* 2008, 321: 974-977; Lutterbuese et al., *Proc. Nat. Acad. Sci.* 2010, 107(28): 12605-12610) were strikingly absent (FIG. 38C). C-reactive protein (CRP; an acute phase reactant and a marker for inflammation) levels remained at the background level. No lymphocyte margination, elevation of liver enzymes (alanine and aspartate aminotransferases; ALT and AST) or elevation of peripheral blood cytokines (not shown) were detected, despite the high TDB dose. Taken together, 4D5-H91A 1Fab-IgG TDB was well tolerated at high dose level and demonstrated limited pharmacological activity in cyno.

TDB exposure was confirmed for both dose levels tested and PK characteristics normal (FIG. 38D). Exposures were close to dose-proportional and maintained until the last evaluated time point (14 days). To confirm that the TDB is not inactivated in cyno, aliquots of serum were recovered from samples harvested 7 days and 14 days after dose with 20 mg/kg of the TDB, and serum dilutions were used to mediate killing of SKBR3 cells in vitro using healthy donor human T cells (FIG. 38E). Measured TDB serum concentrations in undiluted samples were 193 and 65 µg/mL for day-7 and day-14 samples, respectively. 100-fold dilution of serum 14 days after dosing induced similar level of SKBR3 killing, compared to maximal activity achieved in parallel killing assay using fresh TDB at high (1 µg/ml) concentration, which typically is sufficient to saturate activity. Together, this data suggests that the low HER2 expression in normal cyno tissues is not sufficient to trigger T cell activation or off-tumor T cell activity on normal tissues that express low levels of HER2.

WHAT IS CLAIMED IS:

1. A bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein:

(a) the monovalent arm comprises a first antigen-binding moiety, wherein the C-terminus of the first antigen-binding moiety is fused to the N-terminus of a first Fc subunit;

(b) the bivalent arm comprises a second antigen-binding moiety and a third antigen-binding moiety, wherein the C-terminus of the third antigen-binding moiety is fused to the N-terminus of the second antigen-binding moiety, and the C-terminus of the second antigen-binding moiety is fused to an N-terminus of a second Fc subunit; and

(c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain,

wherein the first antigen-binding moiety is capable of specific binding to CD3, and the second antigen-binding moiety and the third antigen-binding moiety are each capable of specific binding to HER2.

2. The bispecific antigen binding molecule of claim 1, wherein the second and third antigen-binding moieties bind domain IV of HER2.

3. The bispecific antigen-binding molecule of claim 1 or 2, wherein the monovalent binding affinity (K_D) of each of the second antigen-binding moiety and the third antigen-binding moiety is from 10 nM to 100 nM.

4. The bispecific antigen-binding molecule of claim 3, wherein the monovalent K_D of each of the second antigen-binding moiety and the third antigen-binding moiety is from 20 nM to 50 nM.

5. The bispecific antigen-binding molecule of any one of claims 1-4, wherein the monovalent dissociation rate of the second antigen-binding moiety and/or the third antigen-binding moiety is from 10^{-3}/second to 10^{-1}/second.

6. The bispecific antigen-binding molecule of claim 5, wherein the monovalent dissociation rate of each of the second antigen-binding moiety and the third antigen-binding moiety is from 10^{-2}/second to 30^{-2}/second.

7. The bispecific antigen-binding molecule of any one of claims 1-6, wherein the monovalent K_D of the first antigen-binding moiety is from 10 nM to 100 nM.

8. The bispecific antigen-binding molecule of claim 7, wherein the monovalent K_D of the first antigen-binding moiety is from 20 nM to 80 nM.

9. The bispecific antigen-binding molecule of claim 8, wherein the monovalent K_D of the first antigen-binding moiety is from 40 nM to 60 nM.

10. The bispecific antigen-binding molecule of any of claims 1-9, wherein the monovalent K_D of the second antigen-binding moiety is the K_D of the second antigen-binding moiety in Fab format, measured using surface plasmon resonance, and wherein the monovalent K_D of the third antigen-binding moiety is the K_D of the third antigen-binding moiety in Fab format, measured using surface plasmon resonance.

11. The bispecific antigen-binding molecule of any one of claims 1-10, wherein the first antigen-binding moiety is a Fab molecule (Fab_A) comprising a variable heavy chain (VH_A) region and a variable light chain (VL_A) region.

12. The bispecific antigen-binding molecule of any one of claims 1-11, wherein the second antigen-binding moiety is a Fab molecule (Fab_{B1}) comprising a variable heavy chain (VH_{B1}) region and a variable light chain (VL_{B1}) region and/or the third antigen-binding moiety is a Fab molecule (Fab_{B2}) comprising a variable heavy chain (VH_{B2}) region and a variable light chain (VL_{B2}) region.

13. The bispecific antigen-binding molecule of claim 12, wherein the first antigen-binding moiety is a Fab_A comprising a VH_A region and a VL_A region, the second antigen-binding moiety is a Fab_{B1} comprising a VH_{B1} region and a VL_{B1} region, and the third antigen-binding moiety is a Fab_{B2} comprising a VH_{B2} region and a VL_{B2} region.

14. The bispecific antigen-binding molecule of any one of claims 12-13, wherein (a) the VH_{B1} and the VH_{B2} share at least 95% sequence identity; (b) the VL_{B1} and the VL_{B2} share at least 95% sequence identity; or (c) the VH_{B1} and the VH_{B2} share at least 95% sequence identity and the VL_{B1} and the VL_{B2} share at least 95% sequence identity.

15. The bispecific antigen-binding molecule of any one of claims 12-14, wherein the VH_{B1} region and/or the VH_{B2} region comprises an amino acid substitution at one, two, three, or all four residues of N54, D98, F100, and/or Y102, according to the Kabat numbering system.

16. The bispecific antigen-binding molecule of claim 15, wherein the VH_{B1} region and/or the VH_{B2} region comprises an amino acid substitution at one, two, three, four, or all five of the following residues: N54E, D98A, D98T, F100A, and/or Y102V, according to the Kabat numbering system.

17. The bispecific antigen-binding molecule of any one of claims 12-15, wherein the VL_{B1} region and/or the VL_{B2} region comprises an amino acid substitution at one, two, or all three residues of N30, Y55, and/or H91, according to the Kabat numbering system.

18. The bispecific antigen-binding molecule of claim 17, wherein the VL_{B1} region and/or the VL_{B2} region comprises an amino acid substitution at one, two, or all three of the following residues: N30S, Y55E, and/or H91A.

19. The bispecific antigen-binding molecule of any one of claims 12-18, wherein the VH_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20.

20. The bispecific antigen-binding molecule of any one of claims 12-19, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24.

21. The bispecific antigen-binding molecule of claim 20, wherein the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 24.

22. The bispecific antigen-binding molecule of any one of claims 12-21, wherein the VL_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

23. The bispecific antigen-binding molecule of any one of claims 12-22, wherein the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27.

24. The bispecific antigen-binding molecule of claim 23, wherein the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 27.

25. The bispecific antigen-binding molecule of any one of claims 12-24, wherein the VH_{B1} comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20;

and the VL_{B1} comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

26. The bispecific antigen-binding molecule of any one of claims 12-25, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24, and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27.

27. The bispecific antigen-binding molecule of claim 26, wherein the VH_{B1} region comprises the amino acid of SEQ ID NO: 24, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 27.

28. The bispecific antigen-binding molecule of any one of claims 12-27, wherein the VH_{B2} region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20.

29. The bispecific antigen-binding molecule of any one of claims 12-28, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24.

30. The bispecific antigen-binding molecule of claim 29, wherein the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 24.

31. The bispecific antigen-binding molecule of any one of claims 12-30, wherein the VL_{B2} region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

32. The bispecific antigen-binding molecule of any one of claims 12-31, wherein the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27.

33. The bispecific antigen-binding molecule of claim 32, wherein the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 27.

34. The bispecific antigen-binding molecule of any one of claims 12-33, wherein the VH_{B2} comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20;

and the VL_{B2} comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and

(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

35. The bispecific antigen-binding molecule of any one of claims 12-34, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24, and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27.

36. The bispecific antigen-binding molecule of claim 35, wherein the VH_{B2} region comprises the amino acid of SEQ ID NO: 24, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 27.

37. The bispecific antigen-binding molecule of any one of claims 12-36, wherein the VL_{B1} region and/or the VL_{B2} region comprises one or more liability fixed residues.

38. The bispecific antigen-binding molecule of claim 37, wherein the one or more liability fixed residues comprises the amino acid substitution of N30S.

39. The bispecific antigen-binding molecule of any one of claims 12-38, wherein the VH_{B1} region and/or the VH_{B2} region comprises one or more liability fixed residues.

40. The bispecific antigen-binding molecule of claim 39, wherein the one or more liability fixed residues comprises one or more an amino acid substitutions selected from the group consisting of N54E, D98A, and D98T.

41. The bispecific antigen-binding molecule of any one of claims 12-18, wherein the VH_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32.

42. The bispecific antigen-binding molecule of any one of claims 12-18 or 41, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33.

43. The bispecific antigen-binding molecule of claim 42, wherein the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 33.

44. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-43, wherein the VL_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23.

45. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-44, wherein the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25.

46. The bispecific antigen-binding molecule of claim 45, wherein the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 25.

47. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-46, wherein the VH_{B1} comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32;

and the VL_{B1} comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23.

48. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-47, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33; and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25.

49. The bispecific antigen-binding molecule of claim 48, wherein the VH_{B1} region comprises the amino acid of SEQ ID NO: 33, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 25.

50. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-49, wherein the VH_{B2} region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32.

51. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-50, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33.

52. The bispecific antigen-binding molecule of claim 51, wherein the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 33.

53. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-52, wherein the VL_{B2} region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23.

54. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-53, wherein the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25.

55. The bispecific antigen-binding molecule of claim 54, wherein the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 25.

56. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-55, wherein the VH_{B2} comprises the following HVRs:
(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32;
and the VL_{B2} comprises the following HVRs:
(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23.

57. The bispecific antigen-binding molecule of any one of claims 12-18 or 41-56, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33, and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25.

58. The bispecific antigen-binding molecule of claim 57, wherein the VH_{B2} region comprises the amino acid of SEQ ID NO: 33, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 25.

59. The bispecific antigen-binding molecule of any one of claims 12-18, wherein the VH_{B1} region comprises one, two, or all three of the following HVRs:
(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37.

60. The bispecific antigen-binding molecule of any one of claims 12-18 or 59, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41.

61. The bispecific antigen-binding molecule of claim 60, wherein the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 41.

62. The bispecific antigen-binding molecule of any one of claims 12-18 or 59-61, wherein the VL_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

63. The bispecific antigen-binding molecule of any one of claims 12-18 or 59-62, wherein the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

64. The bispecific antigen-binding molecule of claim 63, wherein the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48.

65. The bispecific antigen-binding molecule of any one of claims 12-18 or 59-64, wherein the VH_{B1} comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37;

and the VL_{B1} comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

66. The bispecific antigen-binding molecule of any one of claims 12-18 or 59-65, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

67. The bispecific antigen-binding molecule of claim 66, wherein the VH_{B1} region comprises the amino acid of SEQ ID NO: 41, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48.

68. The bispecific antigen-binding molecule of any one of claims 12-18 or 59-67, wherein the VH_{B2} region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37.

69. The bispecific antigen-binding molecule of any one of claims 12-18 or 59-68, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41.

70. The bispecific antigen-binding molecule of claim 69, wherein the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 41.

71. The bispecific antigen-binding molecule of any one of claims 12-18 or 59-70, wherein the VL$_{B2}$ region comprises one, two, or all three of the following HVRs:
(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

72. The bispecific antigen-binding molecule of any one of claims 12-18 or 59-71, wherein the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

73. The bispecific antigen-binding molecule of claim 72, wherein the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 48.

74. The bispecific antigen-binding molecule of any one of claims 12-17 or 59-73, wherein the VH$_{B2}$ comprises the following HVRs:
(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37;
and the VL$_{B2}$ comprises the following HVRs:
(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

75. The bispecific antigen-binding molecule of any one of claims 12-18 or 59-74, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

76. The bispecific antigen-binding molecule of claim 75, wherein the VH$_{B2}$ region comprises the amino acid of SEQ ID NO: 41, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 48.

77. The bispecific antigen-binding molecule of any one of claims 12-18, wherein the VH$_{B1}$ region comprises one, two, or all three of the following HVRs:
(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43.

78. The bispecific antigen-binding molecule of any one of claims 12-18 or 77, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44.

79. The bispecific antigen-binding molecule of claim 78, wherein the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 44.

80. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-79, wherein the VL_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

81. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-80, wherein the VL region of the second antigen-binding moiety comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

82. The bispecific antigen-binding molecule of claim 81, wherein the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48.

83. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-82, wherein the VH_{B1} comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43;

and the VL_{B1} comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

84. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-83, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

85. The bispecific antigen-binding molecule of claim 84, wherein the VH_{B1} region comprises the amino acid of SEQ ID NO: 44, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48.

86. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-85, wherein the VH$_{\mathrm{B2}}$ region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43.

87. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-86, wherein the VH$_{\mathrm{B2}}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44.

88. The bispecific antigen-binding molecule of claim 87, wherein the VH$_{\mathrm{B2}}$ region comprises the amino acid sequence of SEQ ID NO: 44.

89. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-88, wherein the VL$_{\mathrm{B2}}$ region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

90. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-89, wherein the VL$_{\mathrm{B2}}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

91. The bispecific antigen-binding molecule of claim 90, wherein the VL$_{\mathrm{B2}}$ region comprises the amino acid sequence of SEQ ID NO: 48.

92. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-91, wherein the VH$_{\mathrm{B2}}$ comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43;

and the VL$_{\mathrm{B2}}$ comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

93. The bispecific antigen-binding molecule of any one of claims 12-18 or 77-92, wherein the VH$_{\mathrm{B2}}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{\mathrm{B2}}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

94. The bispecific antigen-binding molecule of claim 93, wherein the VH_{B2} region comprises the amino acid of SEQ ID NO: 44, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48.

95. The bispecific antigen-binding molecule of any one of claims 12-18, wherein the VH_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37.

96. The bispecific antigen-binding molecule of any one of claims 12-18 or 95, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41.

97. The bispecific antigen-binding molecule of claim 96, wherein the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 41.

98. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-97, wherein the VL_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

99. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-98, wherein the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

100. The bispecific antigen-binding molecule of claim 99, wherein the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49.

101. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-100, wherein the VH_{B1} comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37;

and the VL_{B1} comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

102. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-101, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41,

and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

103. The bispecific antigen-binding molecule of claim 102, wherein the VH$_{B1}$ region comprises the amino acid of SEQ ID NO: 41, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 49.

104. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-103, wherein the VH$_{B2}$ region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37.

105. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-104, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41.

106. The bispecific antigen-binding molecule of claim 105, wherein the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 41.

107. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-106, wherein the VL$_{B2}$ region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

108. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-107, wherein the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

109. The bispecific antigen-binding molecule of claim 108, wherein the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 49.

110. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-109, wherein the VH$_{B2}$ comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37;

and the VL$_{B2}$ comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

111. The bispecific antigen-binding molecule of any one of claims 12-18 or 95-110, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

112. The bispecific antigen-binding molecule of claim 111, wherein the VH_{B2} region comprises the amino acid of SEQ ID NO: 41, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 49.

113. The bispecific antigen-binding molecule of any one of claims 12-18, wherein the VH_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43.

114. The bispecific antigen-binding molecule of any one of claims 12-18 or 113, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44.

115. The bispecific antigen-binding molecule of claim 114, wherein the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 44.

116. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-115, wherein the VL_{B1} region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

117. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-116, wherein the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

118. The bispecific antigen-binding molecule of claim 117, wherein the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49.

119. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-118, wherein the VH_{B1} comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43;

and the VL_{B1} comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

120. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-119, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

121. The bispecific antigen-binding molecule of claim 120, wherein the VH_{B1} region comprises the amino acid of SEQ ID NO: 44, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 49.

122. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-121, wherein the VH_{B2} region comprises one, two, or all three of the following HVRs:
(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, or
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43.

123. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-122, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44.

124. The bispecific antigen-binding molecule of claim 123, wherein the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 44.

125. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-124, wherein the VL_{B2} region comprises one, two, or all three of the following HVRs:
(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,
(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, or
(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

126. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-125, wherein the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

127. The bispecific antigen-binding molecule of claim 126, wherein the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 49.

128. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-127, wherein the VH_{B2} comprises the following HVRs:
(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,

(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36, and

(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43;

and the VL$_{B2}$ comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,

(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and

(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

129. The bispecific antigen-binding molecule of any one of claims 12-18 or 113-128, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

130. The bispecific antigen-binding molecule of claim 129, wherein the VH$_{B2}$ region comprises the amino acid of SEQ ID NO: 41, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 49.

131. The bispecific antigen-binding molecule of any one of claims 11-130, wherein the VH$_{A}$ region comprises one, two, or all three of the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1,

(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, or

(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3.

132. The bispecific antigen-binding molecule of any one of claims 11-131, wherein the VH$_{A}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7.

133. The bispecific antigen-binding molecule of claim 132, wherein the VH$_{A}$ region comprises the amino acid sequence of SEQ ID NO: 7.

134. The bispecific antigen-binding molecule of any one of claims 11-133, wherein the VL$_{A}$ region comprises one, two, or all three of the following HVRs:

(a) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4,

(b) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, or

(c) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6.

135. The bispecific antigen-binding molecule of any one of claims 11-134, wherein the VL$_{A}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8.

136. The bispecific antigen-binding molecule of claim 135, wherein the VL$_{A}$ region comprises the amino acid sequence of SEQ ID NO: 8.

137. The bispecific antigen-binding molecule of any one of claims 11-136, wherein the VH$_A$ comprises the following HVRs:

(a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1,
(b) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2, and
(c) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3;

and the VL$_A$ comprises the following HVRs:

(d) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4,
(e) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and
(f) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6.

138. The bispecific antigen-binding molecule of any one of claims 11-137, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8.

139. The bispecific antigen-binding molecule of claim 138, wherein the VH$_A$ region comprises the amino acid of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8.

140. A bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein:

(a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6;

(b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 20,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 22,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and

(c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain.

141. The bispecific antigen-binding molecule of claim 140, wherein:

(a) the Fab_{A} comprises a VH_{A} region and a VL_{A} region, wherein the VH_{A} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL_{A} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8;

(b) the Fab_{B1} comprises a VH_{B1} region and a VL_{B1} region, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24, and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27; and

(c) the Fab_{B2} comprises a VH_{B2} region and a VL_{B2} region, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 24, and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 27.

142. The bispecific antigen-binding molecule of claim 141, wherein:

(a) the VH_{A} region comprises the amino acid sequence of SEQ ID NO: 7, and the VL_{A} region comprises the amino acid sequence of SEQ ID NO: 8;

(b) the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 24, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 27; and

(c) the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 24, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 27.

143. A bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein:

(a) the monovalent arm comprises a Fab_{A}, wherein the C-terminus of the Fab_{A} is fused to an N-terminus of a first Fc subunit, and wherein the Fab_{A} comprises the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6;

(b) the bivalent arm comprises a Fab_{B1} and a Fab_{B2}, wherein the C-terminus of the Fab_{B2} is fused to the N-terminus of the Fab_{B1}, and the C-terminus of the Fab_{B1} is fused to an N-terminus of a second Fc subunit, wherein the Fab_{B1} and the Fab_{B2} each comprise the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 19,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 32,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 22, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 23; and

(c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain.

144. The bispecific antigen-binding molecule of claim 143, wherein:

(a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8;

(b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25; and

(c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 33, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 25.

145. The bispecific antigen-binding molecule of claim 144, wherein:

(a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8;

(b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 33, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 25; and

(c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 33, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 25.

146. A bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein:

(a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6;

(b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and

(c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain.

147. The bispecific antigen-binding molecule of claim 146, wherein:

(a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8;

(b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48; and

(c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

148. The bispecific antigen-binding molecule of claim 147, wherein:

(a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8;

(b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 41, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 48; and

(c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 41, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 48.

149. A bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein:

(a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6;

(b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 21,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and

(c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain.

150. The bispecific antigen-binding molecule of claim 149, wherein:

(a) the Fab_A comprises a VH_A region and a VL_A region, wherein the VH_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL_A region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8;

(b) the Fab_{B1} comprises a VH_{B1} region and a VL_{B1} region, wherein the VH_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL_{B1} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48; and

(c) the Fab_{B2} comprises a VH_{B2} region and a VL_{B2} region, wherein the VH_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL_{B2} region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 48.

151. The bispecific antigen-binding molecule of claim 150, wherein:

(a) the VH_A region comprises the amino acid sequence of SEQ ID NO: 7, and the VL_A region comprises the amino acid sequence of SEQ ID NO: 8;

(b) the VH_{B1} region comprises the amino acid sequence of SEQ ID NO: 44, and the VL_{B1} region comprises the amino acid sequence of SEQ ID NO: 48; and

(c) the VH_{B2} region comprises the amino acid sequence of SEQ ID NO: 44, and the VL_{B2} region comprises the amino acid sequence of SEQ ID NO: 48.

152. A bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein:

(a) the monovalent arm comprises a Fab_A, wherein the C-terminus of the Fab_A is fused to an N-terminus of a first Fc subunit, and wherein the Fab_A comprises the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1,

(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2,

(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3,

(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4,

(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and

(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6;

(b) the bivalent arm comprises a Fab_{B1} and a Fab_{B2}, wherein the C-terminus of the Fab_{B2} is fused to the N-terminus of the Fab_{B1}, and the C-terminus of the Fab_{B1} is fused to an N-terminus of a second Fc subunit, wherein the Fab_{B1} and the Fab_{B2} each comprise the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,

(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36,

(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 37,

(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,

(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and

(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and

(c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain.

153. The bispecific antigen-binding molecule of claim 152, wherein:

(a) the Fab$_A$ comprises a VH$_A$ region and a VL$_A$ region, wherein the VH$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_A$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8;

(b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49; and

(c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 41, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

154. The bispecific antigen-binding molecule of claim 153, wherein:

(a) the VH$_A$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_A$ region comprises the amino acid sequence of SEQ ID NO: 8;

(b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 41, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 49; and

(c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 41, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 49.

155. A bispecific antigen-binding molecule comprising a monovalent arm and a bivalent arm, wherein:

(a) the monovalent arm comprises a Fab$_A$, wherein the C-terminus of the Fab$_A$ is fused to an N-terminus of a first Fc subunit, and wherein the Fab$_A$ comprises the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 1,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 2,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 3,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 4,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 6;

(b) the bivalent arm comprises a Fab$_{B1}$ and a Fab$_{B2}$, wherein the C-terminus of the Fab$_{B2}$ is fused to the N-terminus of the Fab$_{B1}$, and the C-terminus of the Fab$_{B1}$ is fused to an N-terminus of a second Fc subunit, wherein the Fab$_{B1}$ and the Fab$_{B2}$ each comprise the following HVRs:

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 11,
(ii) an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 36,
(iii) an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 43,
(iv) an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 38,
(v) an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 29, and
(vi) an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and

(c) the first Fc subunit is associated with the second Fc subunit to form an Fc domain.

156. The bispecific antigen-binding molecule of claim 155, wherein:

(a) the Fab$_{A}$ comprises a VH$_{A}$ region and a VL$_{A}$ region, wherein the VH$_{A}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 7, and the VL$_{A}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 8;

(b) the Fab$_{B1}$ comprises a VH$_{B1}$ region and a VL$_{B1}$ region, wherein the VH$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{B1}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49; and

(c) the Fab$_{B2}$ comprises a VH$_{B2}$ region and a VL$_{B2}$ region, wherein the VH$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 44, and the VL$_{B2}$ region comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 49.

157. The bispecific antigen-binding molecule of claim 156, wherein:

(a) the VH$_{A}$ region comprises the amino acid sequence of SEQ ID NO: 7, and the VL$_{A}$ region comprises the amino acid sequence of SEQ ID NO: 8;

(b) the VH$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 44, and the VL$_{B1}$ region comprises the amino acid sequence of SEQ ID NO: 49; and

(c) the VH$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 44, and the VL$_{B2}$ region comprises the amino acid sequence of SEQ ID NO: 49.

158. The bispecific antigen-binding molecule of any one of claims 1-157, wherein the Fc domain is an IgG Fc domain.

159. The bispecific antigen-binding molecule of claim 158, wherein the IgG Fc domain is an IgG$_1$ or IgG$_4$ Fc domain.

160. The bispecific antigen-binding molecule of any one of claims 1-159, wherein the Fc domain is a human Fc domain.

161. The bispecific antigen-binding molecule of any one of claims 1-160, wherein the Fc domain comprises one or more amino acid substitution that reduces binding to an Fc receptor and/or effector function.

162. The bispecific antigen-binding molecule of claim 161, wherein the one or more amino acid substitutions that reduces binding to an Fc receptor and/or effector function is at N297.

163. The bispecific antigen-binding molecule of claim 162, wherein the first Fc subunit and the second Fc subunit each comprise the amino acid substitution of N297G.

164. The bispecific antigen-binding molecule of any one of claims 161-163, wherein the Fc receptor is an Fcγ receptor.

165. The bispecific antigen-binding molecule of any one of claims 161-164, wherein the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC).

166. The bispecific antigen-binding molecule of any one of claims 1-165, wherein the Fc domain comprises a modification configured to promote the association of the first Fc subunit with the second Fc subunit.

167. The bispecific antigen-binding molecule of claim 166, wherein an amino acid residue in the CH3 domain of the second Fc subunit is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the second Fc subunit which is positionable in a cavity within the CH3 domain of the first Fc subunit, and an amino acid residue in the CH3 domain of the first Fc subunit is replaced with an amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the first Fc subunit within which the protuberance within the CH3 domain of the second Fc subunit is positionable.

168. The bispecific antigen-binding molecule of claim 167, wherein the CH3 domain of the second Fc subunit comprises the amino acid substitution of T366, and the CH3 domain of the first Fc subunit comprises amino acid substitutions at one, two, or all three of T366, L368, and/or Y407.

169. The bispecific antigen-binding molecule of claim 168, wherein the CH3 domain of the second Fc subunit comprises the amino acid substitution of T366W, and the CH3 domain of the first Fc subunit comprises one, two, or all three amino acid substitutions of T366S, L368A, and/or Y407V.

170. The bispecific antigen-binding molecule of any one of claims 1-169, wherein the C-terminus of the third antigen-binding moiety is fused to the N-terminus of the second antigen-binding moiety via a peptide linker.

171. The bispecific antigen-binding molecule of claim 170, wherein the peptide linker is from 5 to 20 amino acids in length.

172. The bispecific antigen-binding molecule of claim 171, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 50.

173. The bispecific antigen-binding molecule of claim 172, wherein the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 53.

174. The bispecific antigen-binding molecule of claim 173, wherein the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity to any one of SEQ ID NOs: 57, 61, 81, 83, 85, and 87.

175. The bispecific antigen-binding molecule of claim 174, wherein the antigen-binding molecule comprises the amino acid sequence of any one of SEQ ID NOs: 57, 61, 81, 83, 85, and 87.

176. The bispecific antigen-binding molecule of claim 175, wherein the peptide linker comprises the amino acid sequence of SEQ ID NO: 51.

177. The bispecific antigen-binding molecule of claim 176, wherein the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO: 56.

178. The bispecific antigen-binding molecule of claim 177, wherein the antigen-binding molecule comprises an amino acid sequence having at least 95% sequence identity to any one of SEQ ID NOs: 58, 62, 82, 84, 86, and 88.

179. The bispecific antigen-binding molecule of claim 178, wherein the antigen-binding molecule comprises the amino acid sequence of any one of SEQ ID NOs: 58, 62, 82, 84, 86, and 88.

180. An isolated nucleic acid encoding the bispecific antigen-binding molecule of any one of claims 1-179.

181. A vector comprising the isolated nucleic acid of claim 180.

182. A host cell comprising the isolated nucleic acid of claim 180 or the vector of claim 181.

183. The host cell of claim 182, wherein the host cell is a mammalian cell.

184. The host cell of claim 183, wherein the mammalian cell is a Chinese hamster ovary (CHO) cell.

185. The host cell of claim 184, wherein the host cell is a prokaryotic cell.

186. The host cell of claim 185, wherein the prokaryotic cell is an *E. coli* cell.

187. A method of producing the bispecific antigen-binding molecule of any one of claims 1-179, the method comprising culturing the host cell of any one of claims 182-186 in a culture medium.

188. The method of claim 187, wherein the method further comprises recovering the bispecific antigen-binding molecule from the host cell or the culture medium.

189. A set of isolated nucleic acids encoding the bispecific antigen-binding molecule of any one of claims 1-179.

190. A set of vectors, wherein each vector of the set comprises an isolated nucleic acid of the set of isolated nucleic acids of claim 189.

191. A set of host cells, wherein each host cell of the set comprises an isolated nucleic acid of the set of isolated nucleic acids of claim 189 or a vector of the set of vectors of claim 190.

192. The set of host cells of claim 191, wherein the host cells comprise mammalian cells.

193. The set of host cells of claim 192, wherein the mammalian cells are CHO cells.

194. The set of host cells of claim 191, wherein the host cells comprise prokaryotic cells.

195. The set of host cells of claim 194, wherein the prokaryotic cells are *E. coli* cells.

196. A method of producing the bispecific antigen-binding molecule of any one of claims 1-179, the method comprising culturing the set of host cells of any one of claims 191-195 in culture medium.

197. The method of claim 196, wherein the method further comprises recovering the bispecific antigen-binding molecule from the set of host cells or the culture medium.

198. An immunoconjugate comprising the bispecific antigen-binding molecule of any one of claims 1-179 and a cytotoxic agent.

199. A composition comprising the bispecific antigen-binding molecule of any one of claims 1-179.

200. The composition of claim 199, further comprising a pharmaceutically acceptable carrier, excipient, or diluent.

201. The composition of claim 199 or 200, wherein the composition is a pharmaceutical composition.

202. The composition of any one of claims 199-201, wherein the composition further comprises a PD-1 axis binding antagonist or an additional therapeutic agent.

203. The bispecific antigen-binding molecule of any one of claims 1-179 for use as a medicament.

204. The bispecific antigen-binding molecule of any one of claims 1-179 for use in treating or delaying progression of a HER2-positive cancer in a subject in need thereof.

205. The bispecific antigen-binding molecule of any one of claims 1-179 for use in enhancing immune function in a subject having a HER2-positive cancer.

206. The bispecific antigen-binding molecule of claim 205, wherein the HER2-positive cancer is selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, renal cancer, bladder cancer, pancreatic cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, glioblastoma, endometrial cancer, and osteosarcoma.

207. The bispecific antigen-binding molecule of any one of claims 204-206, wherein the HER2-positive cancer is characterized by tumor cells that express HER2 at an average copy number of 200,000 or more copies per cell.

208. Use of the bispecific antigen-binding molecule of any one of claims 1-179 in the manufacture of a medicament for treating or delaying progression of a HER2-positive cancer.

209. Use of the bispecific antigen-binding molecule of any one of claims 1-179 in the manufacture of a medicament for enhancing immune function in a subject having a HER2-positive cancer.

210. The use of claim 208 or 209, wherein the HER2-positive cancer is selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, renal cancer, bladder cancer, pancreatic cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, glioblastoma, endometrial cancer, and osteosarcoma.

211. The use of any one of claims 208-210, wherein the HER2-positive cancer is characterized by tumor cells that express HER2 at an average copy number of 200,000 or more copies per cell.

212. A method of treating or delaying the progression of a HER2-positive cancer in a subject in need thereof, the method comprising administering to the subject the bispecific antigen-binding molecule of any one of claims 1-179.

213. A method of enhancing immune function in a subject having a HER2-positive cancer, the method comprising administering to the subject the bispecific antigen-binding molecule of any one of claims 1-179.

214. The method of claim 212 or 213, wherein the HER2-positive cancer is selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, renal cancer, bladder cancer, pancreatic cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, glioblastoma, endometrial cancer, and osteosarcoma.

215. The method of any one of claims 212-214, wherein the HER2-positive cancer is characterized by tumor cells that express HER2 at an average copy number of 200,000 or more copies per cell.

216. A method of treating or delaying the progression of a HER2-positive cancer in a subject in need thereof, the method comprising:

(a) determining an expression of HER2 on a tumor cell, wherein the tumor cell expresses HER2 at an average copy number of 200,000 or more copies per cell; and

(b) administering to the subject the bispecific antigen-binding molecule of any one of claims 1-179.

217. A method of enhancing immune function in a subject having a HER2-positive cancer, the method comprising:

(a) determining an expression of HER2 on a tumor cell, wherein the tumor cell expresses HER2 at an average copy number of 200,000 or more copies per cell; and

(b) administering to the subject the bispecific antigen-binding molecule of any one of claims 1-179.

218. The method of claim 216 or 217, wherein the HER2-positive cancer is selected from the group consisting of breast cancer, gastric cancer, colorectal cancer, non-small cell lung cancer, renal cancer, bladder cancer, pancreatic cancer, prostate cancer, liver cancer, head and neck cancer, melanoma, ovarian cancer, mesothelioma, glioblastoma, endometrial cancer, and osteosarcoma.

219. The method of any one of claims 212-218, wherein the bispecific antigen-binding molecule is administered to the subject in a dosage of about 0.01 mg/kg to about 10 mg/kg.

220. The method of claim 219, wherein the bispecific antigen-binding molecule is administered to the subject in a dosage of about 0.1 mg/kg to about 10 mg/kg.

221. The method of claim 220, wherein the bispecific antigen-binding molecule is administered to the subject in a dosage of about 1 mg/kg.

222. The method of any one of claims 212-221, further comprising administering to the subject a PD-1 axis binding antagonist and/or an additional therapeutic agent.

223. The method of claim 222, wherein the PD-1 axis binding antagonist or additional therapeutic agent is administered prior to or subsequent to the administration of the bispecific antigen-binding molecule.

224. The method of claim 222, wherein the PD-1 axis binding antagonist additional therapeutic agent is administered concurrently with the bispecific antigen-binding molecule.

225. The method of any one of claims 212-224, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist, a PD-1 binding antagonist, and a PD-L2 binding antagonist.

226. The method of claim 225, wherein the PD-1 axis binding antagonist is a PD-L1 binding antagonist.

227. The method of claim 226, wherein the PD-L1 binding antagonist is selected from the group consisting of MPDL3280A (atezolizumab), YW243.55.S70, MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab).

228. The method of claim 225, wherein the PD-1 axis binding antagonist is a PD-1 binding antagonist.

229. The method of claim 228, wherein the PD-1 binding antagonist is selected from the group consisting of MDX-1106 (nivolumab), MK-3475 (pembrolizumab), MEDI-0680 (AMP-514), PDR001 (spartalizumab), REGN2810 (cemiplimab), and BGB-108.

230. The method of claim 225, wherein the PD-1 axis binding antagonist is a PD-L2 binding antagonist.

231. The method of claim 230, wherein the PD-L2 binding antagonist is an antibody or an immunoadhesin.

232. The method of any one of claims 212-231, wherein the bispecific antigen-binding molecule is administered subcutaneously, intravenously, intramuscularly, topically, orally, transdermally, intraperitoneally, intraorbitally, by implantation, by inhalation, intrathecally, intraventricularly, or intranasally.

233. The method of claim 232, wherein the bispecific antigen-binding molecule is administered subcutaneously.

234. The method of claim 232, wherein the bispecific antigen-binding molecule is administered intravenously.

235. The method of any one of claims 212-234, wherein the subject is a human.

236. A kit comprising:

(a) the composition of any one of claims 199-202; and

(b) a package insert comprising instructions for administering the composition to a subject to treat or delay progression of a HER2-positive cancer.

237. The kit of claim 236, wherein the HER2-positive cancer is characterized by tumor cells that express HER2 at an average copy number of 200,000 or more copies per cell.

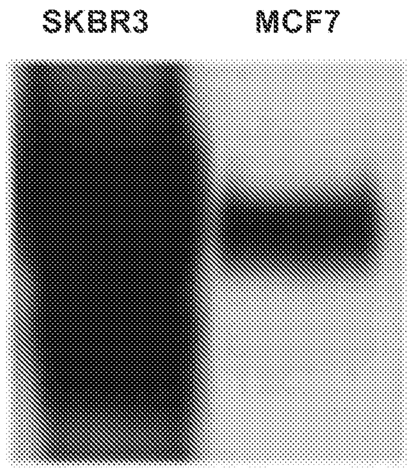

SKBR3
MCF7

FIG. 1

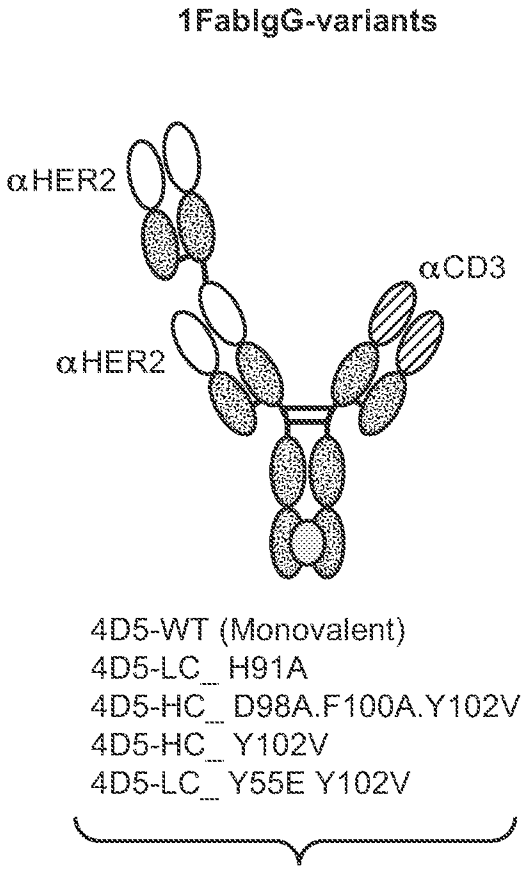

1FabIgG-variants
αHER2
αHER2
αCD3
4D5-WT (Monovalent)
4D5-LC_ H91A
4D5-HC_ D98A.F100A.Y102V
4D5-HC_ Y102V
4D5-LC_ Y55E Y102V

FIG. 3

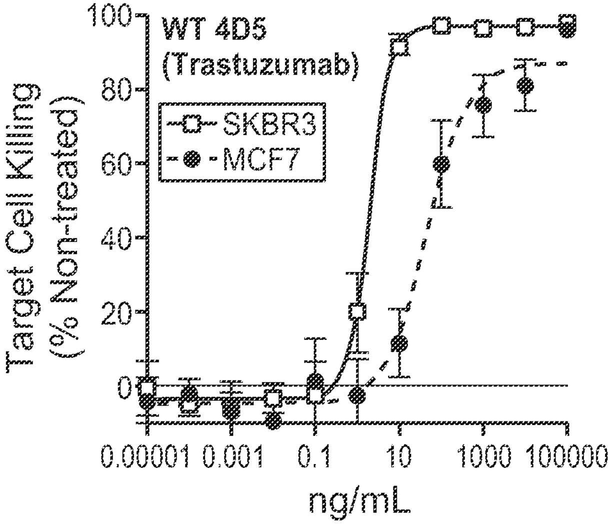


FIG. 2A

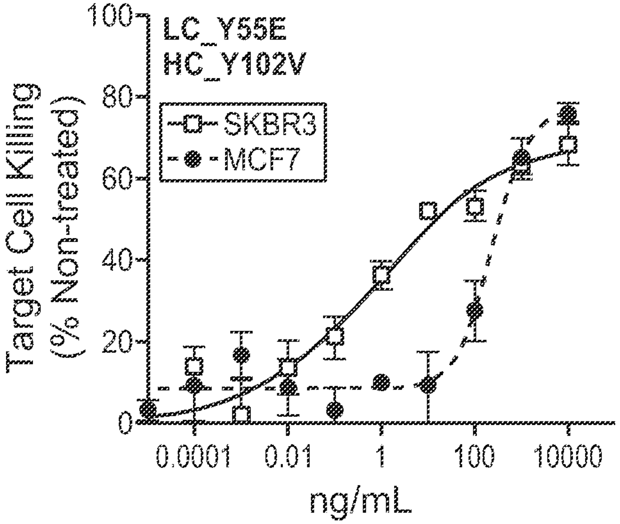


FIG. 2B

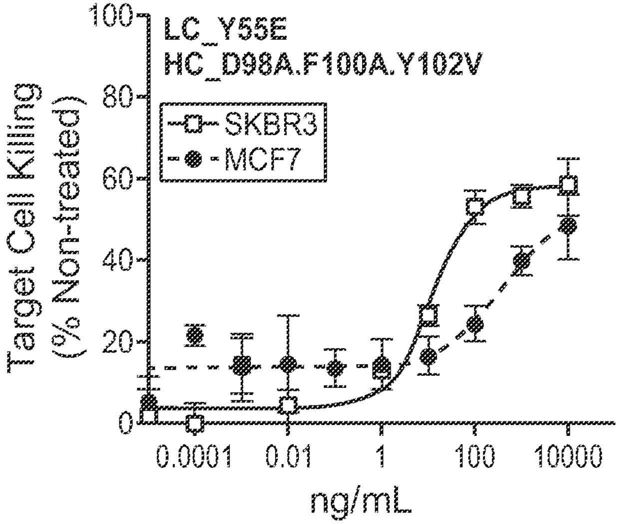


FIG. 2C

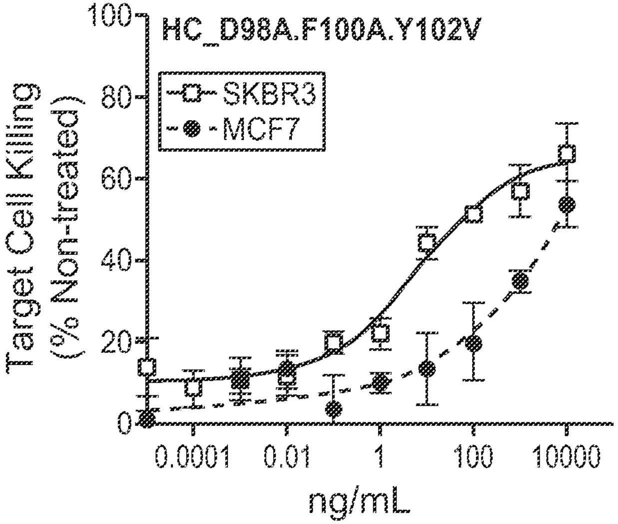


FIG. 2D

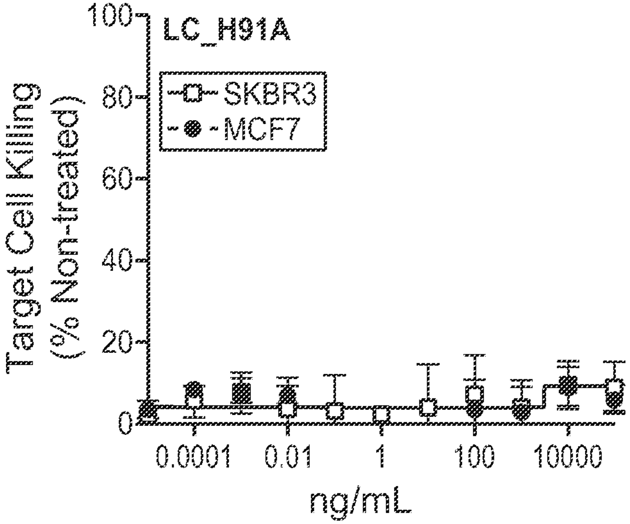


FIG. 2E

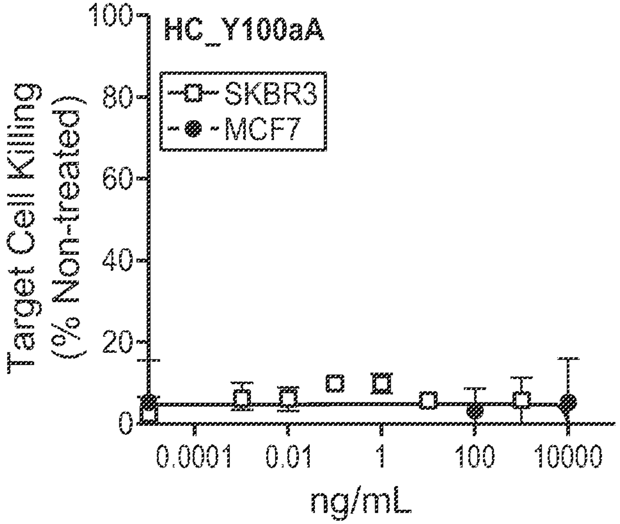


FIG. 2F

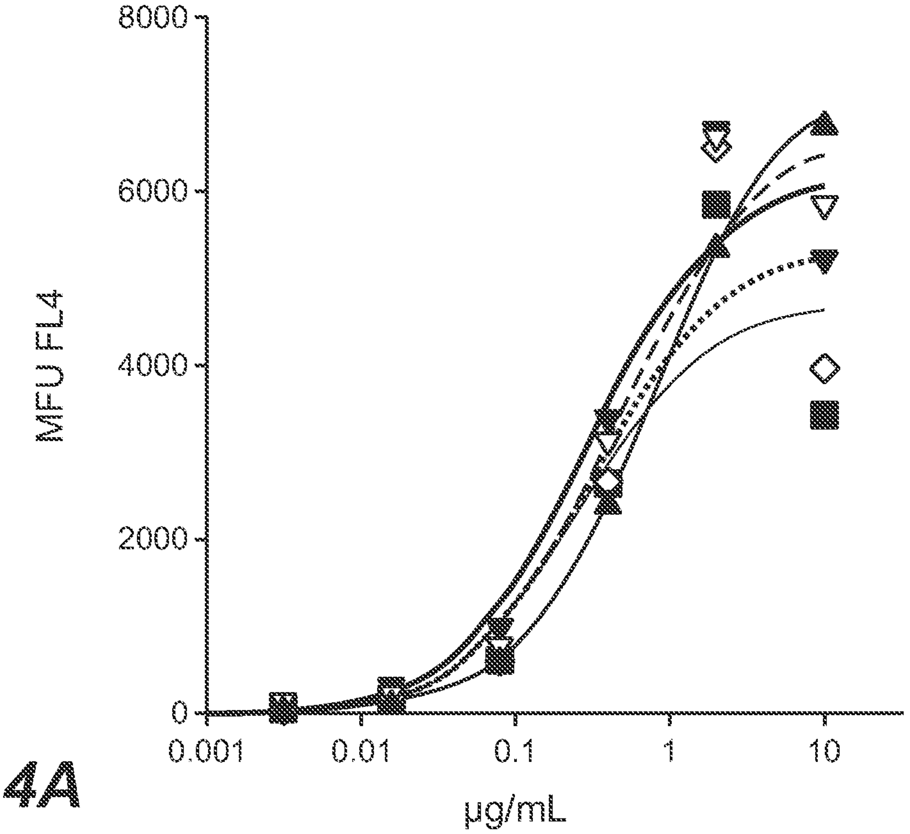

8000
6000
4000
2000
0
MFU FL4
0.001
0.01
0.1
1
10
µg/mL

FIG. 4A

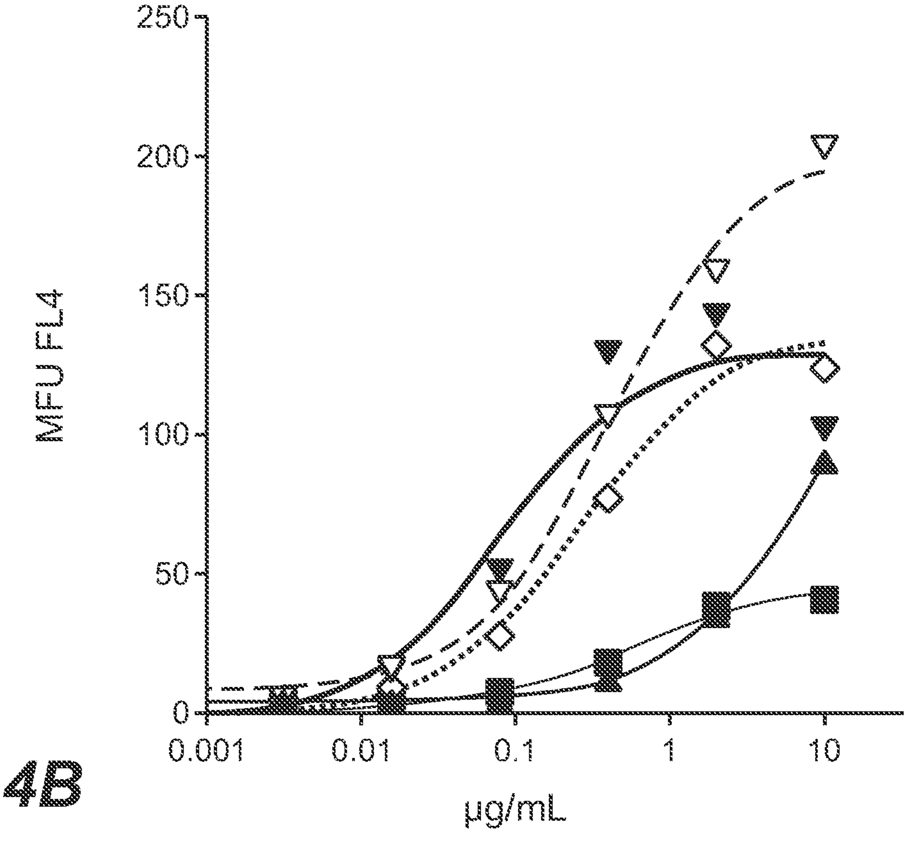

250
200
150
100
50
0
MFU FL4
0.001
0.01
0.1
1
10
µg/mL

FIG. 4B

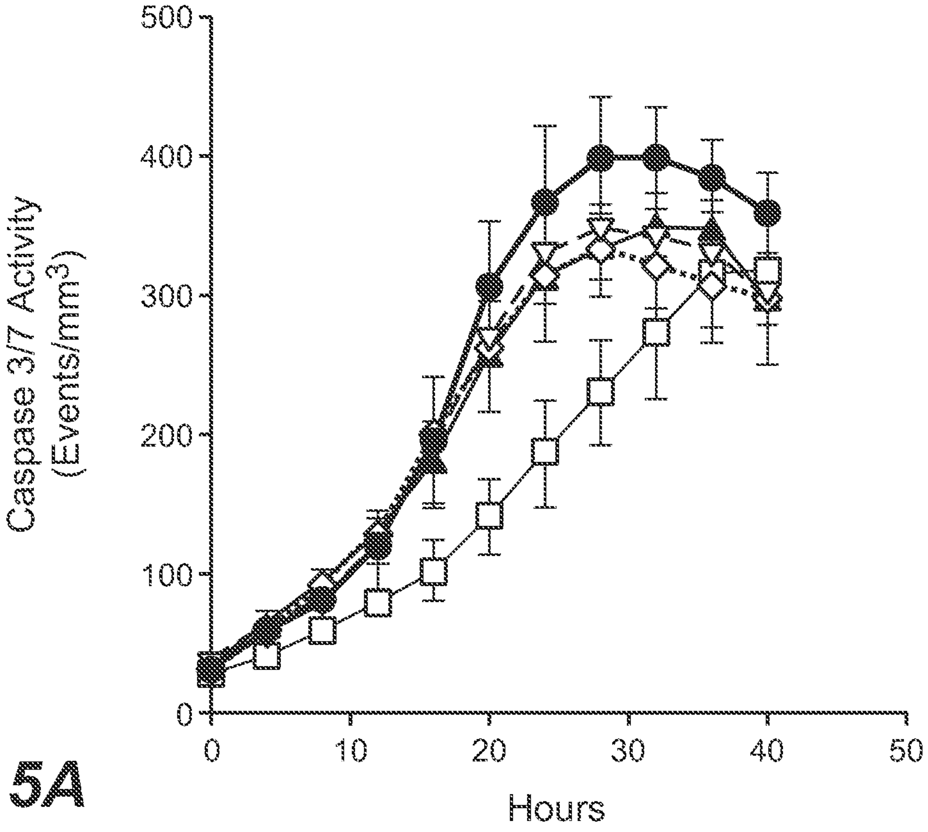

Caspase 3/7 Activity
(Events/mm³)
500
400
300
200
100
0
0
10
20
30
40
50
Hours

FIG. 5A

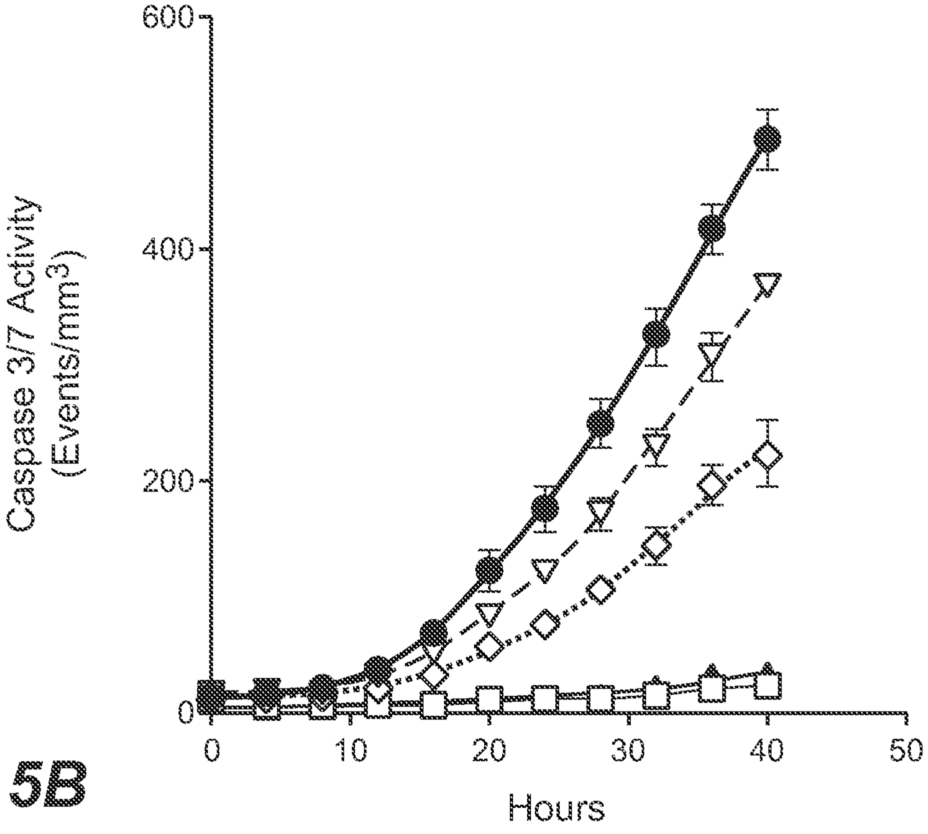

Caspase 3/7 Activity
(Events/mm³)
600
400
200
0
0
10
20
30
40
50
Hours

FIG. 5B

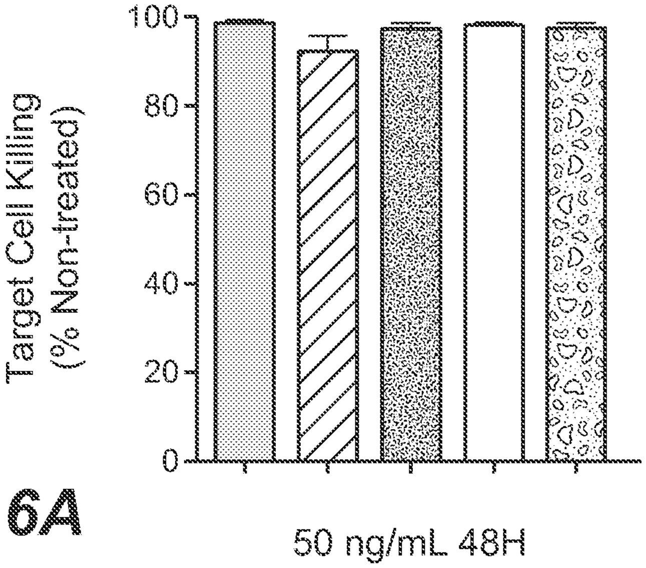


FIG. 6A

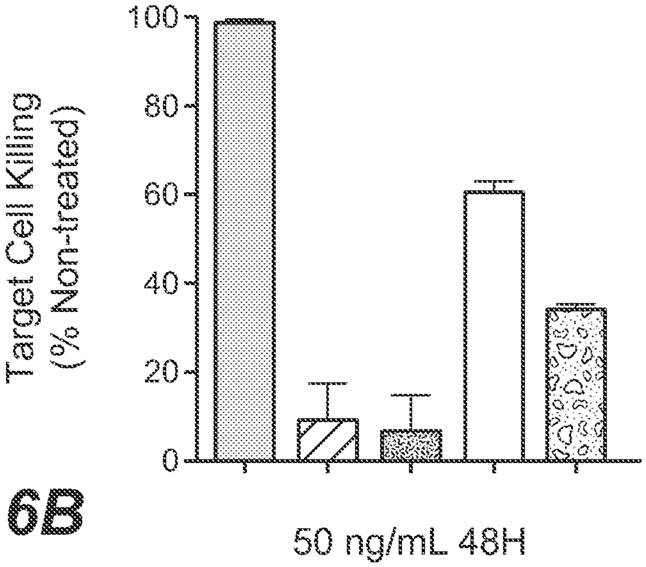


FIG. 6B

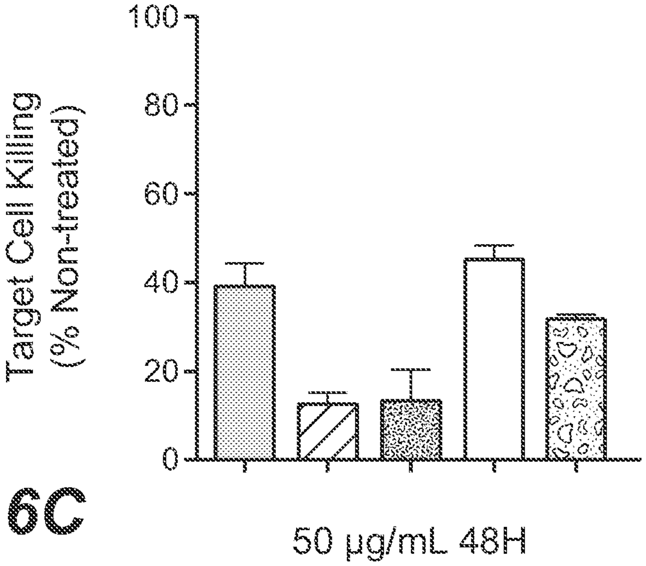


FIG. 6C

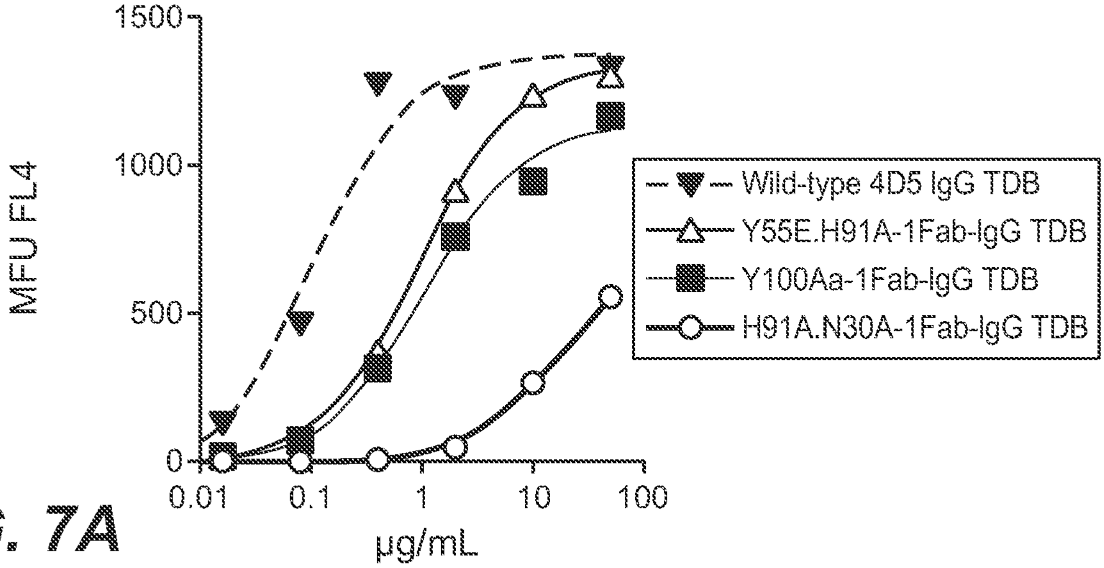


FIG. 7A

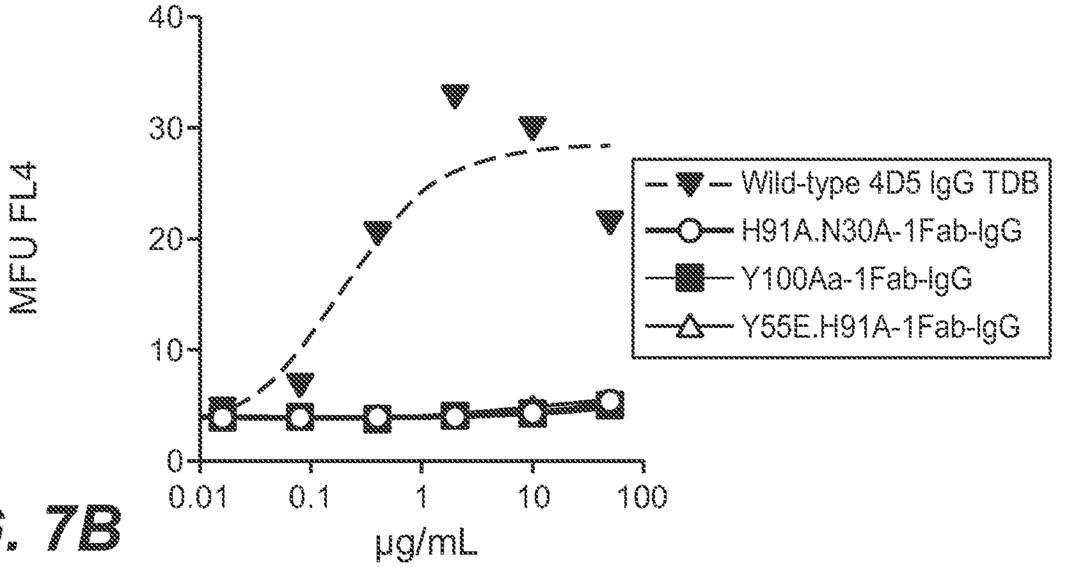


FIG. 7B

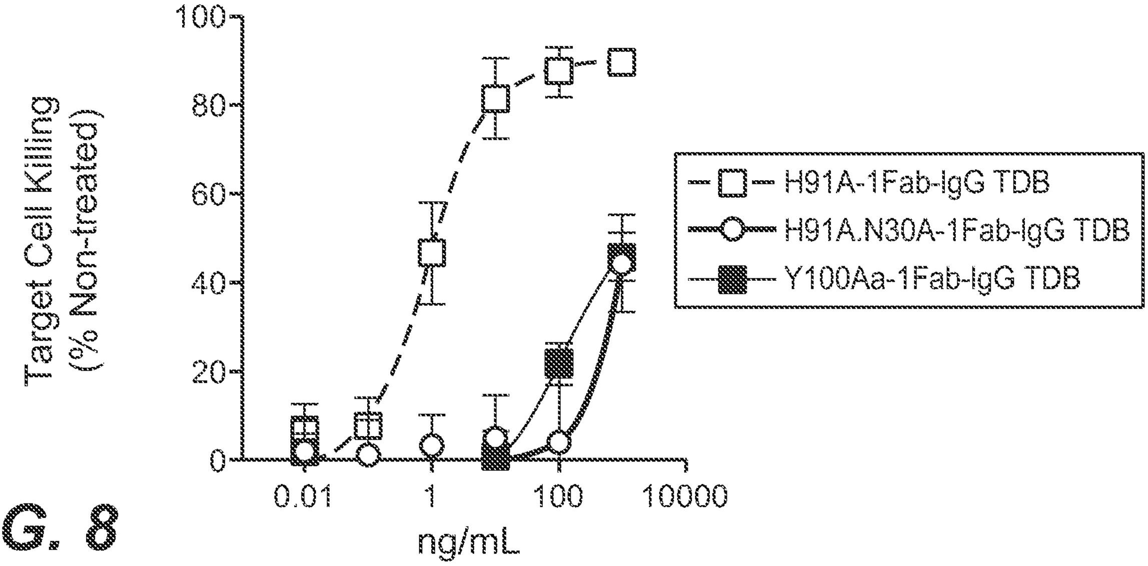


FIG. 8

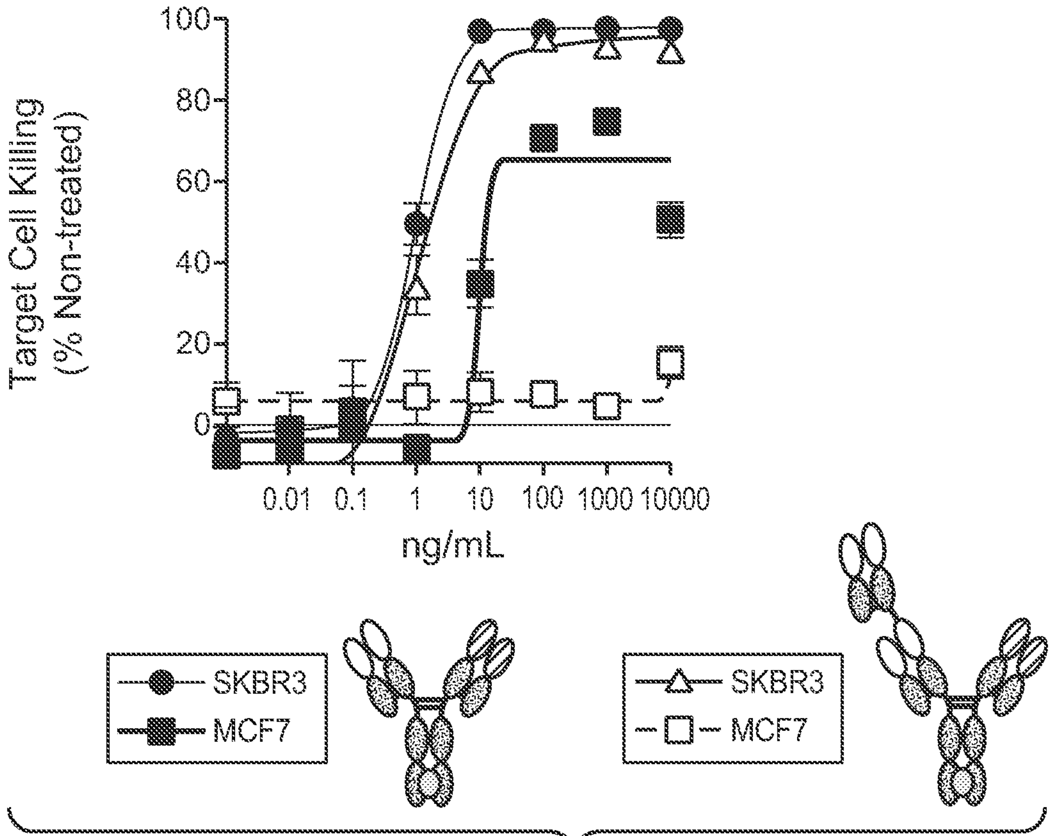

Target Cell Killing
(% Non-treated)
100
80
60
40
20
0
0.01
0.1
1
10
100
1000
10000
ng/mL
SKBR3
MCF7
SKBR3
MCF7

FIG. 9

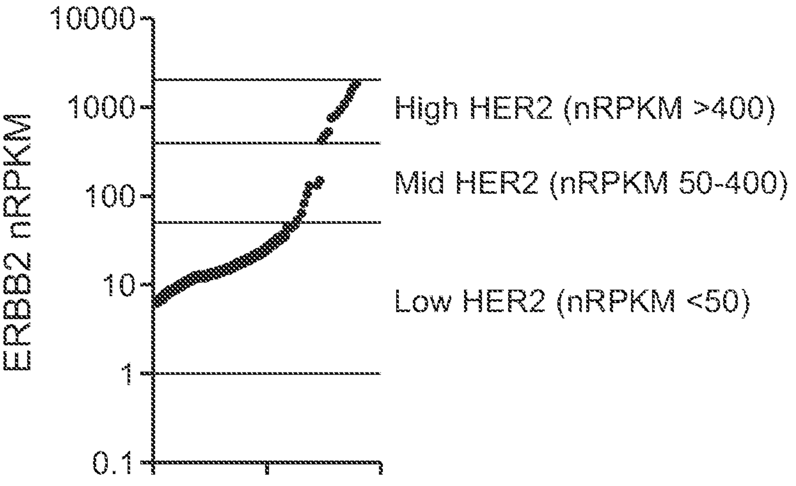

ERBB2 nRPKM
10000
1000
100
10
1
0.1
0
50
100
Cell Line #
High HER2 (nRPKM >400)
Mid HER2 (nRPKM 50-400)
Low HER2 (nRPKM <50)

FIG. 10

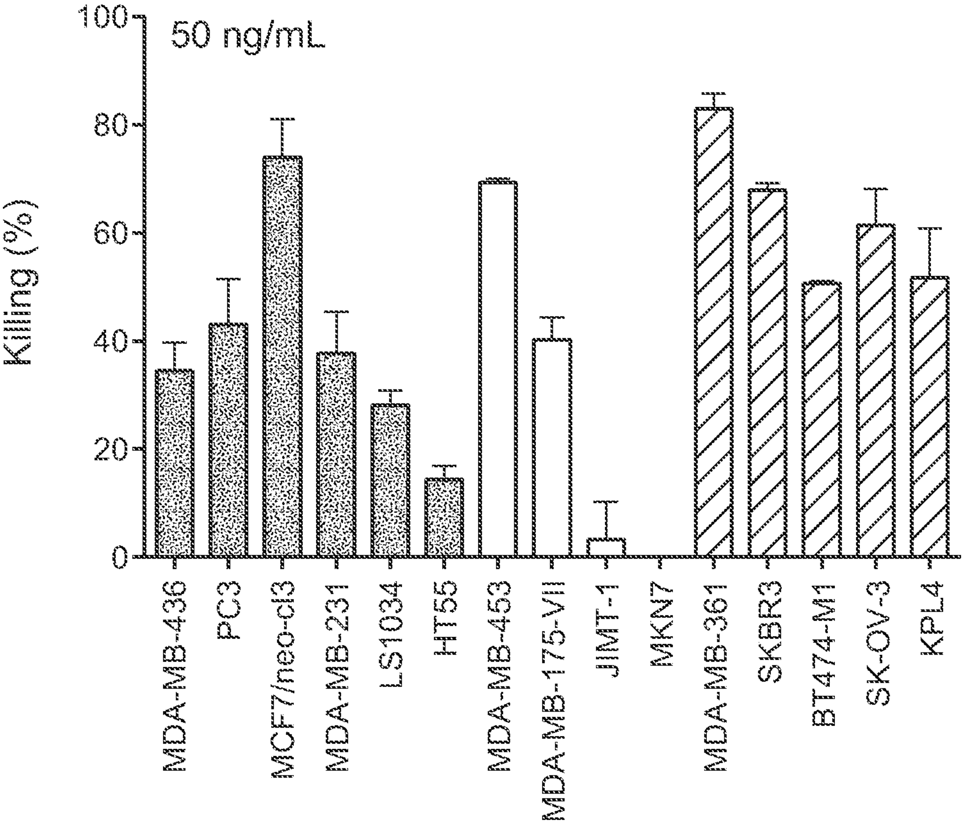


FIG. 11A

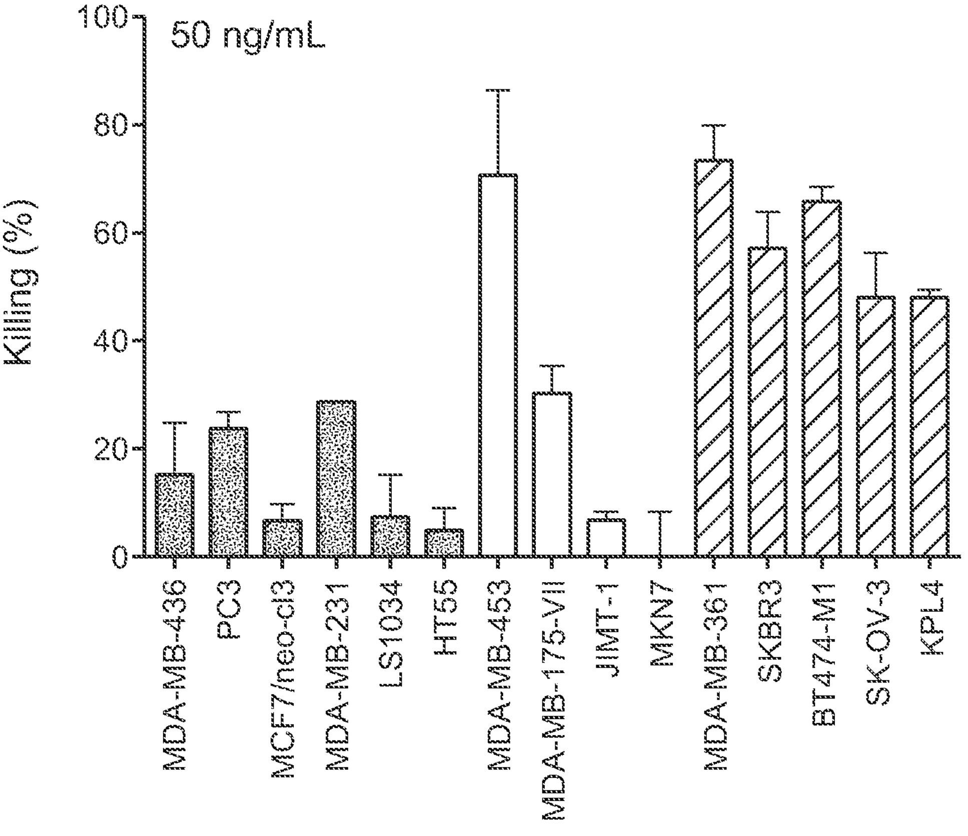


FIG. 11B

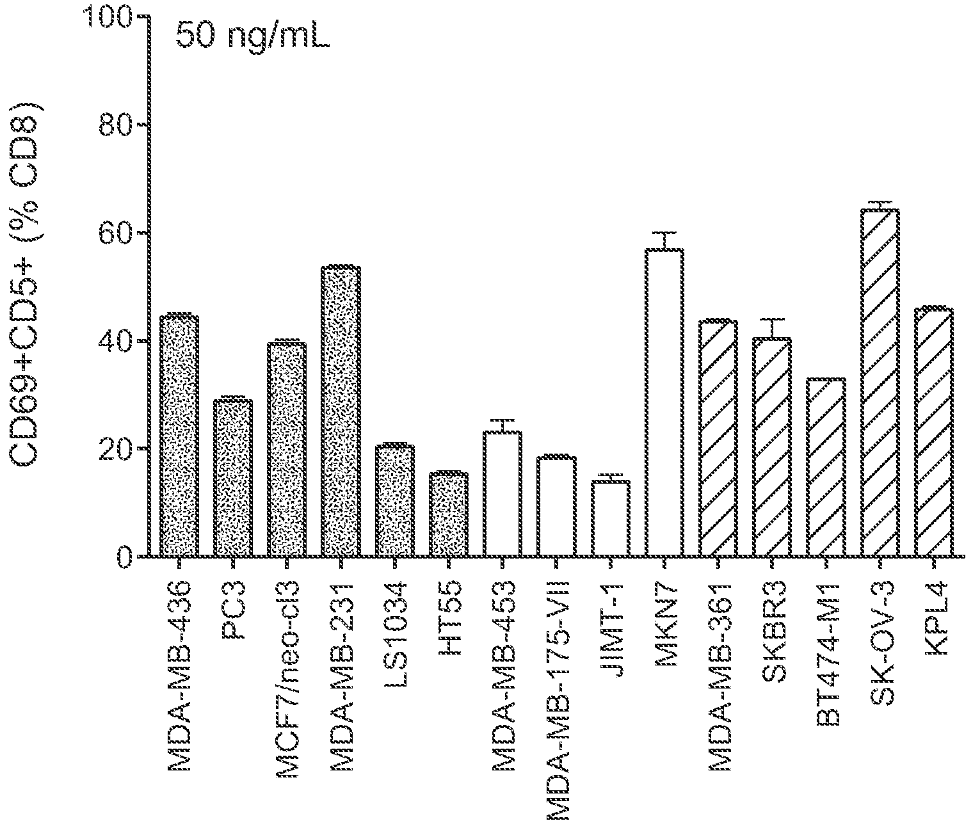


FIG. 11C

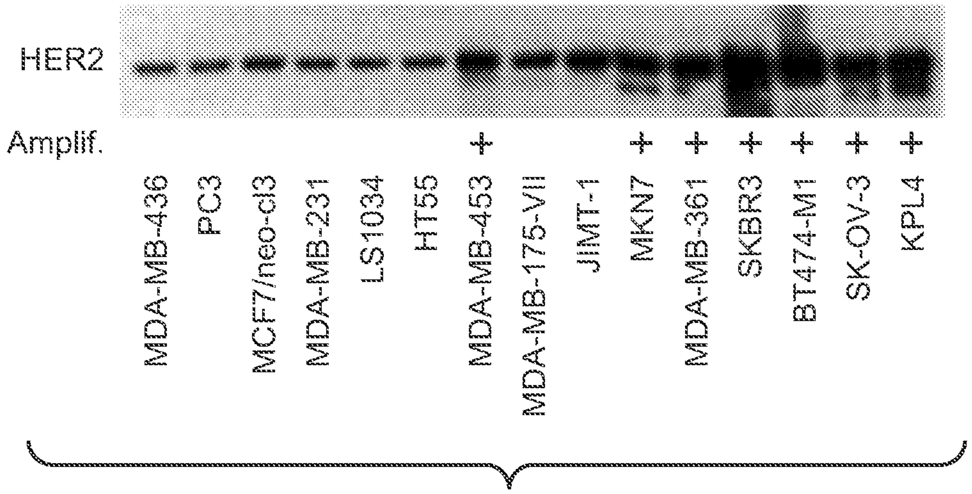


FIG. 11D

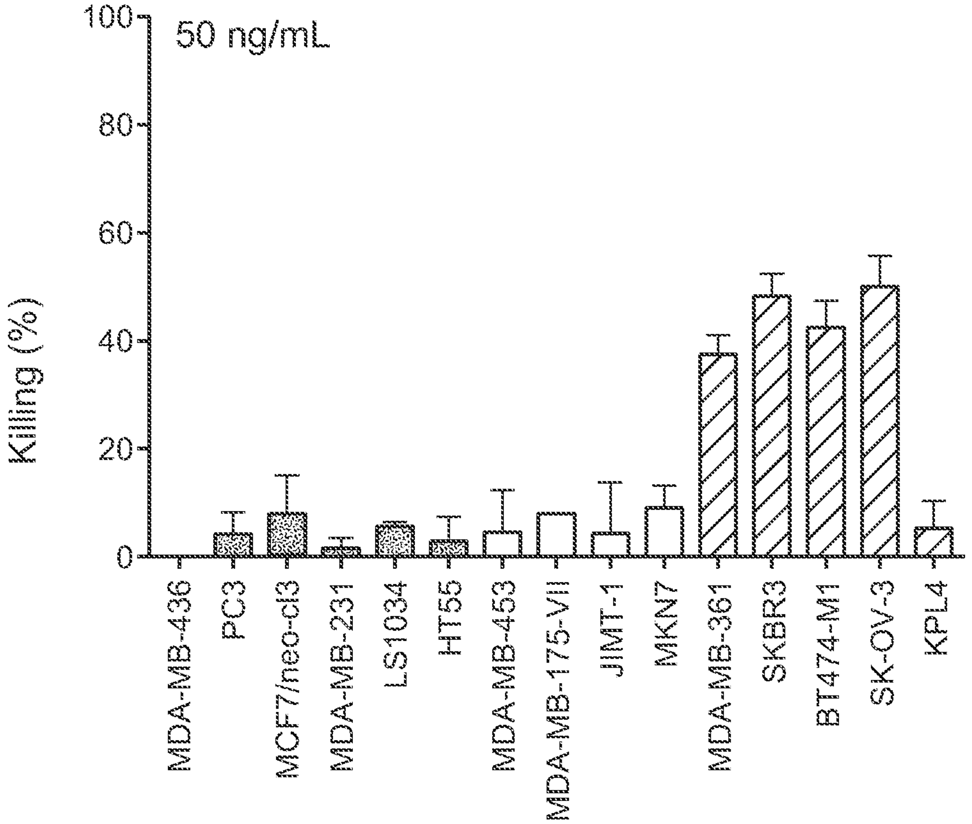


FIG. 12A

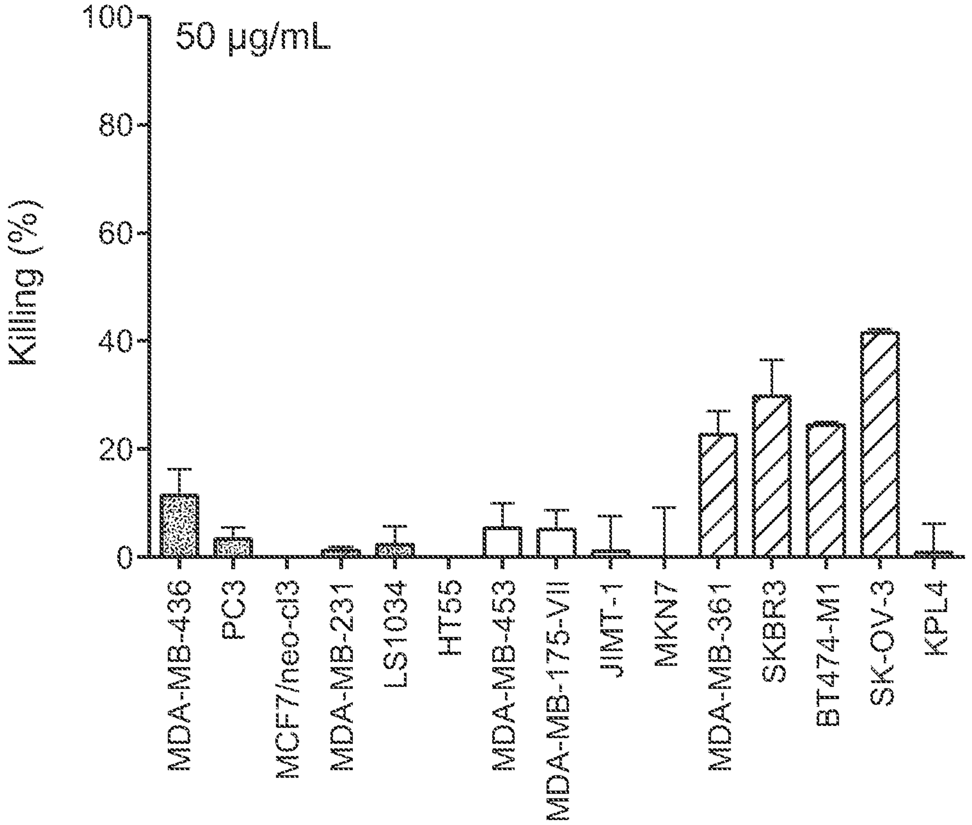


FIG. 12B

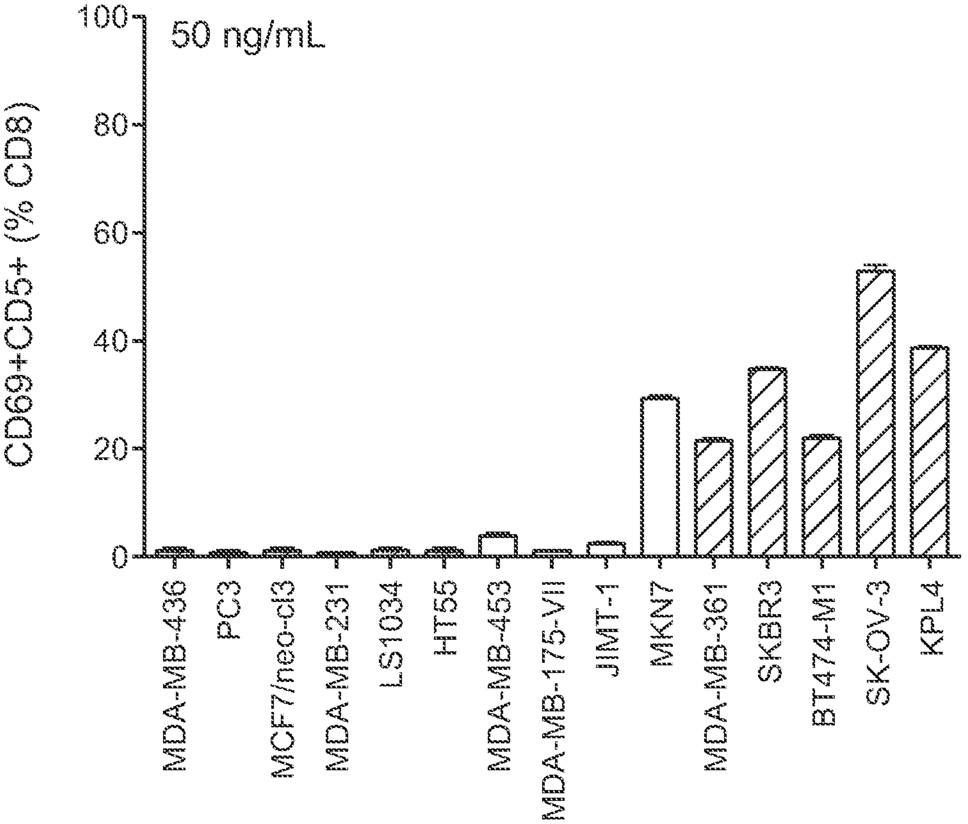


FIG. 12C

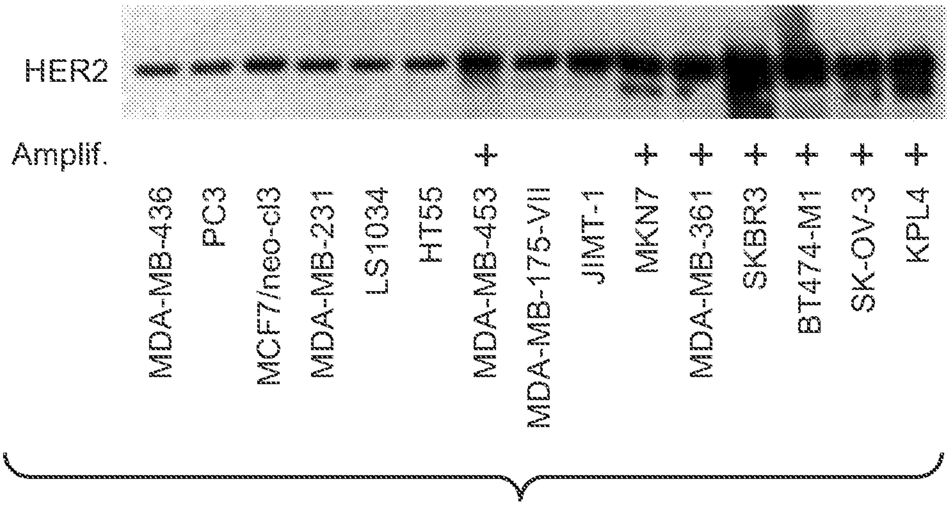


FIG. 12D

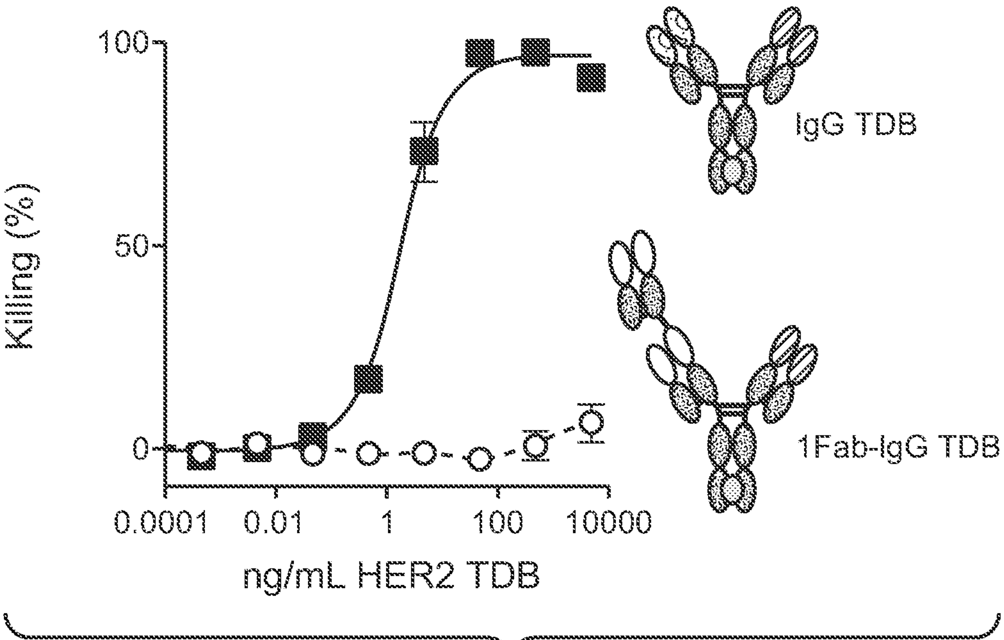

100
50
0
Killing (%)
0.0001
0.01
1
100
10000
ng/mL HER2 TDB
IgG TDB
1Fab-IgG TDB

FIG. 13

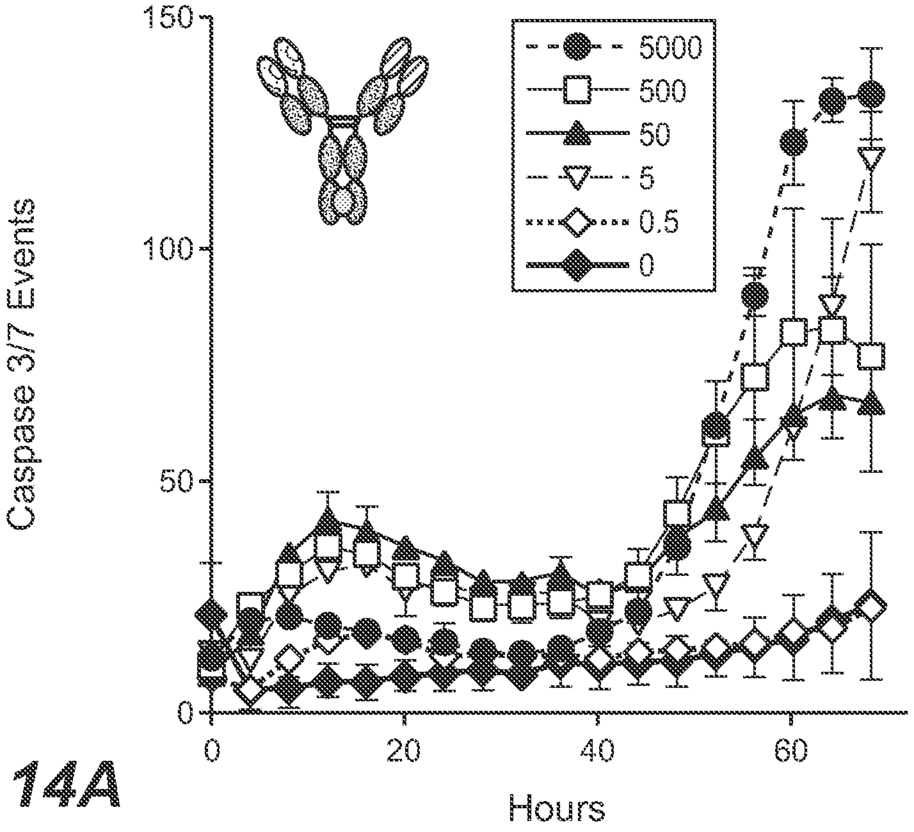


FIG. 14A

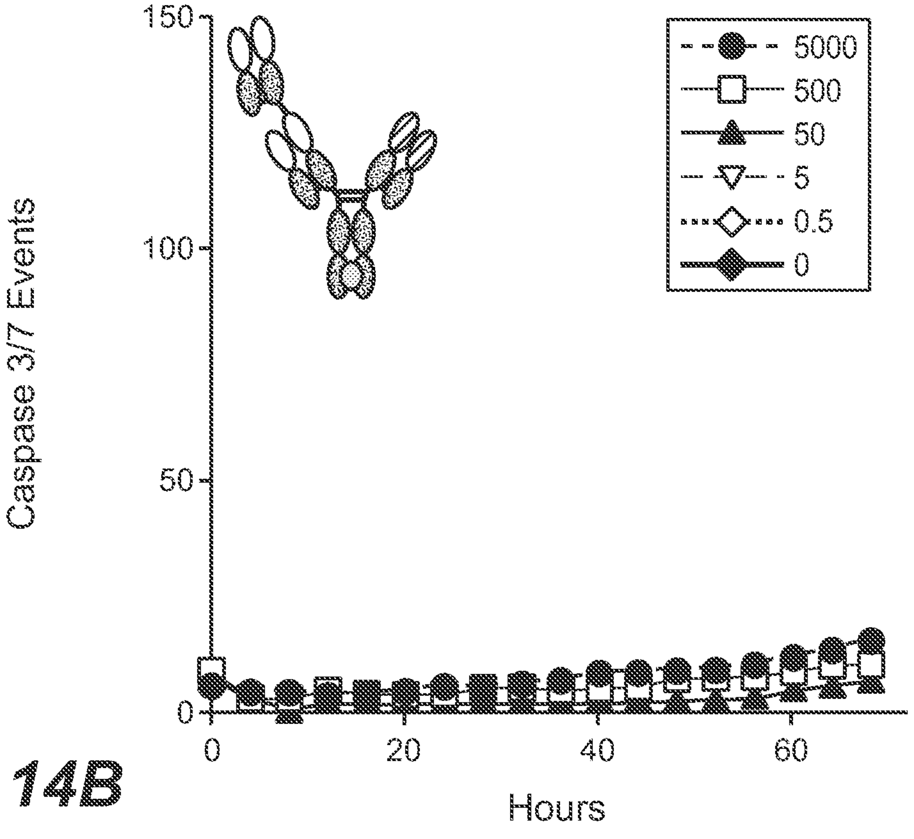


FIG. 14B

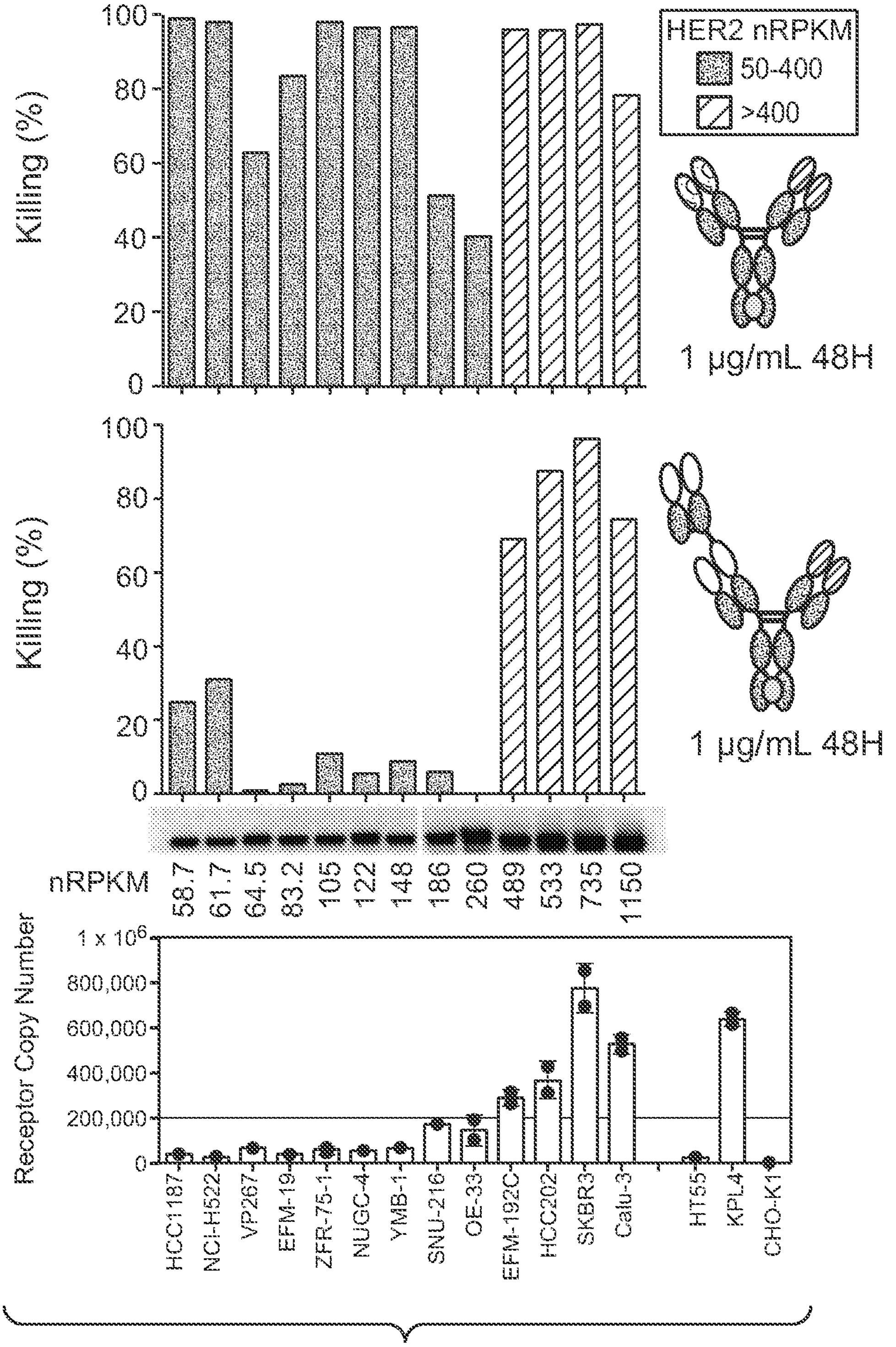

Killing (%)
0
20
40
60
80
100
HER2 nRPKM
▨ 50-400
▨ >400
1 µg/mL 48H
Killing (%)
0
20
40
60
80
100
1 µg/mL 48H
nRPKM
58.7
61.7
64.5
83.2
105
122
148
186
260
489
533
735
1150
Receptor Copy Number
0
200,000
400,000
600,000
800,000
1 x 10^6
HCC1187
NCI-H522
VP267
EFM-19
ZFR-75-1
NUGC-4
YMB-1
SNU-216
OE-33
EFM-192C
HCC202
SKBR3
Calu-3
HT55
KPL4
CHO-K1

FIG. 15A

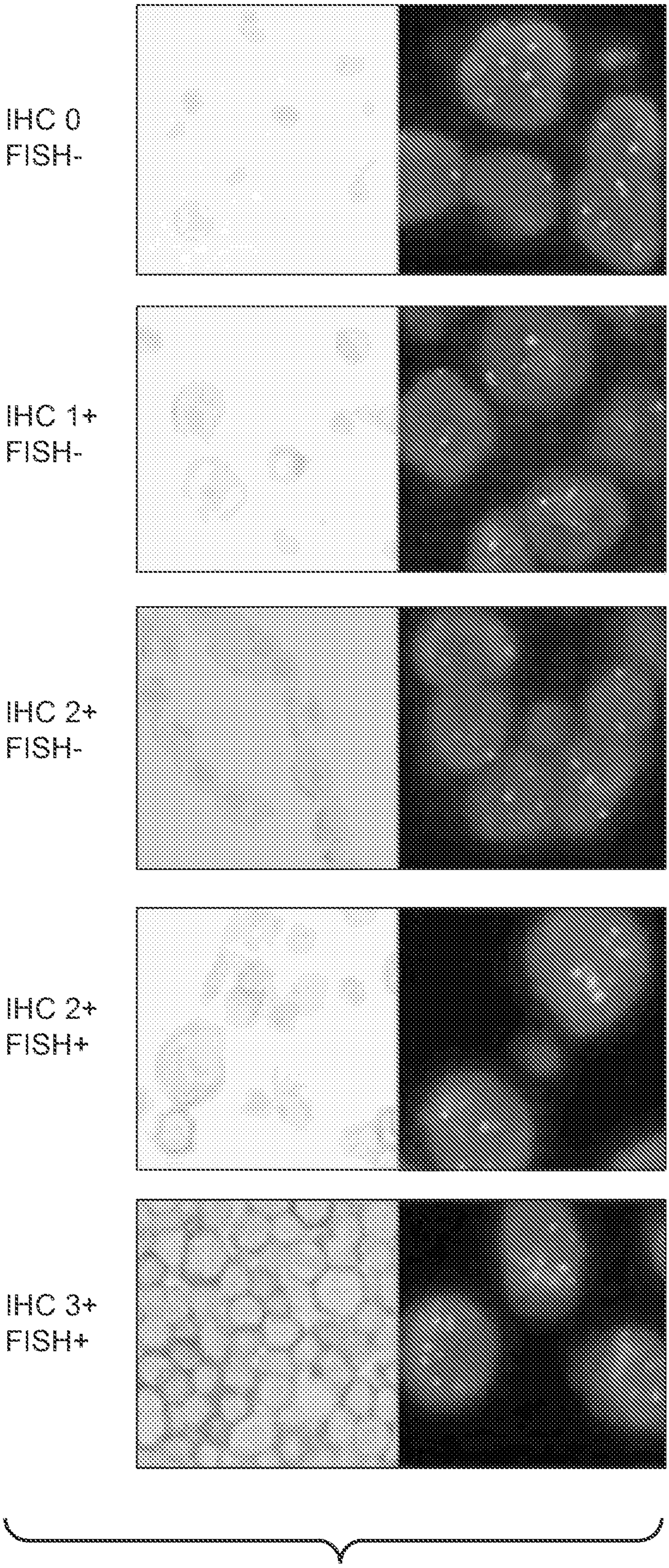


FIG. 15B

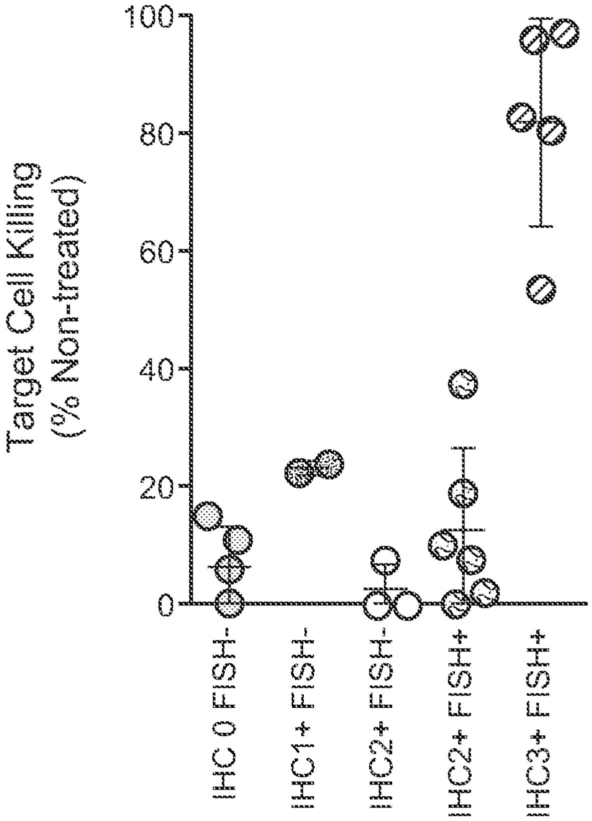


FIG. 15C

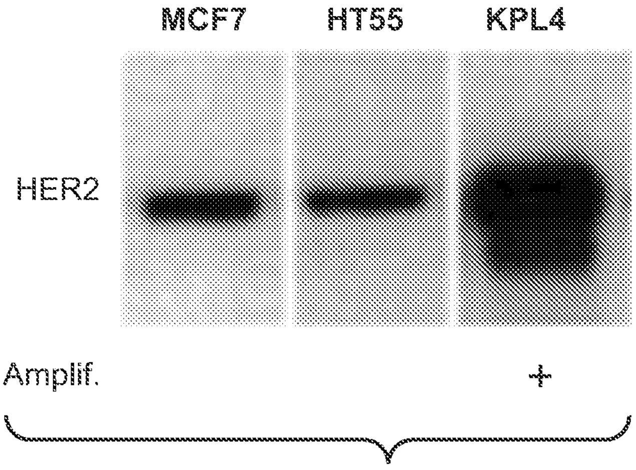


FIG. 16

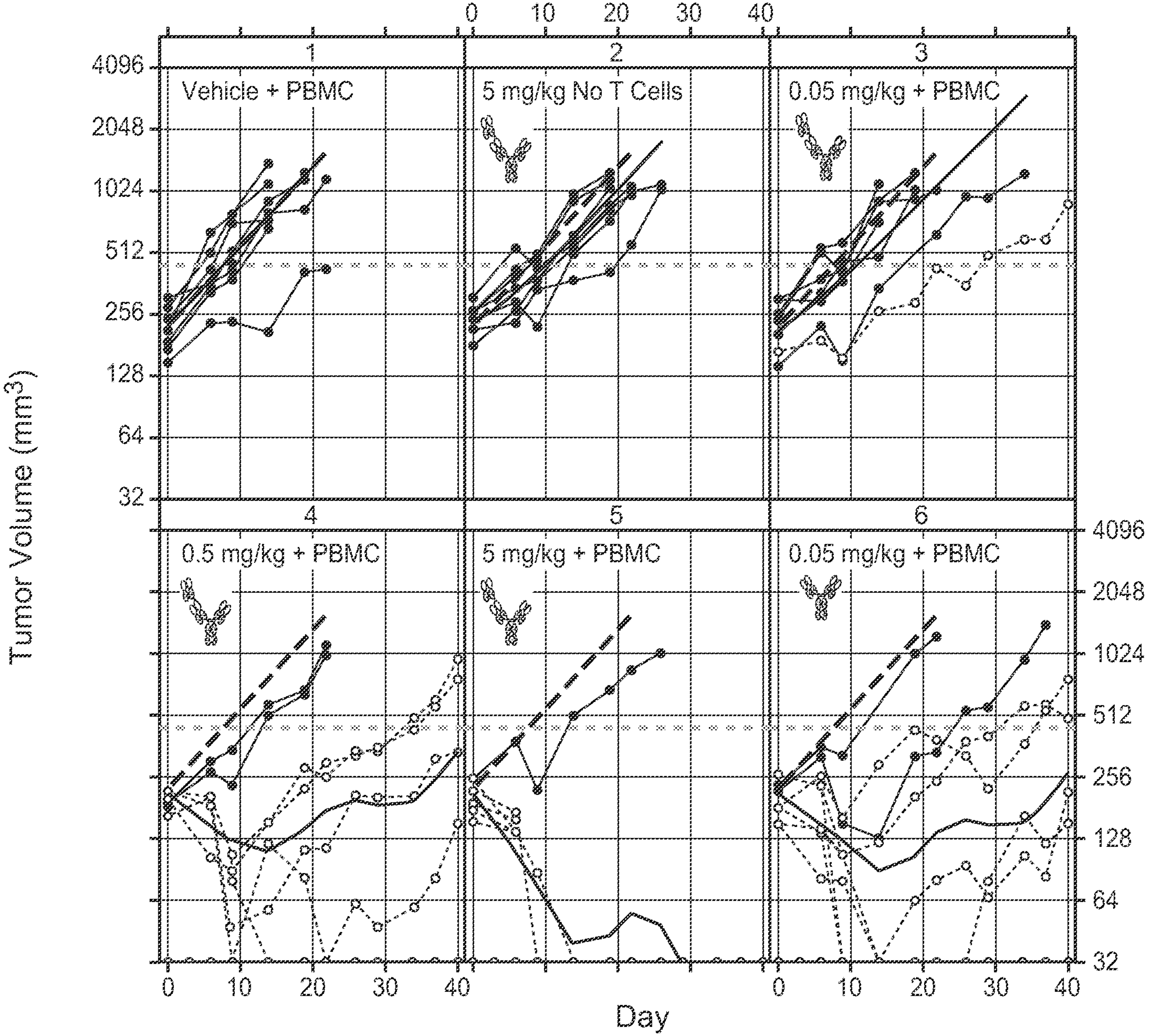

1
Vehicle + PBMC
2
5 mg/kg No T Cells
3
0.05 mg/kg + PBMC
4
0.5 mg/kg + PBMC
5
5 mg/kg + PBMC
6
0.05 mg/kg + PBMC
Tumor Volume (mm³)
4096
2048
1024
512
256
128
64
32
0
10
20
30
40
Day

FIG. 17

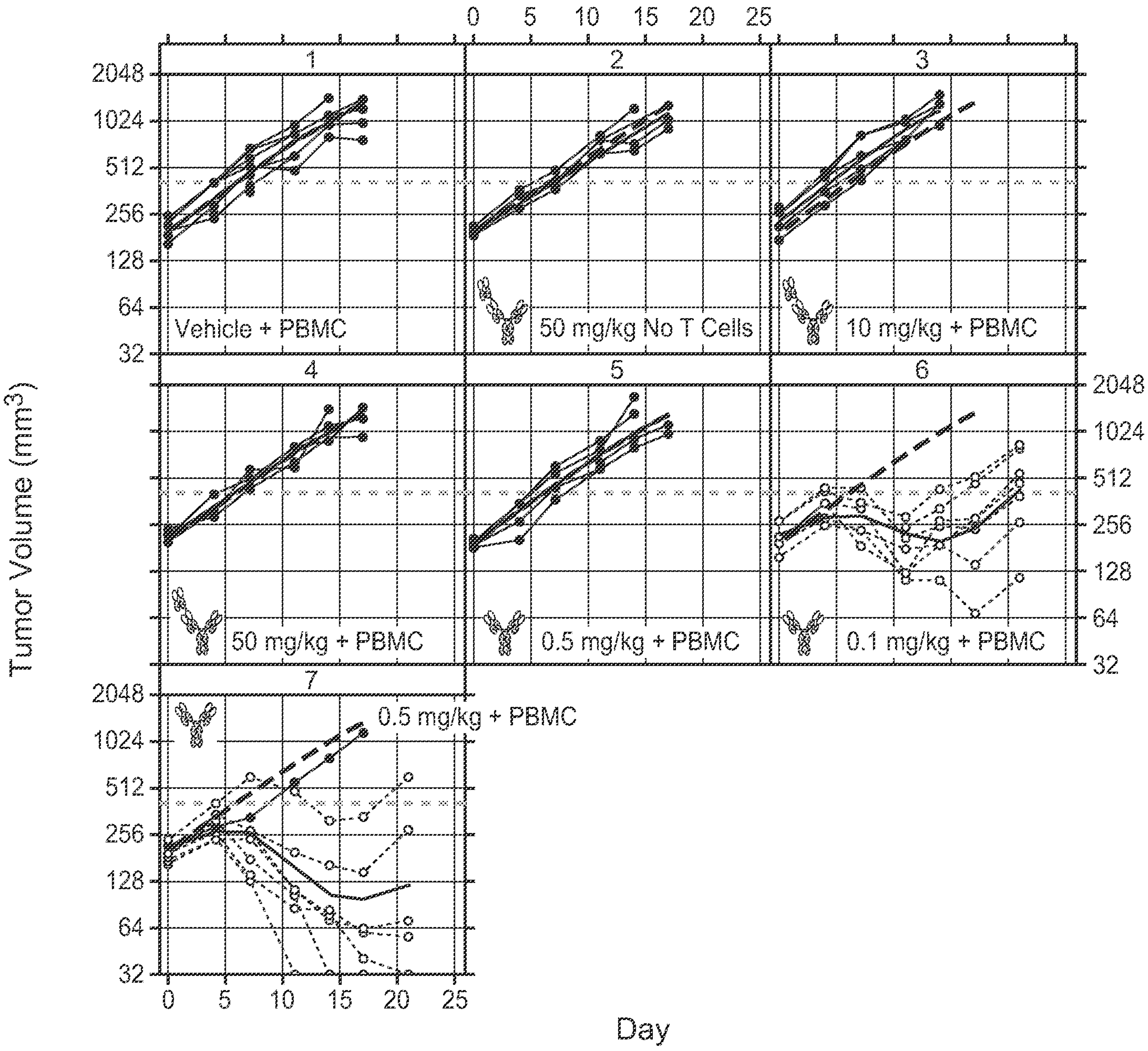
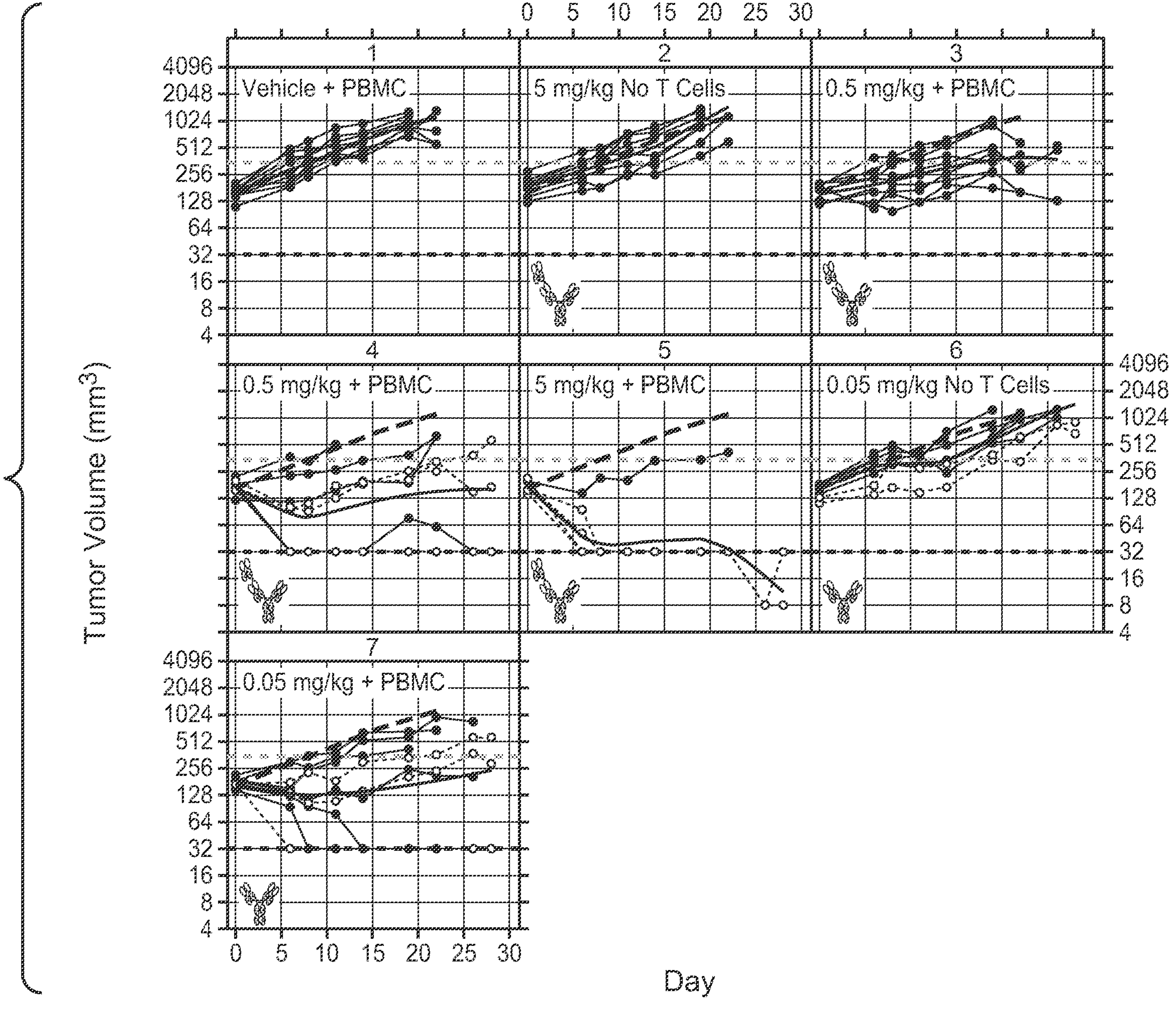

1
Vehicle + PBMC
2
50 mg/kg No T Cells
3
10 mg/kg + PBMC
4
50 mg/kg + PBMC
5
0.5 mg/kg + PBMC
6
0.1 mg/kg + PBMC
7
0.5 mg/kg + PBMC
Tumor Volume (mm3)
2048
1024
512
256
128
64
32
Day
0
5
10
15
20
25

FIG. 18



1
Vehicle + PBMC
2
5 mg/kg No T Cells
3
0.5 mg/kg + PBMC
4
0.5 mg/kg + PBMC
5
5 mg/kg + PBMC
6
0.05 mg/kg No T Cells
7
0.05 mg/kg + PBMC
Tumor Volume (mm^3)
4096
2048
1024
512
256
128
64
32
16
8
4
Day
0
5
10
15
20
25
30

FIG. 19

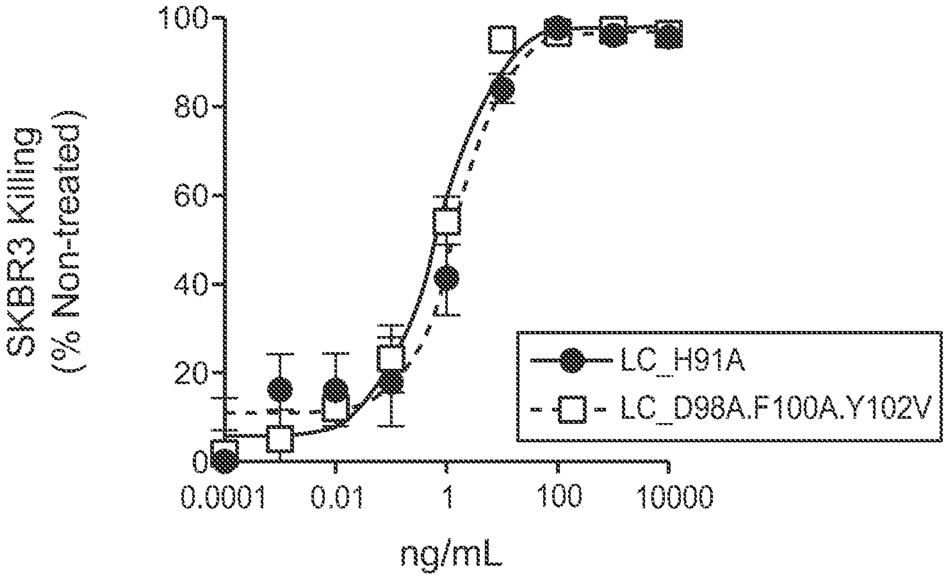

SKBR3 Killing
(% Non-treated)
100
80
60
40
20
0
0.0001
0.01
1
100
10000
ng/mL
LC_H91A
LC_D98A.F100A.Y102V

FIG. 20

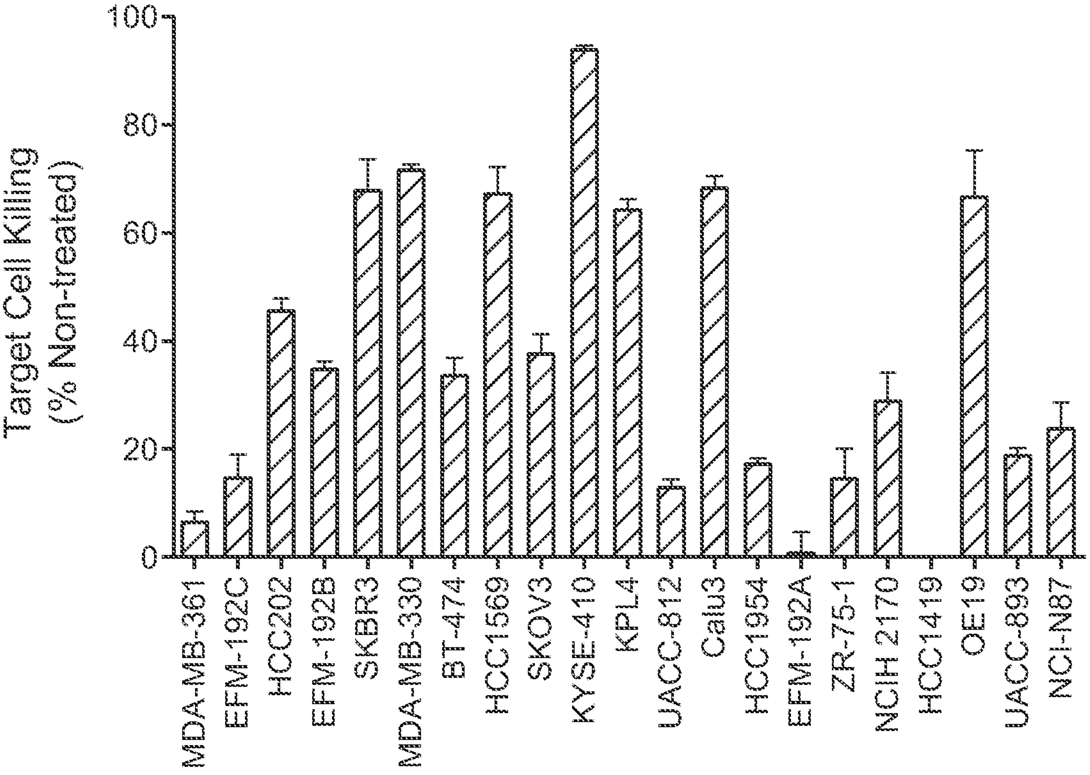


FIG. 21A

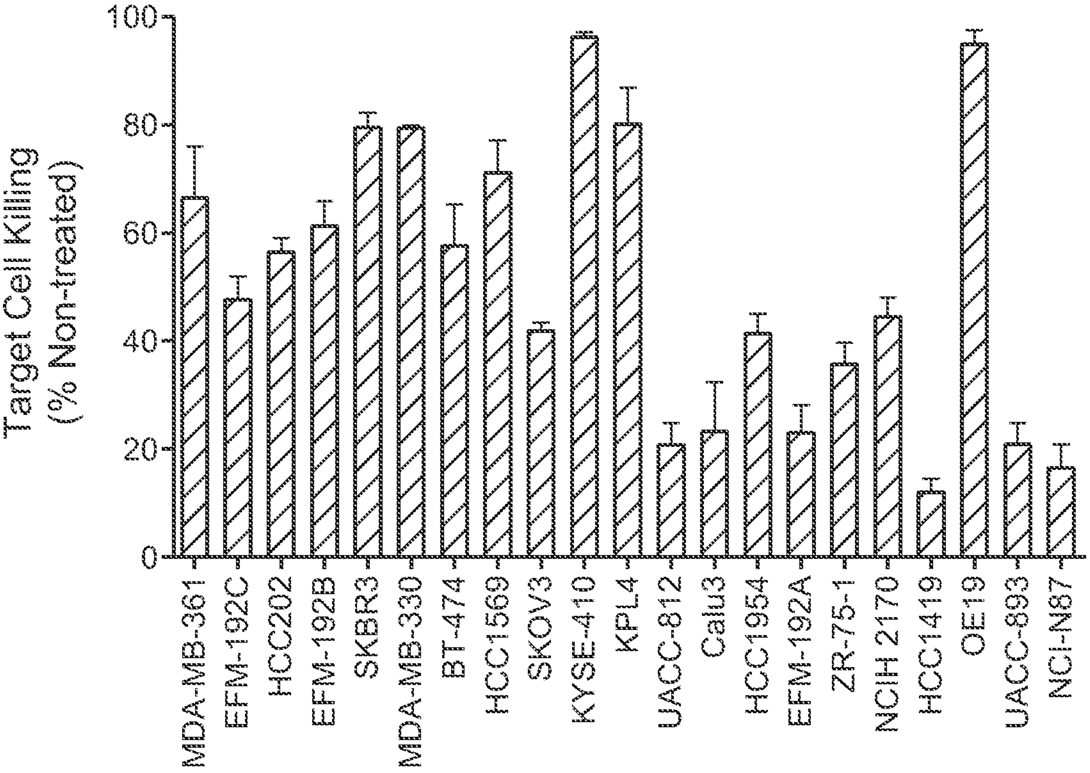


FIG. 21B

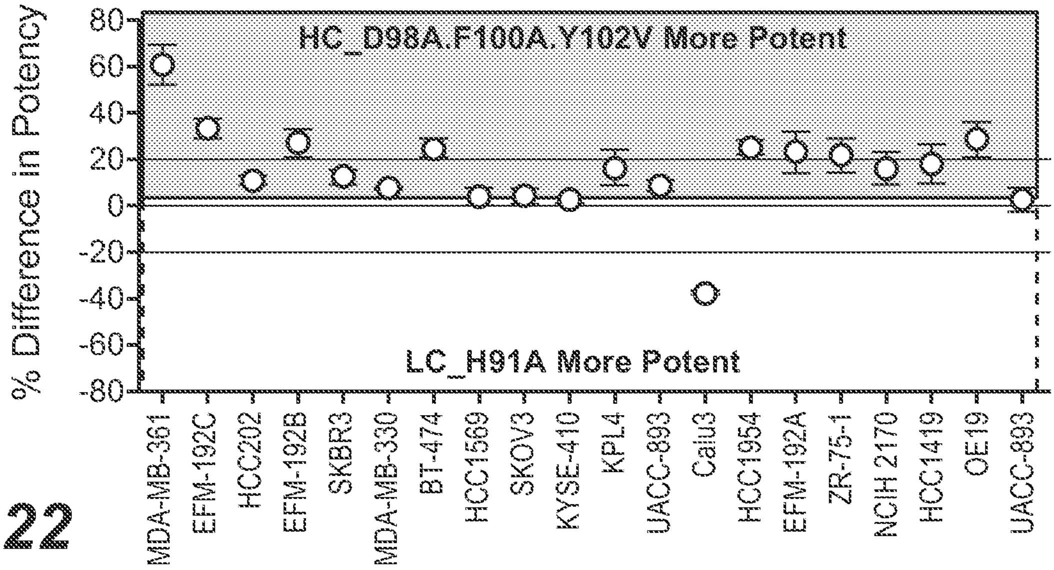


FIG. 22

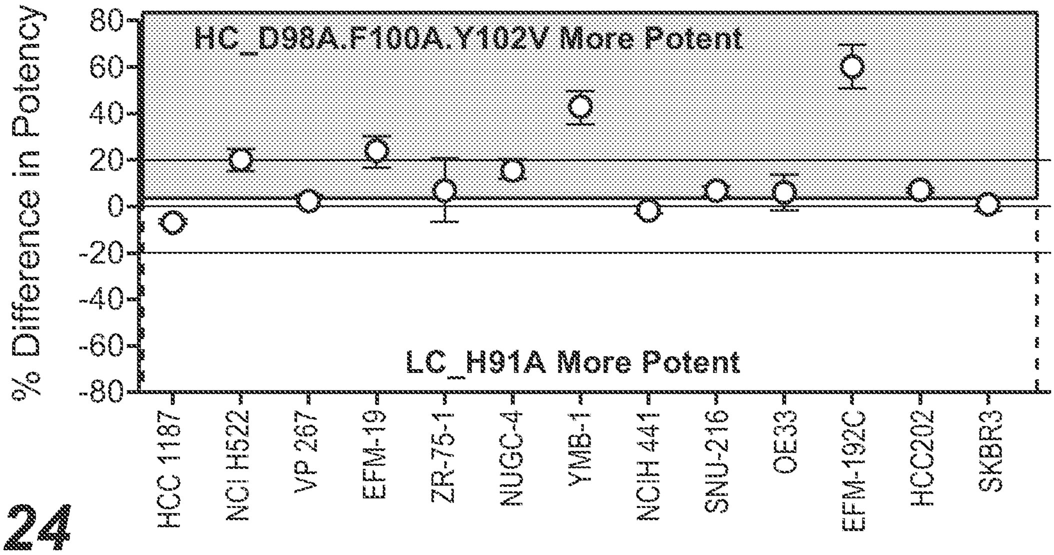


FIG. 24

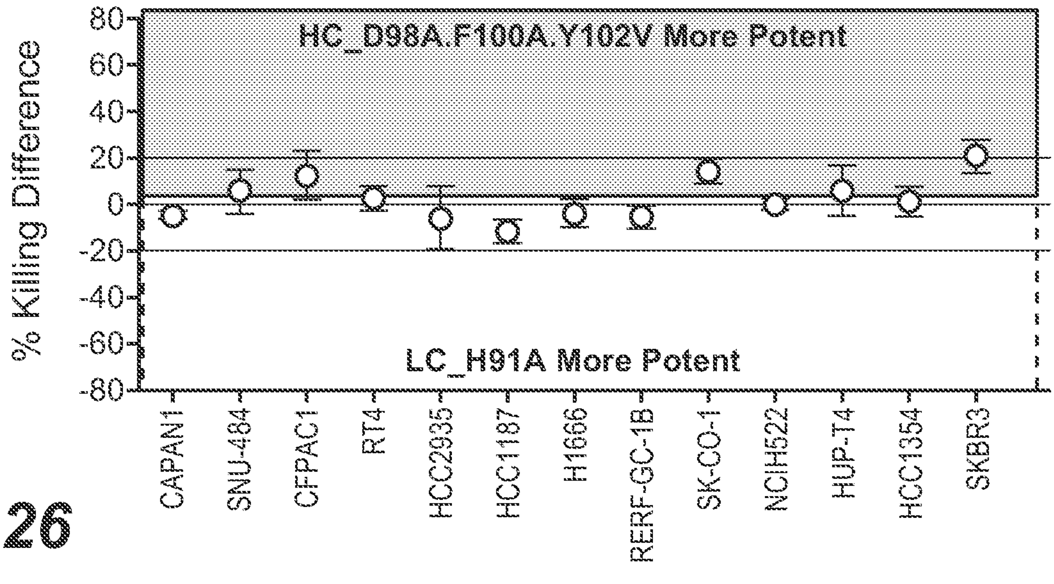


FIG. 26

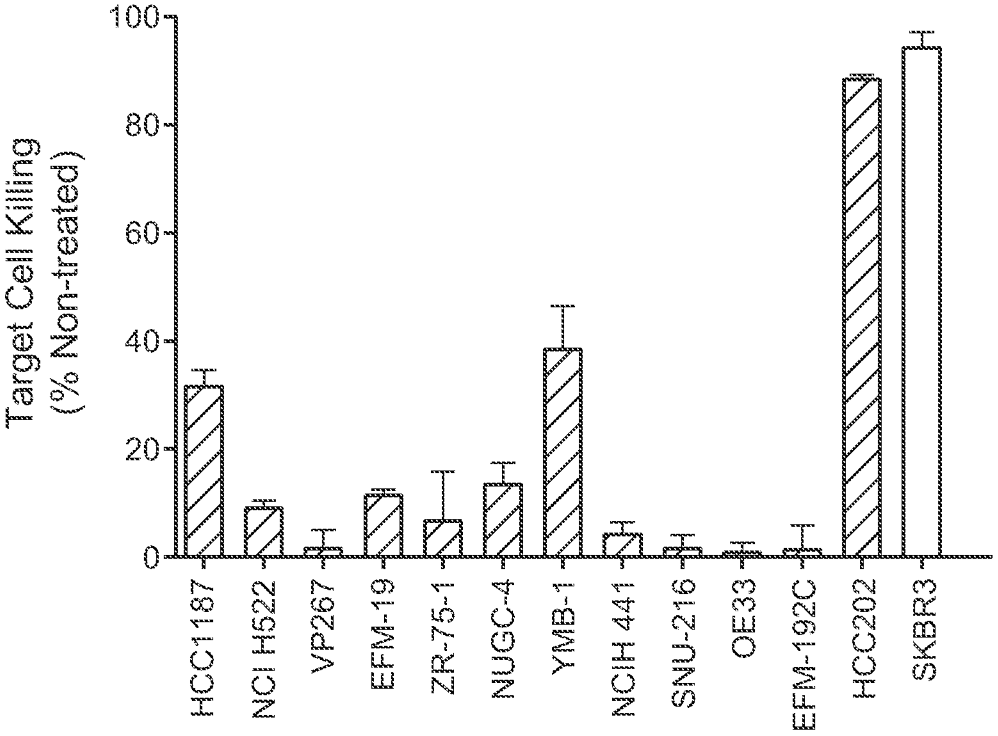


FIG. 23A

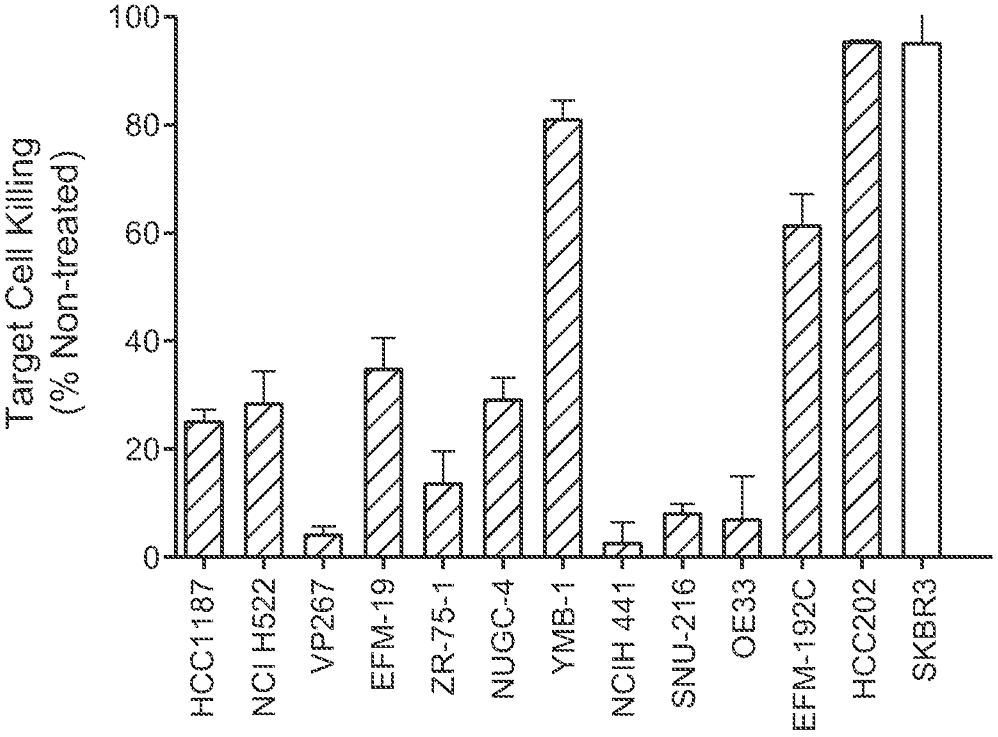


FIG. 23B

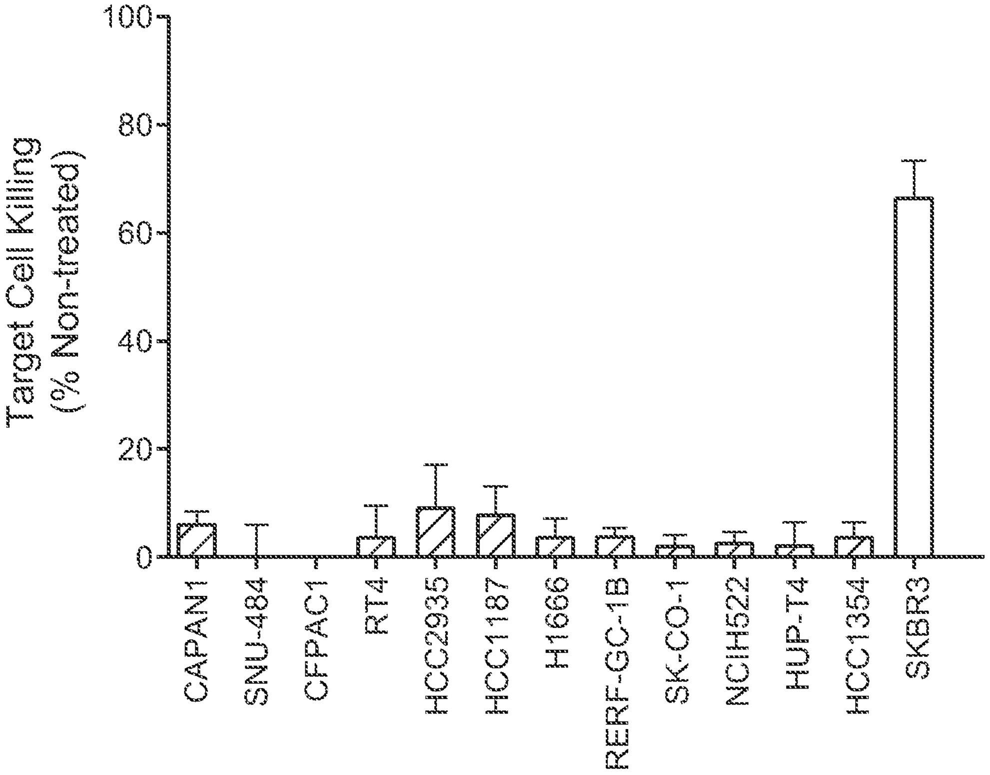


FIG. 25A

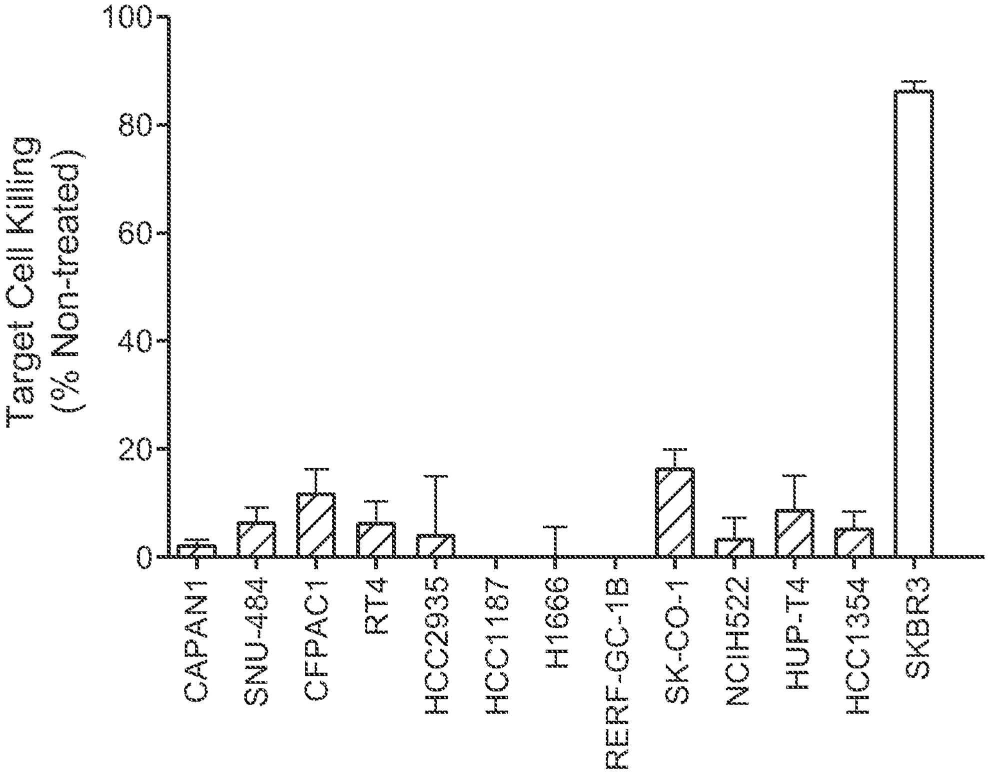
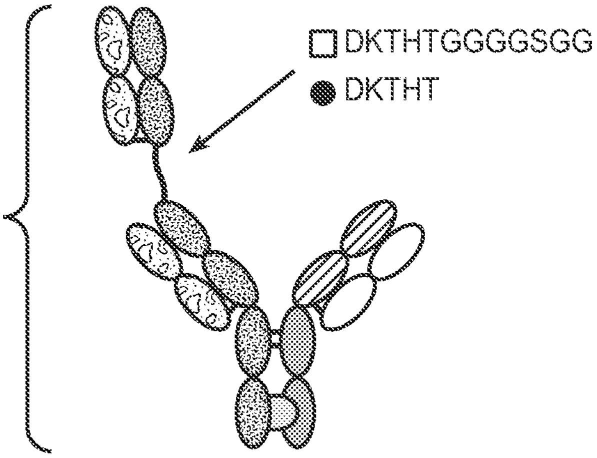


FIG. 25B

FIG. 27A



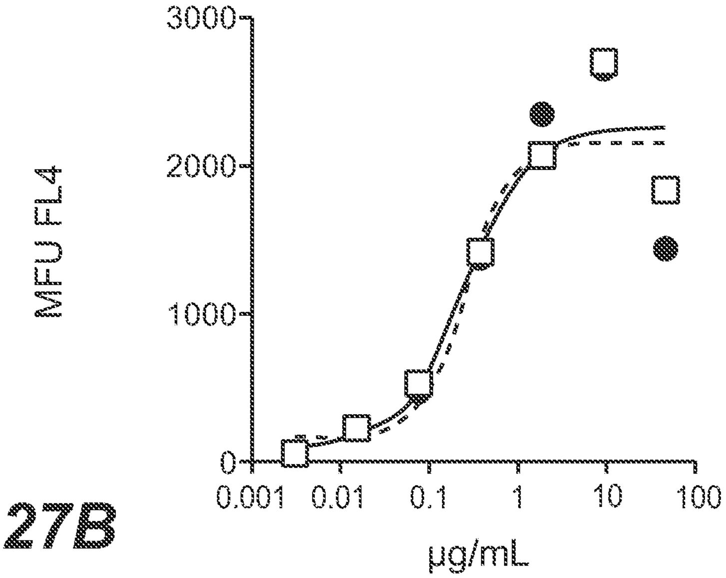


FIG. 27B

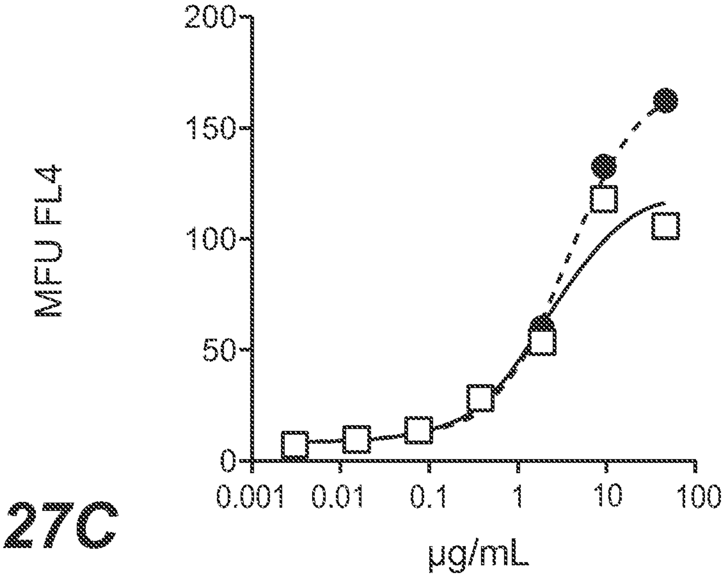


FIG. 27C

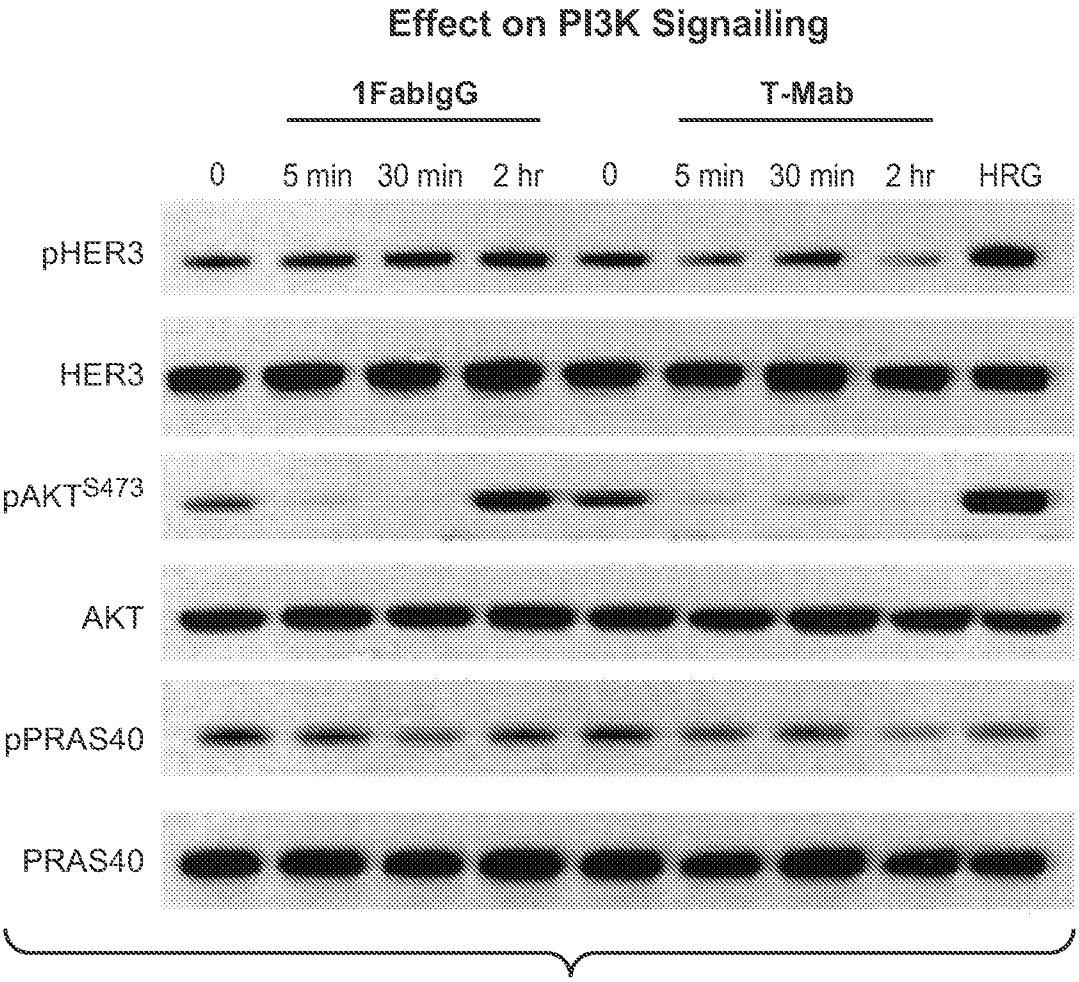


FIG. 28A

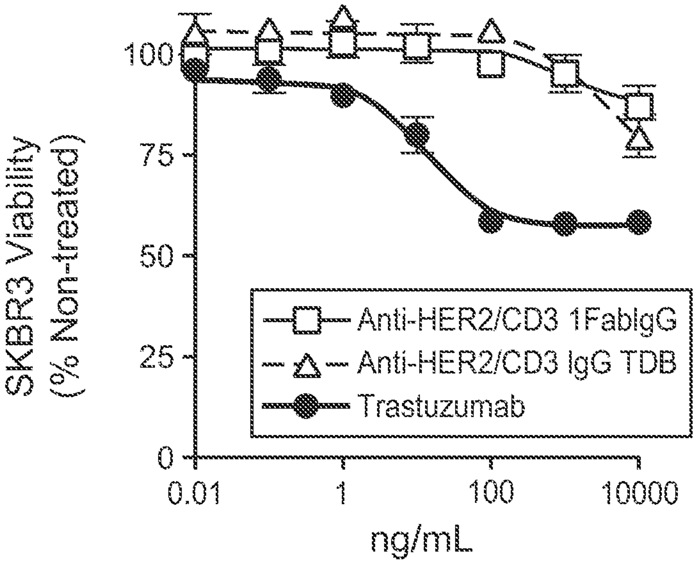


FIG. 28B

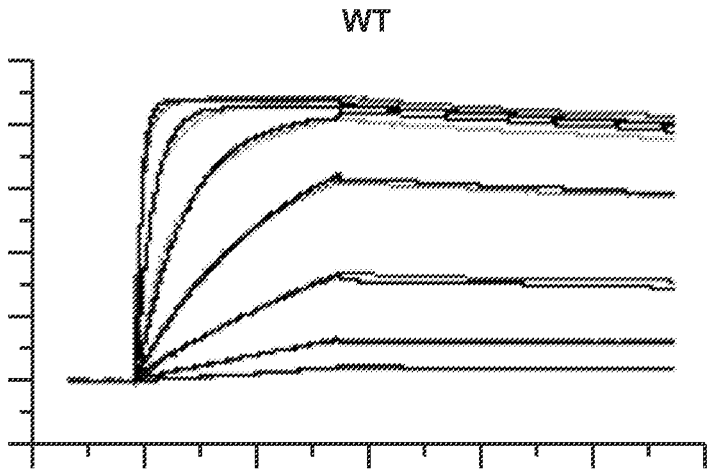


FIG. 29A

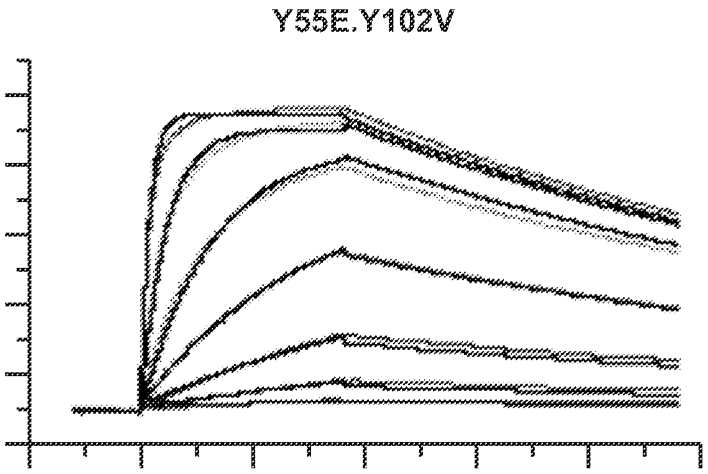


FIG. 29B

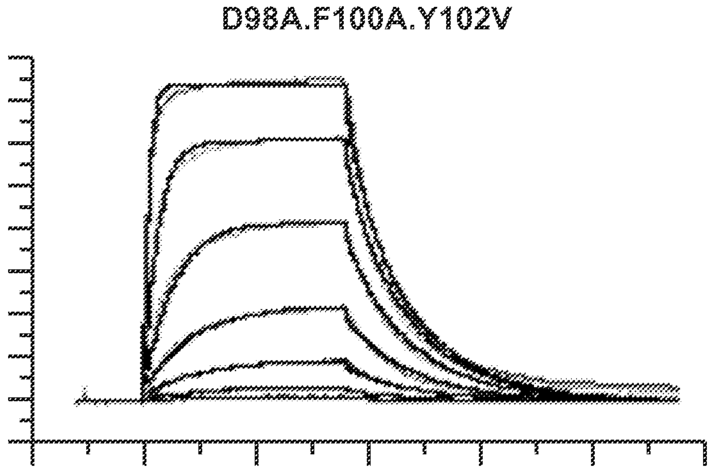


FIG. 29C

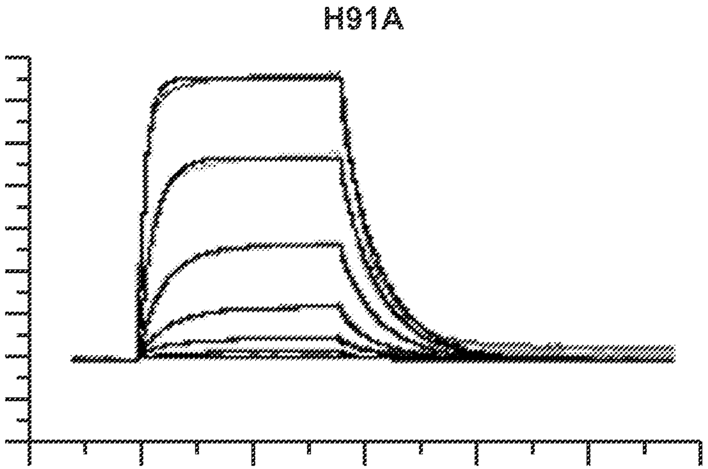


FIG. 29D

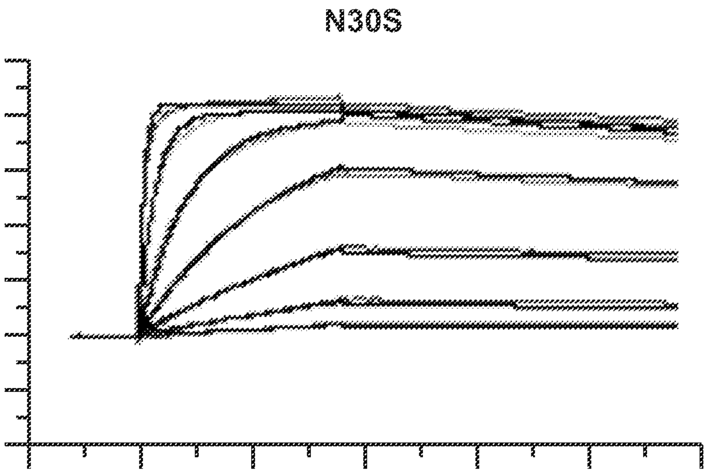


FIG. 30A

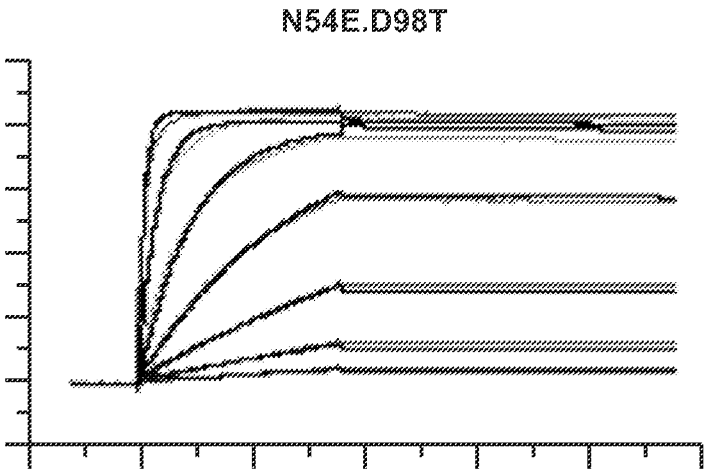


FIG. 30B

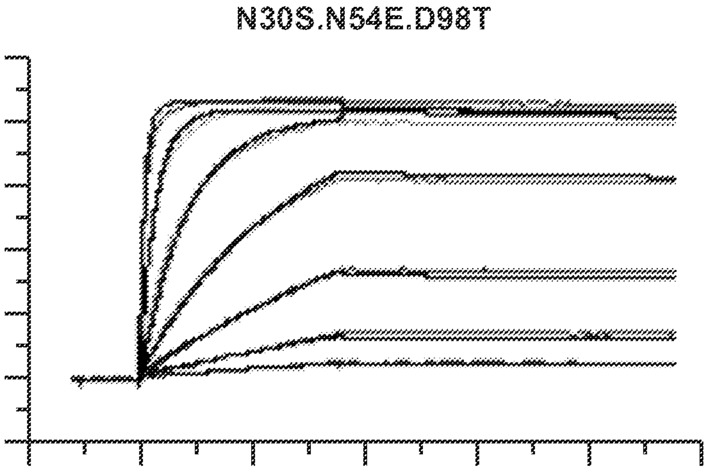
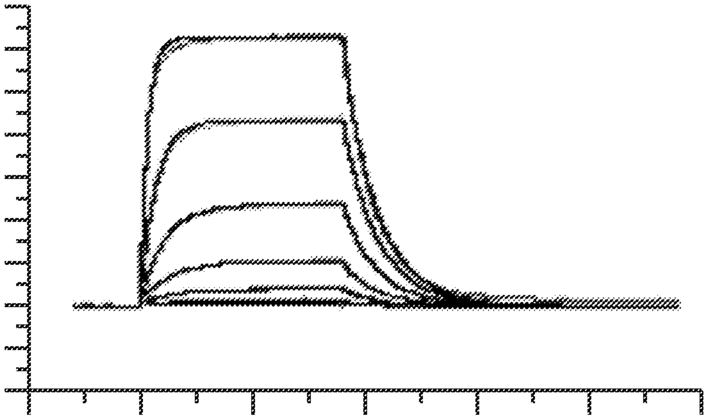
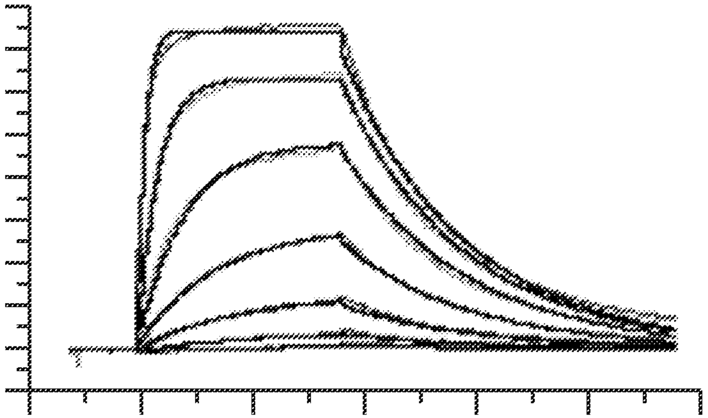


FIG. 30C



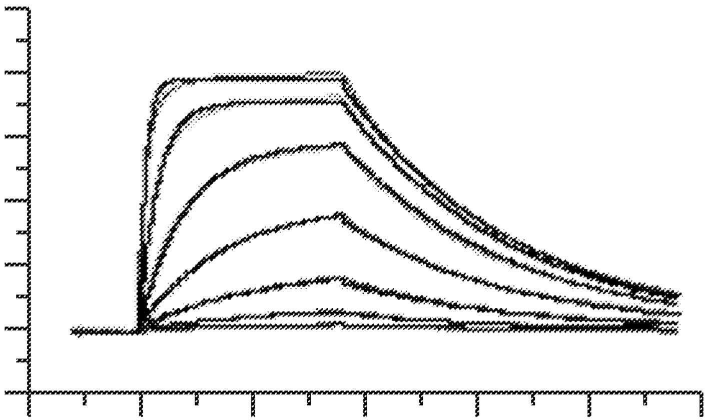
N30S.Y55E.N54E.D98T.Y102V

FIG. 31A



N30S.Y54E.D98T.F100A.Y102V

FIG. 31B



N30S.H91A.N54E.D98T

FIG. 31C

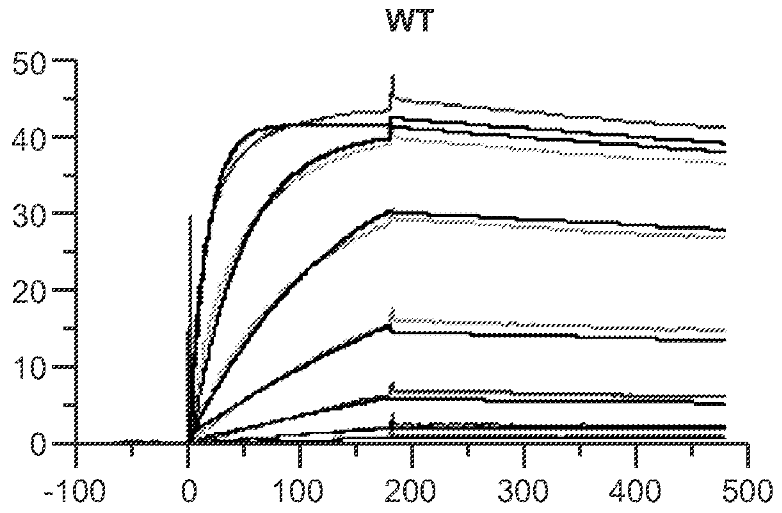


FIG. 32A

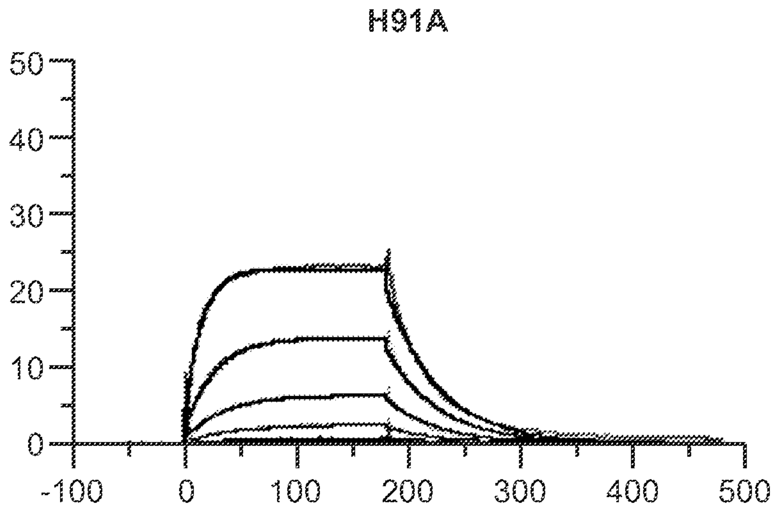


FIG. 32B

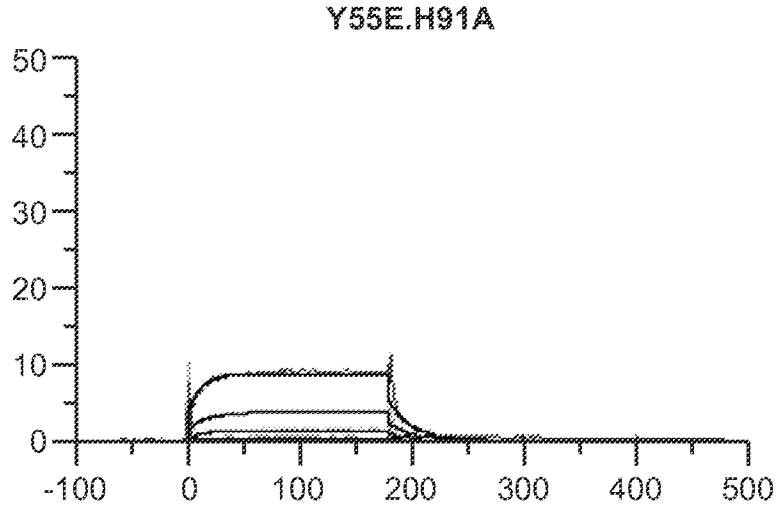


FIG. 32C

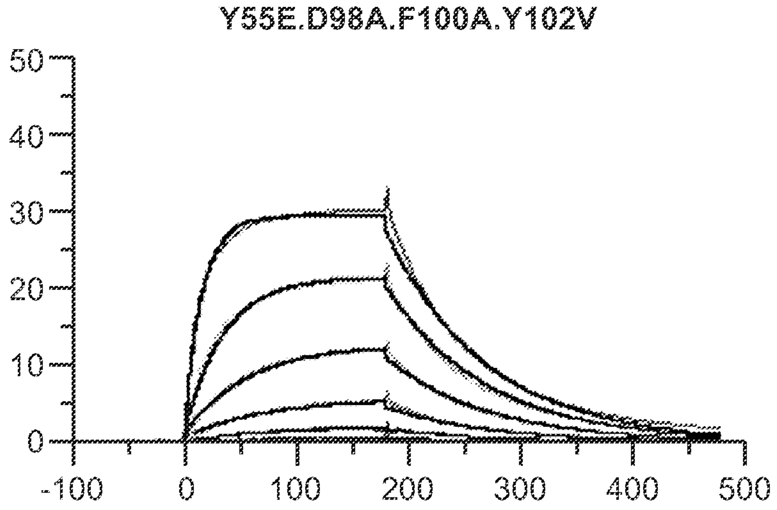


FIG. 32D

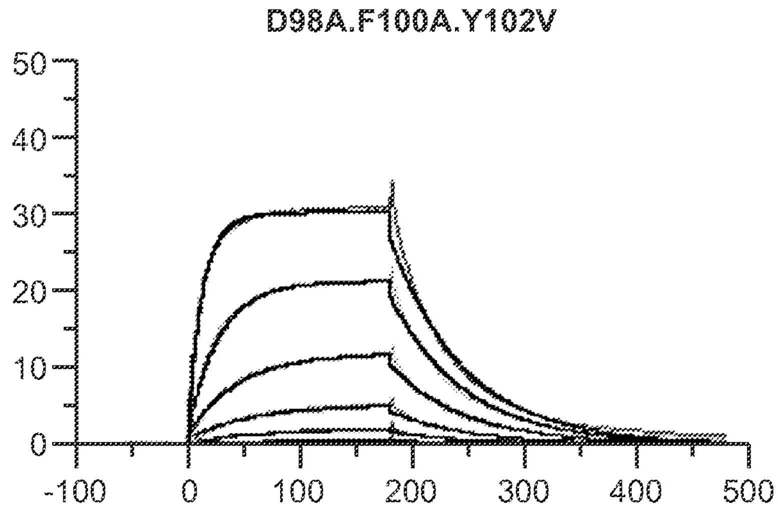


FIG. 32E

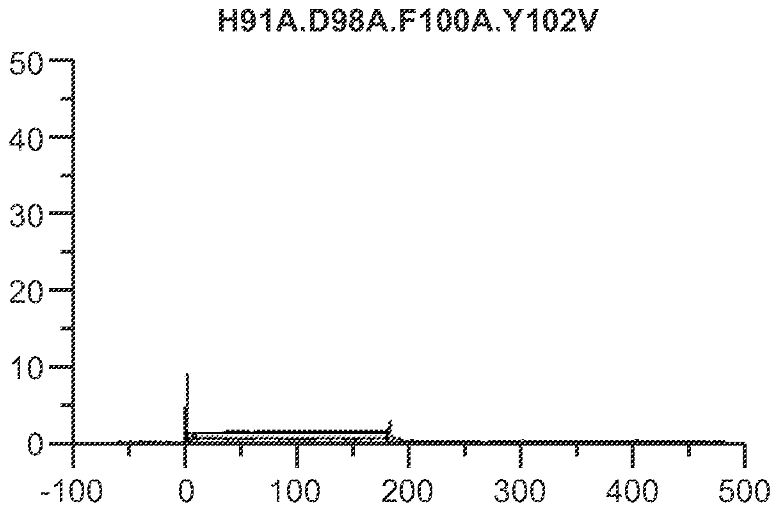


FIG. 32F

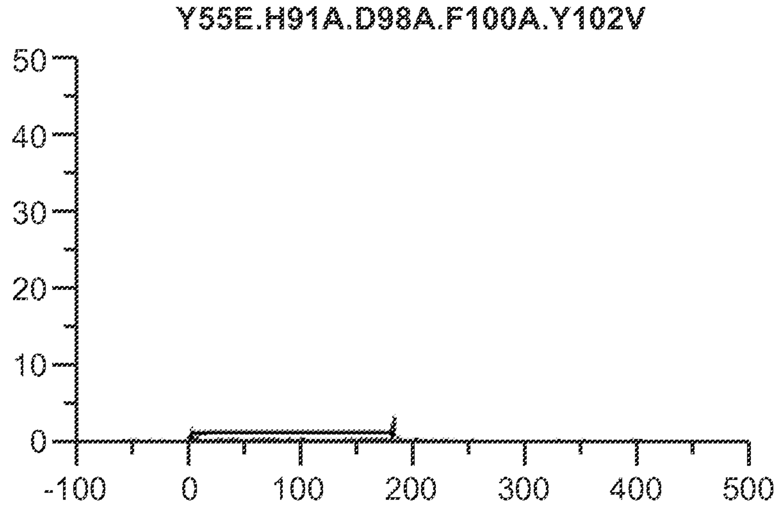


FIG. 32G

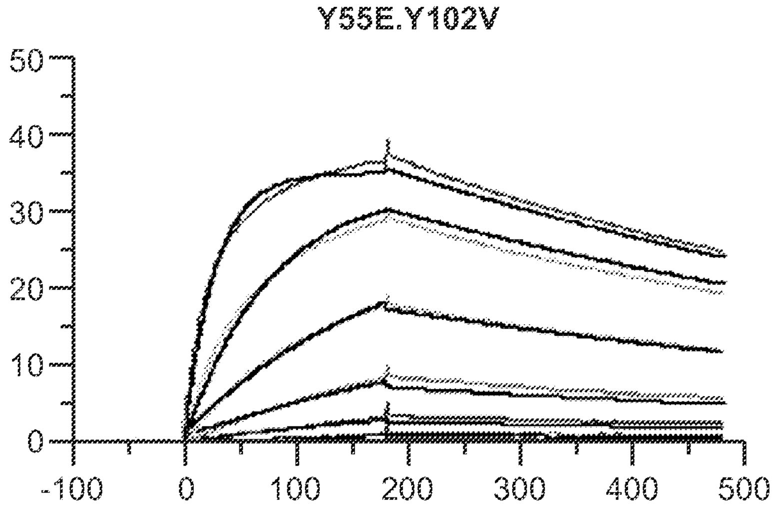


FIG. 32H

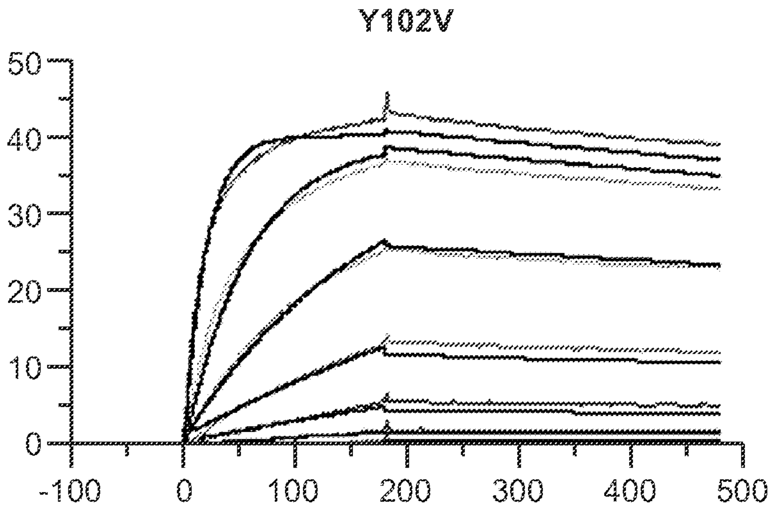


FIG. 32I

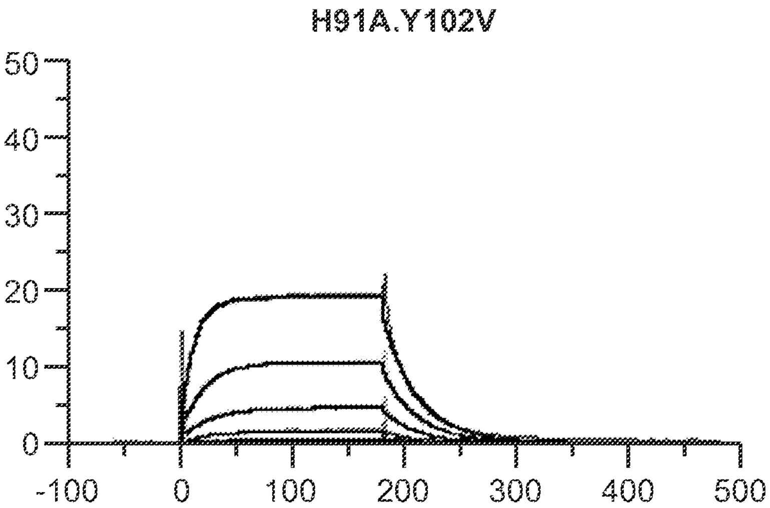


FIG. 32J

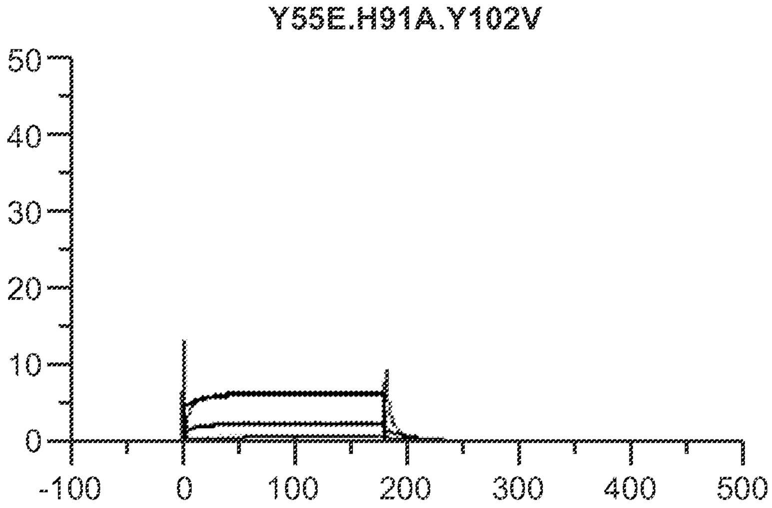


FIG. 32K

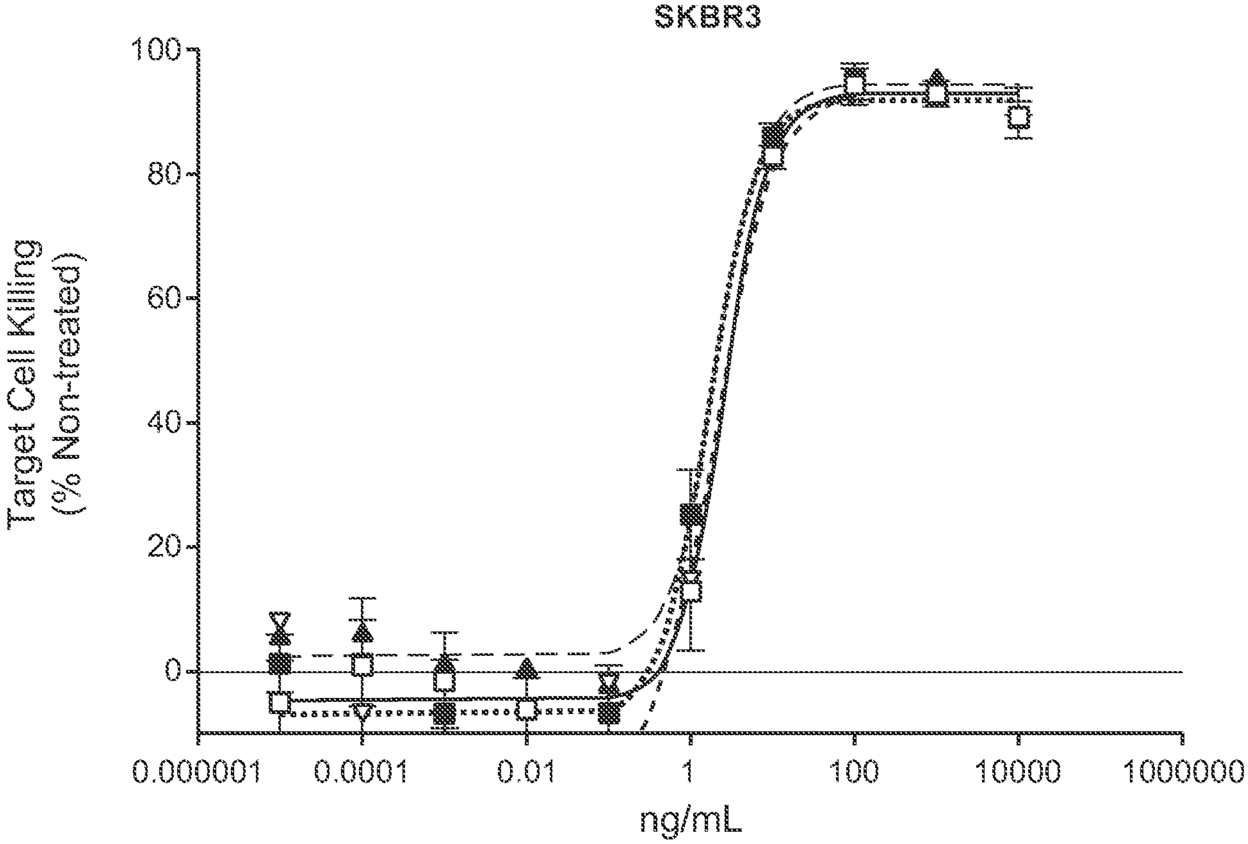


FIG. 33A

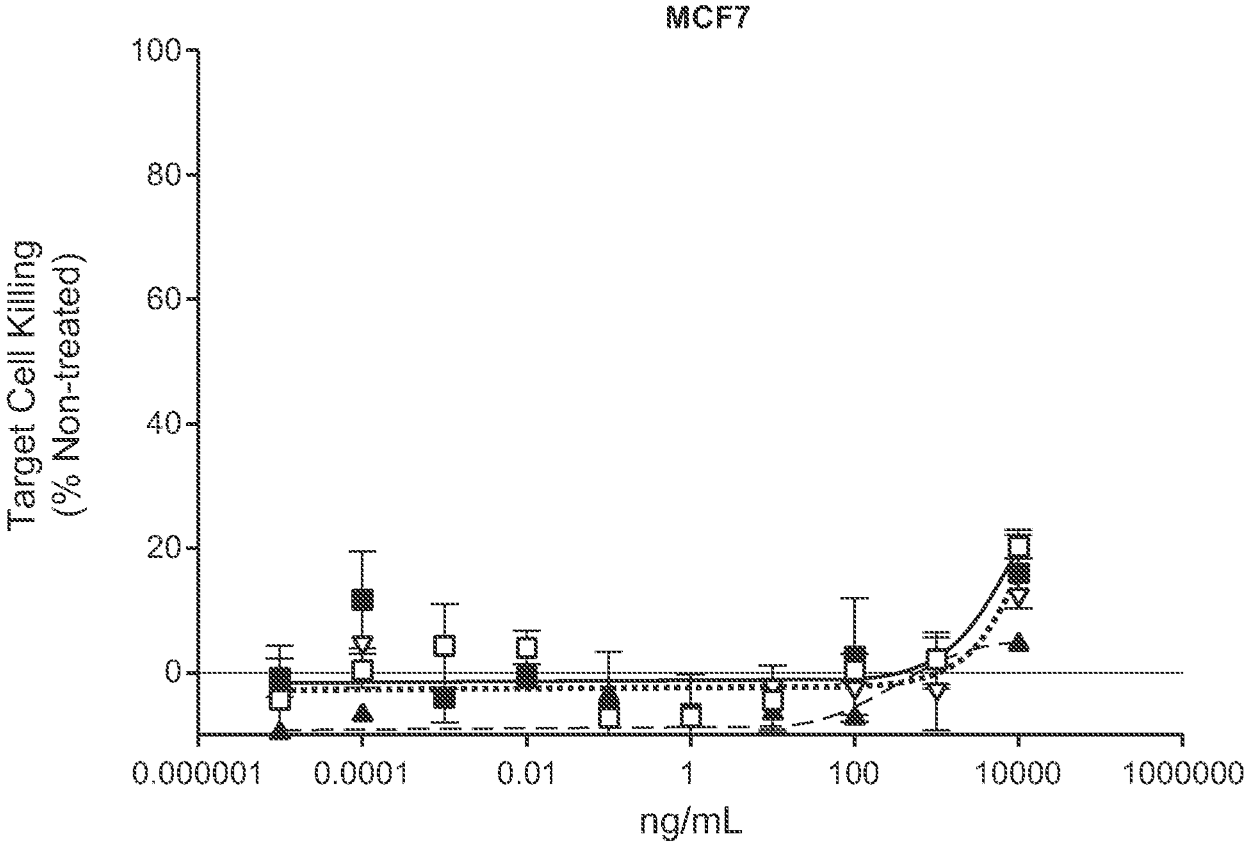


FIG. 33B

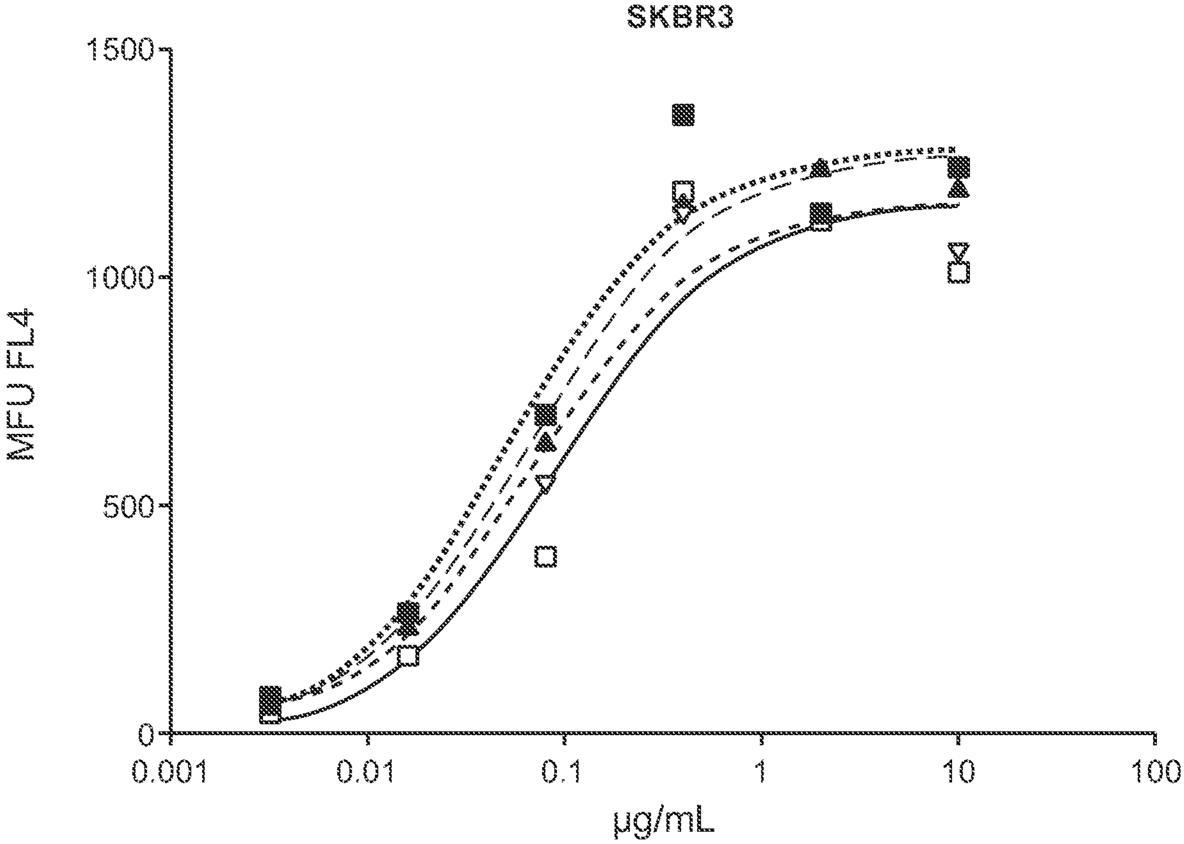


FIG. 34A

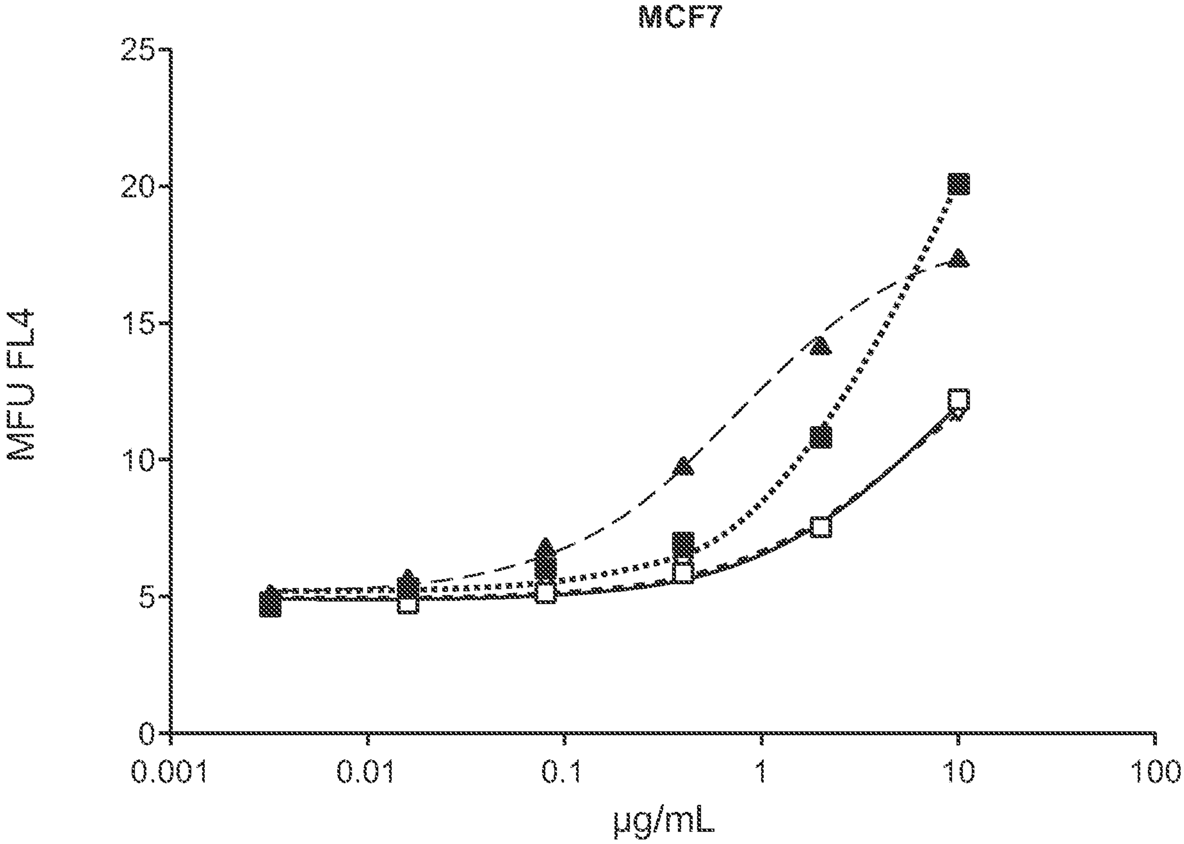


FIG. 34B

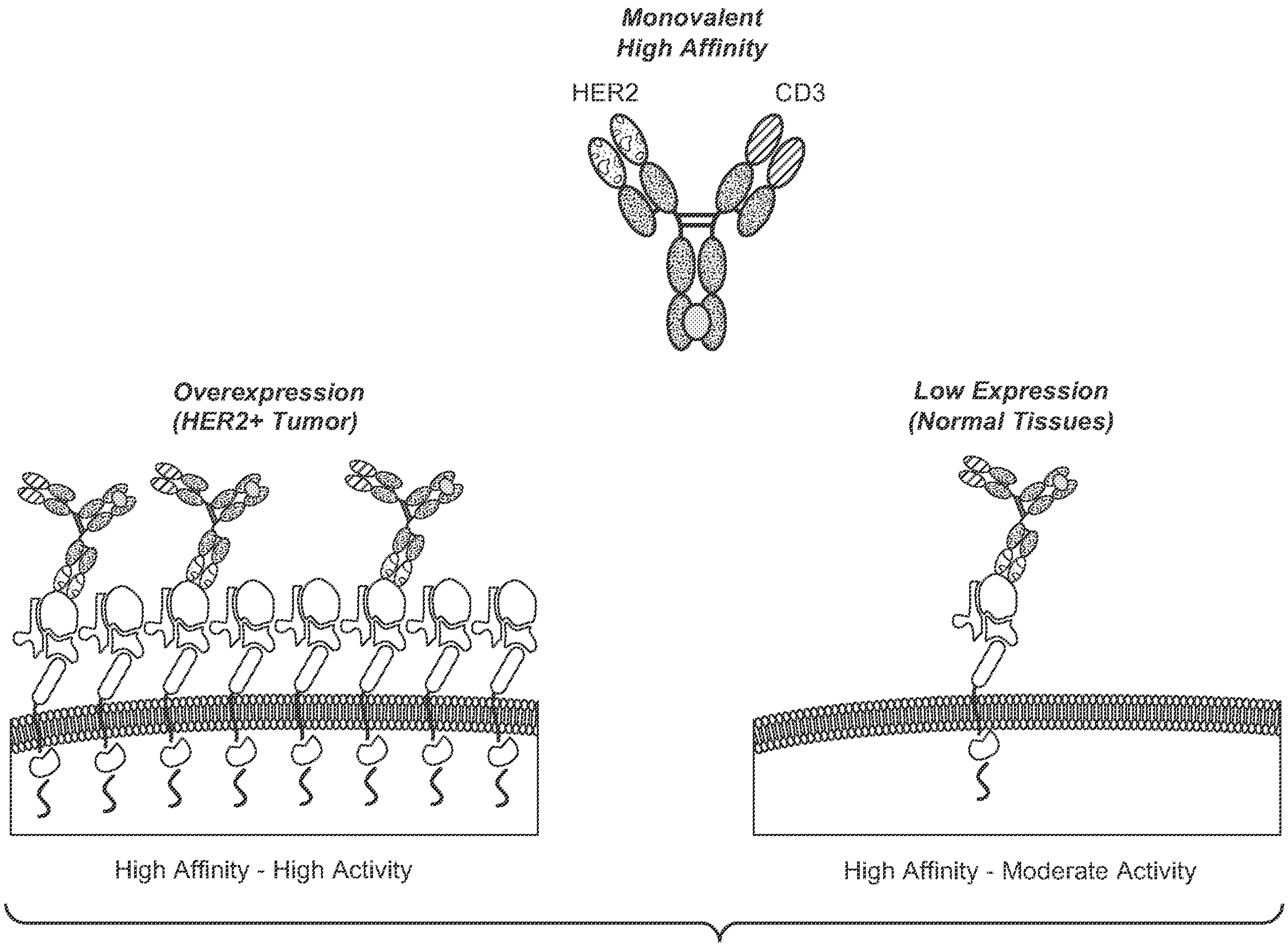

Monovalent
High Affinity
HER2
CD3
Overexpression
(HER2+ Tumor)
Low Expression
(Normal Tissues)
High Affinity - High Activity
High Affinity - Moderate Activity

FIG. 35A

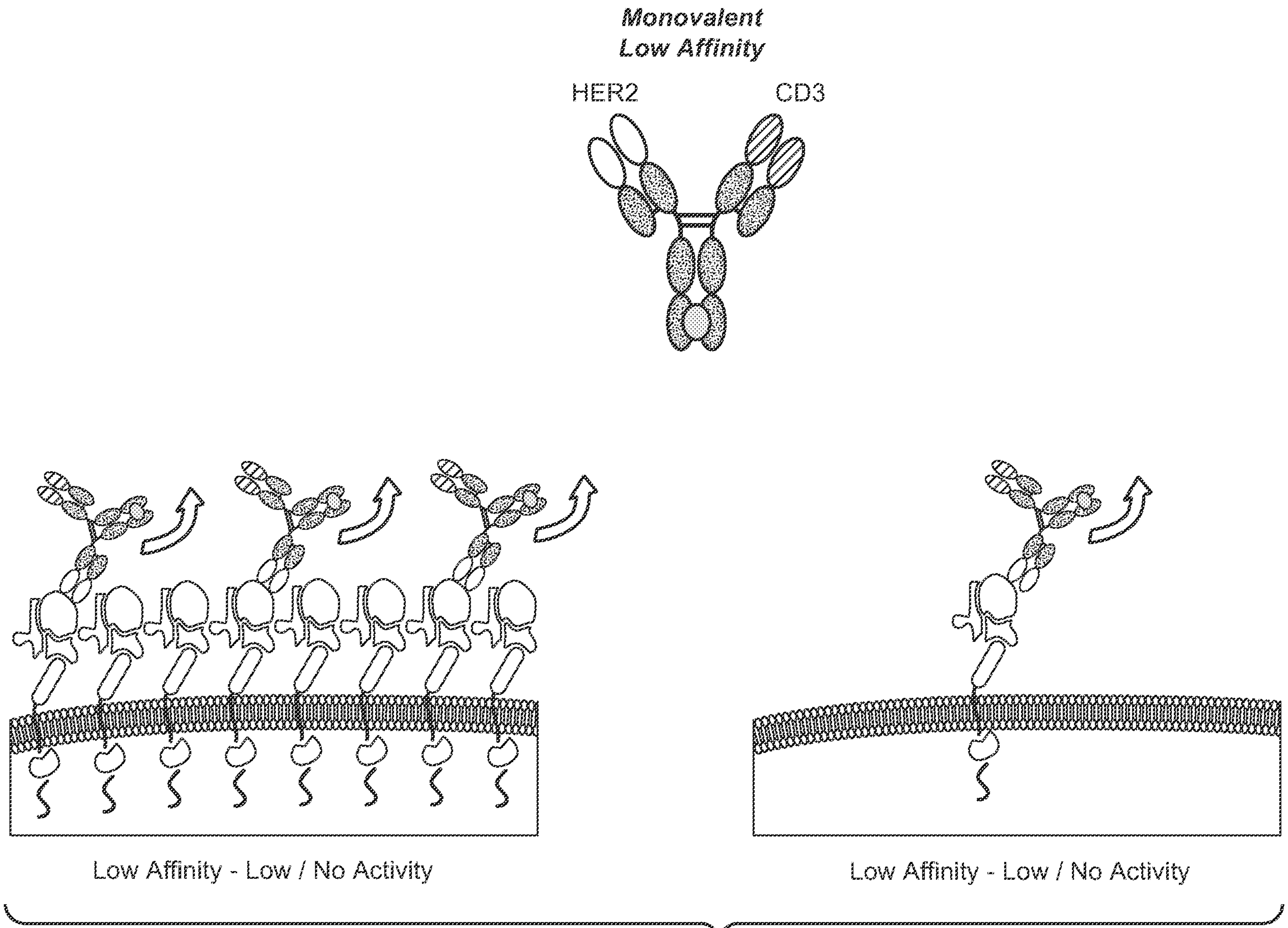

Monovalent
Low Affinity
HER2
CD3
Low Affinity - Low / No Activity
Low Affinity - Low / No Activity

FIG. 35B

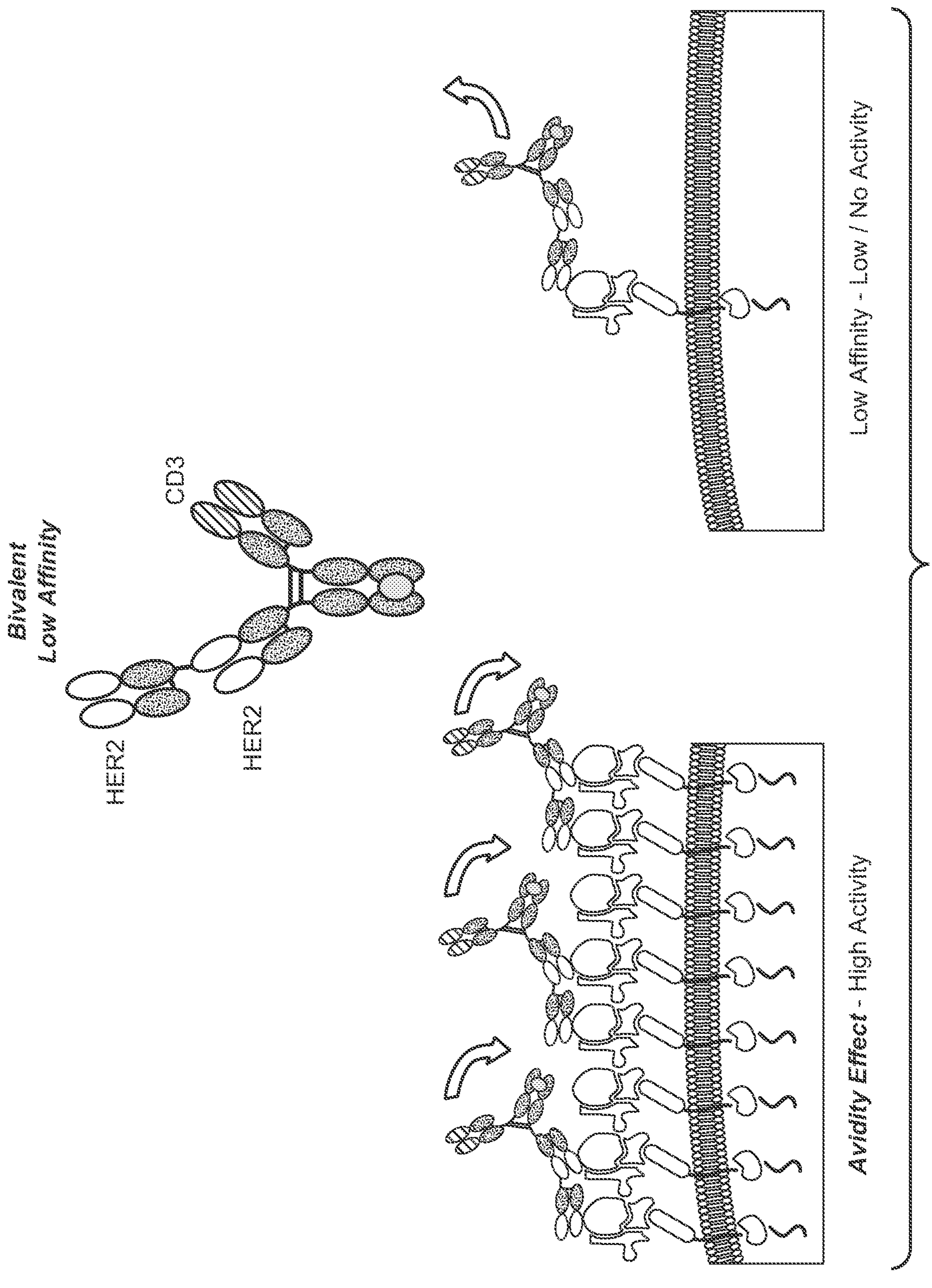

Bivalent
Low Affinity
HER2
HER2
CD3
Avidity Effect - High Activity
Low Affinity - Low / No Activity

FIG. 35C

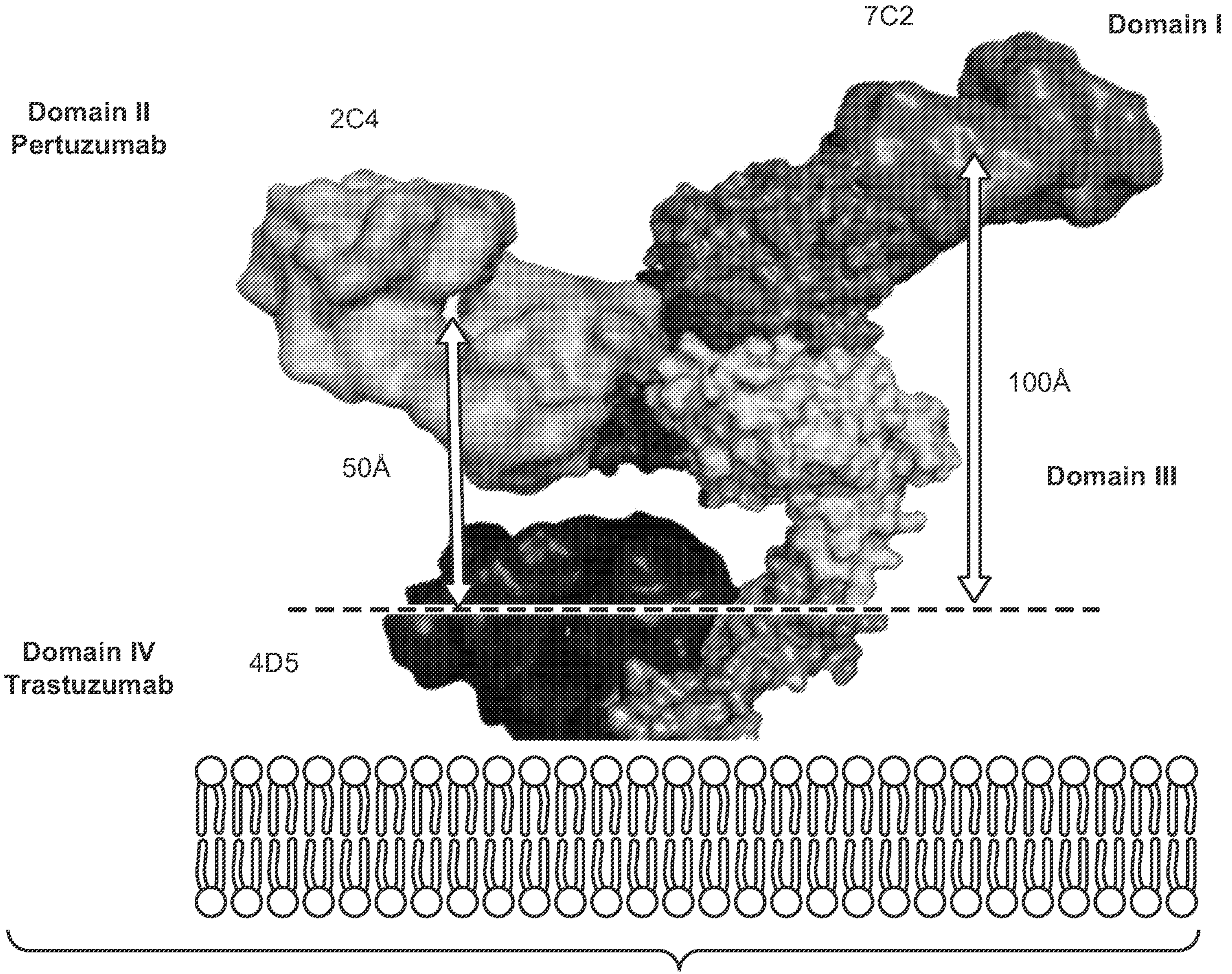

7C2
Domain I
Domain II
Pertuzumab
2C4
100Å
50Å
Domain III
Domain IV
Trastuzumab
4D5

FIG. 36

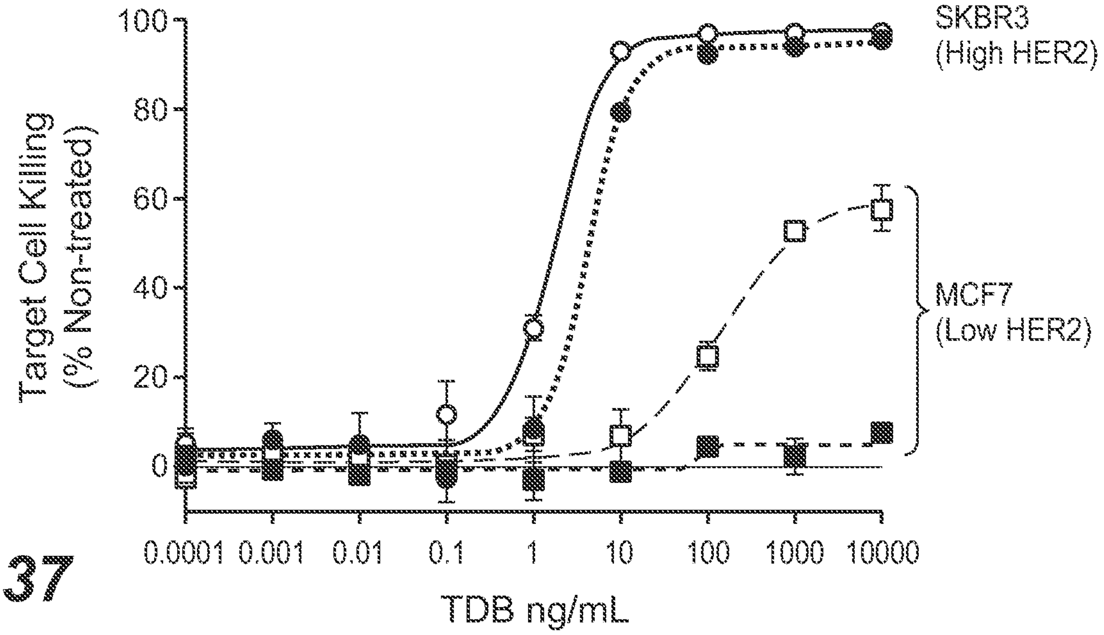


FIG. 37

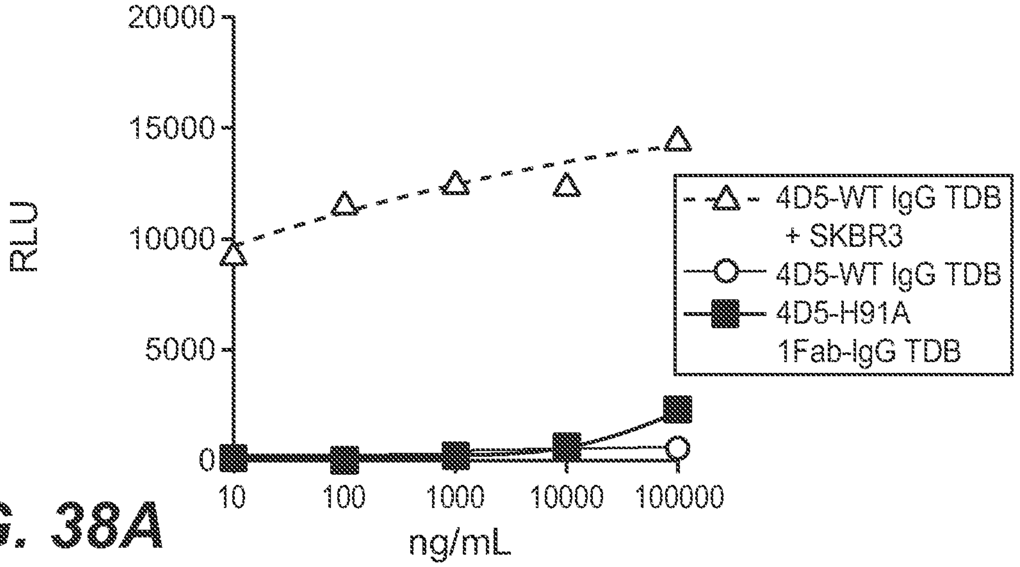


FIG. 38A

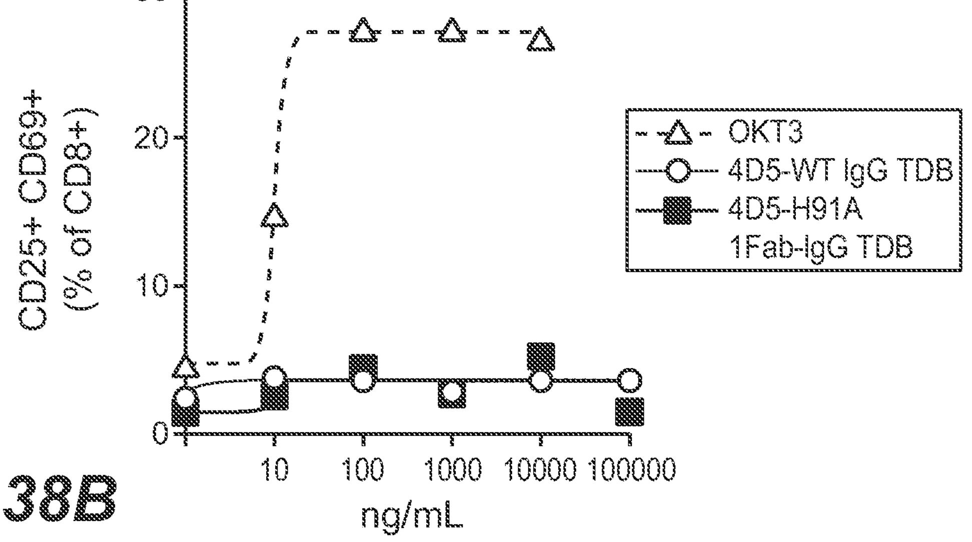


FIG. 38B

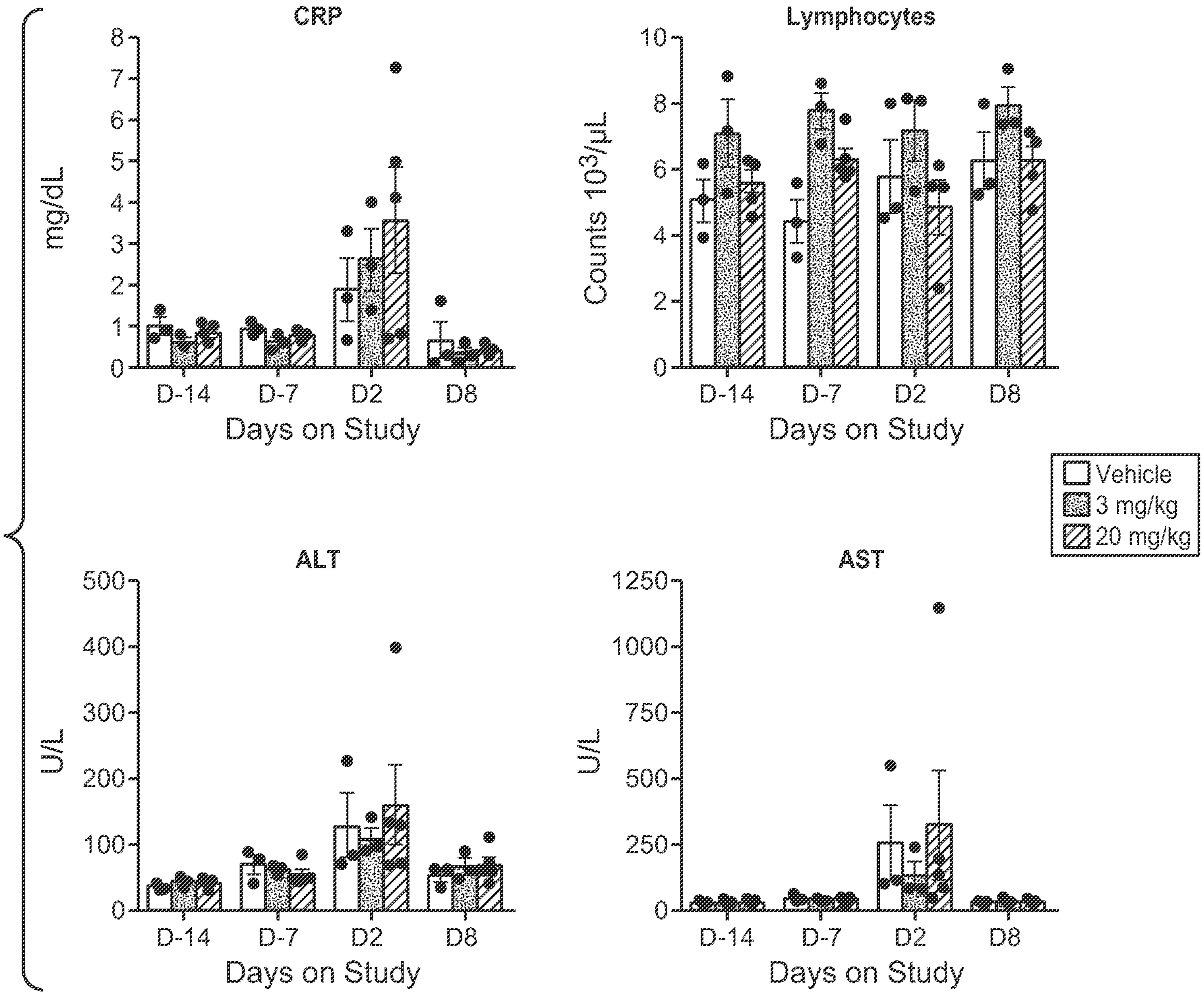
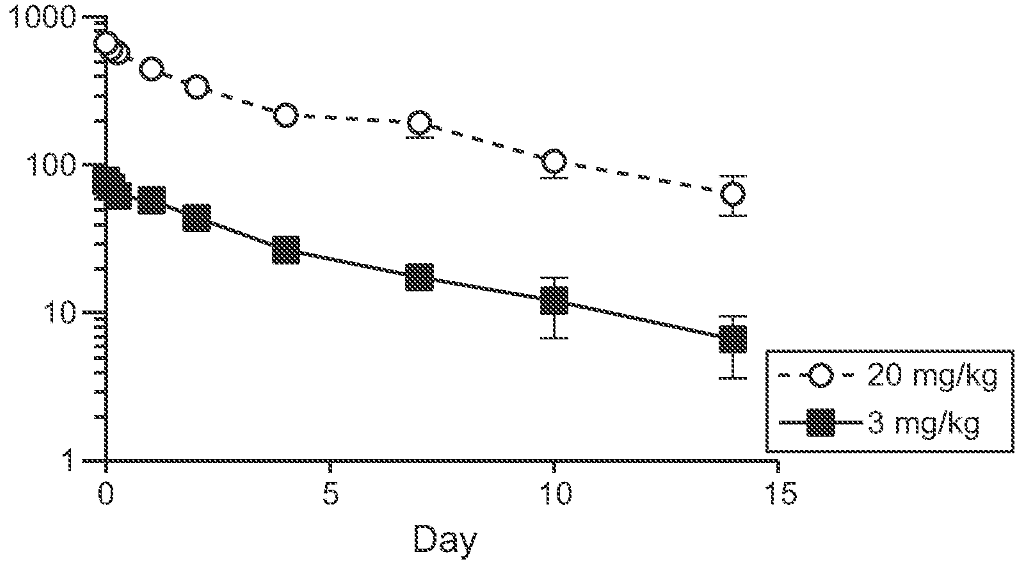

CRP
mg/dL
0
1
2
3
4
5
6
7
8
D-14
D-7
D2
D8
Days on Study
Lymphocytes
Counts 10³/μL
0
2
4
6
8
10
D-14
D-7
D2
D8
Days on Study
Vehicle
3 mg/kg
20 mg/kg
ALT
U/L
0
100
200
300
400
500
D-14
D-7
D2
D8
Days on Study
AST
U/L
0
250
500
750
1000
1250
D-14
D-7
D2
D8
Days on Study

FIG. 38C



Dose Level	Cmax (µg/mL)	AUC (µg/mL-day)	CL (mL/day/kg)
3 mg/kg	74.8 ± 1.0	379 ± 65	8.1 ± 1.3
20 mg/kg	716 ± 38	3351 ± 538	6.1 ± 1.0

FIG. 38D

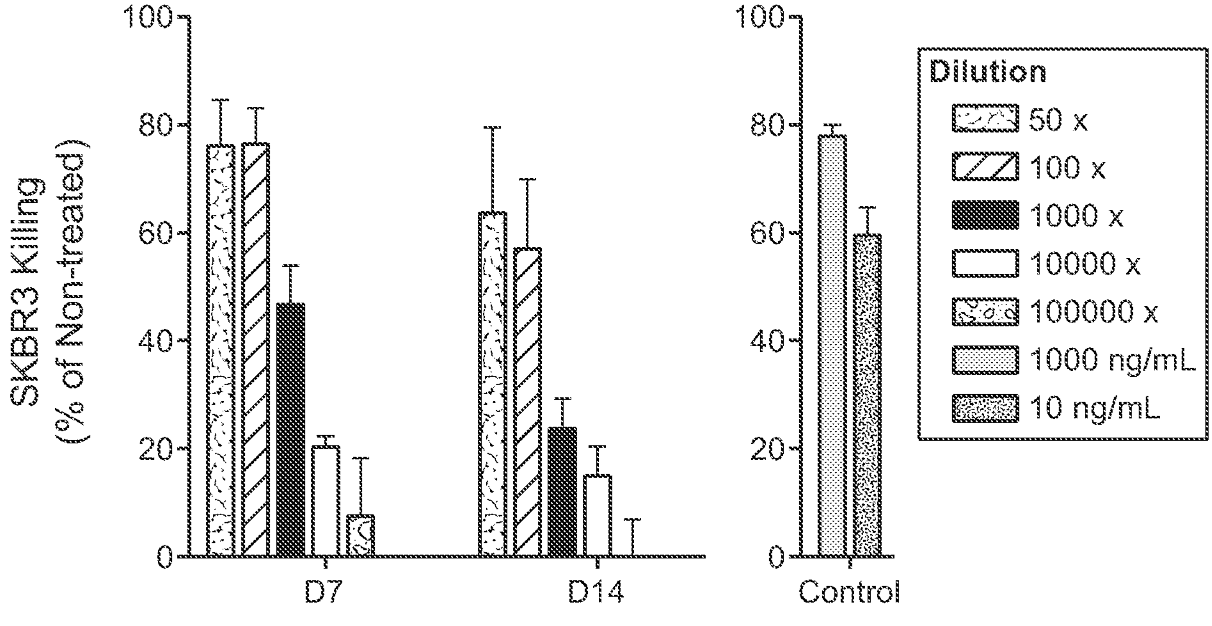


FIG. 38E

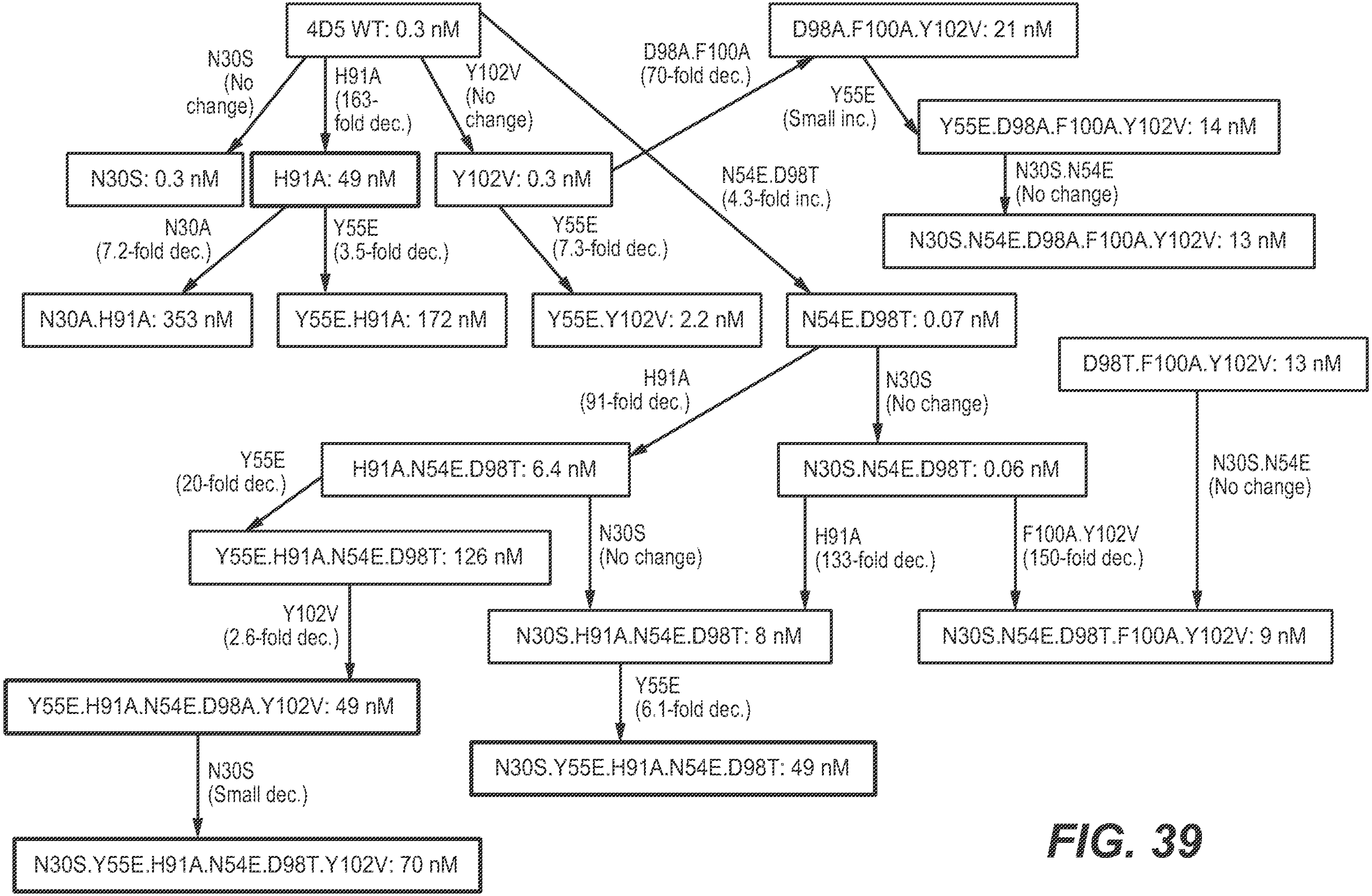

4D5 WT: 0.3 nM
N30S
(No
change)
H91A
(163-
fold dec.)
Y102V
(No
change)
N30S: 0.3 nM
H91A: 49 nM
Y102V: 0.3 nM
N30A
(7.2-fold dec.)
Y55E
(3.5-fold dec.)
Y55E
(7.3-fold dec.)
N30A.H91A: 353 nM
Y55E.H91A: 172 nM
Y55E.Y102V: 2.2 nM
D98A.F100A
(70-fold dec.)
D98A.F100A.Y102V: 21 nM
Y55E
(Small inc.)
Y55E.D98A.F100A.Y102V: 14 nM
N30S.N54E
(No change)
N30S.N54E.D98A.F100A.Y102V: 13 nM
N54E.D98T
(4.3-fold inc.)
N54E.D98T: 0.07 nM
H91A
(91-fold dec.)
N30S
(No change)
D98T.F100A.Y102V: 13 nM
H91A.N54E.D98T: 6.4 nM
N30S.N54E.D98T: 0.06 nM
N30S.N54E
(No change)
Y55E
(20-fold dec.)
Y55E.H91A.N54E.D98T: 126 nM
N30S
(No change)
H91A
(133-fold dec.)
F100A.Y102V
(150-fold dec.)
Y102V
(2.6-fold dec.)
N30S.H91A.N54E.D98T: 8 nM
N30S.N54E.D98T.F100A.Y102V: 9 nM
Y55E.H91A.N54E.D98A.Y102V: 49 nM
Y55E
(6.1-fold dec.)
N30S.Y55E.H91A.N54E.D98T: 49 nM
N30S
(Small dec.)
N30S.Y55E.H91A.N54E.D98T.Y102V: 70 nM

FIG. 39

INTERNATIONAL SEARCH REPORT

International application No	PCT/US2019/017251

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/395 C07K1/113 C07K16/10 C07K16/28 C07K16/32
C07K16/46 A61K47/68 A61P35/02

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K C07K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, CHEM ABS Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2012/143524 A2 (GENMAB AS [DK]; NEIJSEEN JOOST J [NL] ET AL.) 26 October 2012 (2012-10-26) the whole document -----	1-237
Y	WO 2013/026831 A1 (ROCHE GLYCART AG [CH]; AUER JOHANNES [DE] ET AL.) 28 February 2013 (2013-02-28) the whole document -----	1-237

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search 9 April 2019	Date of mailing of the international search report 17/04/2019
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Meyer, Wolfram

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		AU 2017210666 A1	24-08-2017
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		JP 2014526895 A	09-10-2014
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		LT 2748202 T	25-09-2018
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		US 2017198043 A1	13-07-2017
		WO 2013026831 A1	28-02-2013