

**ANTIBODIES, ACTIVATABLE ANTIBODIES, BISPECIFIC ANTIBODIES, AND
BISPECIFIC ACTIVATABLE ANTIBODIES AND METHODS OF USE THEREOF**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/572,468, filed October 14, 2017; U.S. Provisional Application No. 62/577,140, filed October 25, 2017; U.S. Provisional Application No. 62/613,358, filed January 3, 2018; U.S. Provisional Application No. 62/666,065, filed May 2, 2018; and U.S. Provisional Application No. 62/731,622, filed September 14, 2018, the contents of each of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] Provided herein antibodies, activatable antibodies (AAs), bispecific antibodies, and bispecific activatable antibodies (BAAs). Also provided herein are methods of making and methods of use of these antibodies, AAs, bispecific antibodies, and BAAs.

REFERENCE TO SEQUENCE LISTING

[0003] The "Sequence Listing" submitted electronically concurrently herewith pursuant 37 C.F.R. § 1.821 in computer readable form (CRF) via EFS-Web as file name CYTX-045-US_SEQLIST_10-12-18_ST25.txt is incorporated herein by reference. The electronic copy of the Sequence Listing was created on 10-12-18, and the size on disk is 440 kilobytes.

BACKGROUND

[0004] Antibody-based therapies have proven effective treatments for several diseases but in some cases, toxicities due to broad target expression have limited their therapeutic effectiveness. In addition, antibody-based therapeutics have exhibited other limitations such as rapid clearance from the circulation following administration.

[0005] In the realm of small molecule therapeutics, strategies have been developed to provide prodrugs of an active chemical entity. Such prodrugs are administered in a relatively inactive (or significantly less active) form. Once administered, the prodrug is metabolized *in vivo* into the active

compound. Such prodrug strategies can provide for increased selectivity of the drug for its intended target and for a reduction of adverse effects.

[0006] Accordingly, there is a continued need in the field of antibody-based therapeutics for antibodies that mimic the desirable characteristics of the small molecule prodrug.

SUMMARY OF THE INVENTION

[0007] Provided herein are antibodies, bispecific antibodies, activatable antibodies, and bispecific activatable antibodies, methods of making, and methods of use thereof. These find use in therapeutics and diagnostics. The activatable antibodies and bispecific activatable antibodies of the present disclosure may be used to reduce damage to healthy tissue generally caused by an antibody binding to its target on healthy tissue as well as on diseased tissue.

[0008] Accordingly in one aspect, provided herein are bispecific activatable antibodies (BAA), wherein said BAA, when activated, specifically binds to two targets and comprises the following structure:

- a) an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises:
 - a. two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and
 - b. a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein
 - c. the MM1 inhibits the binding of the AB1 to its target; and
 - d. the CM1 is a polypeptide that functions as a substrate for a first protease,
- b) two scFvs (AB2) that each specifically binds to a second target wherein each AB2 comprises:
 - a. a light chain variable region linked to a heavy chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and
 - b. a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of a MM2-CM2 construct is linked to the amino terminus of each AB2 wherein
 - c. the MM2 inhibits the binding of the AB2 to its target; and

- d. the CM2 is a polypeptide that functions as a substrate for a second protease,
- c) and wherein the BAA has at least one of the following characteristics:
 - a. MM2 comprises amino acid sequence SEQ ID NO: 12;
 - b. MM1 comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7;
 - c. AB2 comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4; and
 - d. AB1 comprises an Fc region comprising an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0009] In some embodiments, the BAAs provided herein comprise:

A bispecific activatable antibody (BAA), wherein said BAA, when activated, specifically binds to two targets and comprises the following structure:

- a. an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and wherein the AB1 is linked to a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein
 - the MM1 inhibits the binding of the AB1 to its target; and
 - the CM1 is a polypeptide that functions as a substrate for a first protease,
- b. two scFvs (each an AB2) that each specifically binds to a second target wherein each AB2 comprises a light chain variable region linked to a heavy chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and wherein each AB2 is linked to a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of each MM2-CM2 construct is linked to the amino terminus of each AB2 wherein
 - the MM2 inhibits the binding of the AB2 to its target; and

the CM2 is a polypeptide that functions as a substrate for a second protease, and wherein the BAA has at least one of the following characteristics:

- i. MM2 comprises amino acid sequence SEQ ID NO: 12;
- ii. MM1 comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7;
- iii. AB2 comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4; and
- iv. AB1 comprises an Fc region comprising an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0010] In some embodiments, the BAA comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4. In some embodiments, the AB1 binds a tumor target and the AB2 binds an immune effector target. In some embodiments, the BAA is a T cell-engaging bispecific (TCB) AA (TCBAA). In some embodiments, the AB1 binds EGFR and the AB2 binds CD3. In some embodiments, the MM1 comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7. In some embodiments, the MM1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 85 and SEQ ID NO: 78. In some embodiments, the MM1 comprises SEQ ID NO: 78. In some embodiments, the MM2 comprises the amino acid sequence SEQ ID NO: 12. In some embodiments, the CM comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the CM comprises the amino acid sequence of SEQ ID NO: 17. In some embodiments, the CMs the CM comprises the amino acid sequence of SEQ ID NO: 16. In some embodiments, the CM1 comprises an amino acid sequence selected from the group comprising SEQ ID NO: 14 and SEQ ID NO: 16. In some embodiments, the CM2 comprises an amino acid sequence selected from the group comprising SEQ ID NO: 14 and SEQ ID NO: 17. In some embodiments, provided herein is BAA CI106, comprising the layout and sequence as provided in Table 11 and Example 1. In some embodiments, provided herein is BAA CI107, comprising the layout and sequence as provided in Table 11 and Example 1. In some embodiments, provided herein is BAA CI079, comprising the layout and sequence as provided in Table 11 and

Example 1. In some embodiments, provided herein is BAA CI090, comprising the layout and sequence as provided in Table 11 and Example 1. In some embodiments, provided herein is BAA CI135, comprising the layout and sequence as provided in Table 11 and Example 1. In some embodiments, provided herein is BAA CI136, comprising the layout and sequence as provided in Table 11 and Example 1. In some embodiments, the AB1 comprises amino acid substitutions in at least two of amino acid positions L234, L235, and P331. In some embodiments, the AB1 comprises amino acid substitutions at amino acid positions L234, L235, and P331. In some embodiments, the AB1 comprises L234F, L235E, and P331S amino acid substitutions. In some embodiments, the AB1 comprises an Fc region comprising an amino acid substitution at N297. In some embodiments, the AB1 comprises L234F, L235E, P331S, and N297Q amino acid substitutions. In some embodiments, the heavy chain of the AB1 comprises any one of SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 76, as set forth in Table 6.

[0011] In another aspect, provided herein is a bispecific activatable antibody (BAA) comprising:

- a) an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises:
 - i. two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and
 - ii. a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein
 - the MM1 inhibits the binding of the AB1 to its target; and
 - the CM1 is a polypeptide that functions as a substrate for a first protease,
- b) two scFvs (AB2) that each specifically binds to a second target wherein each AB2 comprises:
 - i. a heavy chain variable region linked to a light chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and
 - ii. a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of a MM2-CM2 construct is linked to the amino terminus of each AB2 wherein

the MM2 inhibits the binding of the AB2 to its target; and

the CM2 is a polypeptide that functions as a substrate for a second protease,

and wherein the AB1 comprises an Fc region comprises an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function. In some embodiments, the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, N297 and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function. In some embodiments, the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function. In some embodiments, the first target is selected from the group consisting of the targets presented in Table 9 and the second target is selected from the group consisting of the targets presented in Table 9.

[0012] In some embodiments, the BAAs provided herein comprise:

- a) A bispecific activatable antibody (BAA) comprising:
 - i) an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and wherein the AB1 is linked to a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein

the MM1 inhibits the binding of the AB1 to its target; and

the CM1 is a polypeptide that functions as a substrate for a first protease,
 - ii) two scFvs (each an AB2) that each specifically binds to a second target wherein each AB2 comprises a heavy chain variable region linked to a light chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and wherein each AB2 is linked to a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of each MM2-CM2 construct is linked to the amino terminus of each AB2 wherein

the MM2 inhibits the binding of the AB2 to its target; and

the CM2 is a polypeptide that functions as a substrate for a second protease,

and wherein the AB1 comprises an Fc region comprises an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function. In some embodiments, the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, N297 and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function. In some embodiments, the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function. In some embodiments, the first target is selected from the group consisting of the targets presented in Table 9 and the second target is selected from the group consisting of the targets presented in Table 9.

[0013] In another aspect, provided herein is an activatable antibody (AA) comprising: (a) an antibody (AB) that specifically binds to Epidermal Growth Factor Receptor (EGFR), wherein the AB is an IgG1 antibody, and wherein the Fc region of the AB comprises an amino acid substitution in at least one of amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the AA has reduced effector function; (b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the EGFR when the AA is in an uncleaved state; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. In some embodiments, the amino acid substitution is any one or more of L234F, L235E, and P331S. In some embodiments, the AB comprises amino acid substitutions in at least two of amino acid positions L234, L235, and P331. In some embodiments, the AB comprises amino acid substitutions at amino acid positions L234, L235, and P331. In some embodiments, the AB comprises L234F, L235E, and P331S amino acid substitutions. In some embodiments, the AB comprises an Fc region comprising an amino acid substitution at N297. In some embodiments, the Fc region comprises an N297Q mutation. In some embodiments, the AB comprises L234F, L235E, P331S, and N297Q amino acid substitutions. In some embodiments, the MM comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7. In some embodiments, the MM comprises the amino acid sequence of SEQ ID NO: 78. In some embodiments, the MM comprises the amino acid sequence of SEQ ID NO: 85. In some embodiments, the CM comprises an amino acid sequence selected from the group consisting of sequences presented in Table 4. In some embodiments, the CM comprises

the amino acid sequence of SEQ ID NO: 14. In some embodiments, the CM comprises the amino acid sequence of SEQ ID NO: 16. In some embodiments, the AA is part of a BAA.

[0014] In another aspect, provided herein is an activatable antibody (AA) comprising: (a) an antibody or an antigen binding fragment thereof (AB) that specifically binds to Epidermal Growth Factor Receptor (EGFR); (b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the EGFR when the AA is in an uncleaved state, and wherein the MM comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. In some embodiments, the MM comprises the amino acid sequence of SEQ ID NO: 78. In some embodiments, the CM comprises a substrate cleavable by a serine protease or an MMP. In some embodiments, the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18-56. In some embodiments, the CM comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the CM comprises the amino acid sequence of SEQ ID NO: 16. In some embodiments, the AA is part of a BAA.

[0015] In another aspect, provided herein is an activatable antibody (AA) comprising:

[0016] (a) an antibody or antigen binding fragment thereof (AB) that specifically binds to the epsilon chain of CD3, wherein the antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or comprises a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4; (b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the CD3 when the AA is in an uncleaved state; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. In some embodiments, the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 2. In some embodiments, the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 3. In some embodiments, the AB comprises a light chain variable domain as set forth in SEQ ID NO: 1. In some embodiments, the AB comprises a light chain variable domain as set forth in SEQ ID NO: 4. In some embodiments, the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain variable domain as set forth in SEQ ID NO: 1. In some embodiments, the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 1. In some embodiments, the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 and a

light chain variable domain as set forth in SEQ ID NO: 4. In some embodiments, the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 4. In some embodiments, the MM comprises any one of the sequences set forth in Table 3. In some embodiments, the CM comprises any one of the sequences set forth in Table 4. In some embodiments, the AA is part of a BAA.

[0017] In another aspect, provided herein is an activatable antibody (AA) comprising: (a) an antibody or antigen binding fragment thereof (AB) that specifically binds to the epsilon chain of CD3; (b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the CD3 when the AA is in an uncleaved state, wherein the MM comprises amino acid sequence SEQ ID NO: 12; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. In some embodiments, the CM comprises any one of the sequences set forth in Table 4. In some embodiments, the CM comprises a substrate cleavable by a serine protease or an MMP. In some embodiments, the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18-56. In some embodiments, the protease is an MMP. In some embodiments, protease is a serine protease. In some embodiments, the AA is part of a BAA.

[0018] In another aspect, provided herein is an activatable antibody (AA) comprising: (a) an antibody (AB) that specifically binds a target, wherein the antibody is an IgG1 antibody, and wherein the Fc region of the antibody comprises an amino acid substitution in at least one of amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the AA has reduced effector function; (b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the target when the AA is in an uncleaved state; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. In some embodiments, the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, N297 and P331, as numbered by the EU index as set forth in Kabat, such that the AA has reduced effector function. In some embodiments, the target is selected from the group consisting of the targets presented in Table 9. In some embodiments, the AA is part of a BAA.

[0019] In another aspect, provided herein is an antibody or antigen binding fragment thereof (AB) that specifically binds to the epsilon chain of CD3, wherein the antibody comprises a heavy chain

variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4. In some embodiments the antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4. In some embodiments the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 2. In some embodiments the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 3. In some embodiments the AB comprises a light chain variable domain as set forth in SEQ ID NO: 1. In some embodiments the AB comprises a light chain variable domain as set forth in SEQ ID NO: 4. In some embodiments the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain variable domain as set forth in SEQ ID NO: 1. In some embodiments the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 1. In some embodiments the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 4. In some embodiments the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain variable domain as set forth in SEQ ID NO: 4. In some embodiments, the antibody is a bispecific AB. In some embodiments the antibody is a scFv. In some embodiments the antibody is an IgG1 antibody. In some embodiments, the antibody is part of an AA or is part of a BAA.

[0020] In another aspect, provided herein is an antibody that specifically binds to EGFR or CD3 (AB), wherein the antibody is an IgG1 antibody or a scFv linked to an Fc domain, wherein the antibody comprises an Fc region comprising an amino acid substitution in at least one of amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the antibody has reduced effector function. In some embodiments, the amino acid substitution is any one or more of L234F, L235E, and P331S. In some embodiments, the antibody comprises amino acid substitutions in at least two of amino acid positions L234, L235, and P331. In some embodiments, the antibody comprises amino acid substitutions at amino acid positions L234, L235, and P331. In some embodiments, the antibody comprises L234F, L235E, and P331S amino acid substitutions. In some embodiments, the antibody comprises an Fc region comprising an amino acid substitution at N297. In some embodiments, the Fc region comprises an N297Q mutation. In some embodiments, the antibody comprises L234F, L235E, P331S, and N297Q amino acid substitutions. In some embodiments, the heavy chain of the antibody comprises any one of SEQ ID

NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 76, as set forth in Table 6. In some embodiments, the heavy chain variable domain of the antibody comprises any one of SEQ ID NO: 2 or SEQ ID NO: 3 or wherein the light chain variable domain of the AB comprises any one of SEQ ID NO: 1 or SEQ ID NO: 4. In some embodiments, the antibody is part of an AA or is part of a BAA.

[0021] In another aspect, also provided herein are pharmaceutical composition comprising any one of the BAAs, AAs, and antibodies described above, and optionally a carrier. In another aspect, also provided herein are pharmaceutical composition comprising any one of the BAAs, AAs, and antibodies described above and a carrier. In some embodiments, the composition comprises an additional agent, for example the additional agent can be a therapeutic agent.

[0022] In another aspect, also provided herein are isolated nucleic acid molecules encoding any one of the BAAs, AAs, and antibodies described above. Also provided are vectors comprising the nucleic acid is provided. In some embodiments, the vector comprises the nucleic acid sequence of pLW289. In some embodiments, the vector comprises the nucleic acid sequence of pLW246. In some embodiments, the vector comprises the nucleic acid sequence of pLW307. In some embodiments, the vector comprises the nucleic acid sequence of pLW291. In some embodiments, the vector comprises the nucleic acid sequence of pLW352. In some embodiments, the vector comprises the nucleic acid sequence of pLW246. In some embodiments, the vector comprises the nucleic acid sequence of pLW353.

[0023] In another aspect, also provided herein is a cell comprising any one of the vectors described above. In some embodiments, provided herein is a cell comprising pLW289 and pLW246. In some embodiments, provided herein is a cell comprising pLW307 and pLW291. In some embodiments, provided herein is a cell comprising pLW352 and pLW246. In some embodiments, provided herein is a cell comprising pLW353 and pLW246.

[0024] In another aspect, provided herein are methods of producing the antibody, AA, or BAA provided above, by culturing a cell under conditions that lead to expression of the antibody, AA, or BAA, wherein the cell comprises the relevant nucleic acid molecule or vectors provided herein.

[0025] In another aspect, provided herein is a method of treating, alleviating a symptom of, or delaying the progression of a disorder or disease comprising administering a therapeutically effective amount of the antibodies/AAs/BAAs/pharmaceutical compositions described above to a

subject in need thereof. In some embodiments, the disorder or disease comprises disease cells expressing EGFR. In some embodiments, the disorder or disease is cancer. In some embodiments, the cancer is anal cancer, basal cell carcinoma, brain cancer, bladder cancer, bone cancer, breast cancer, cervical cancer, cholangiocarcinoma, colorectal cancer, endometrial cancer, esophageal cancer, gall bladder cancer, gastric cancer, glioblastoma, head and neck cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, small bowel carcinoma, skin cancer, testicular cancer, thyroid cancer or uterine cancer. In some embodiments, the disorder is lymphoma, *e.g.* Epstein-Barr virus associated lymphoma, B-cell lymphoma, T-cell lymphoma Hodgkins and non-Hodgkins lymphoma. In some embodiments, the cancer is a squamous cell cancer. In some embodiments, the cancer is a head and neck squamous cell cancer. In some embodiments, the cancer is a skin squamous cell carcinoma. In some embodiments, the cancer is an esophageal squamous cell carcinoma. In some embodiments, the cancer is a head and neck squamous cell carcinoma. In some embodiments, the cancer is a lung squamous cell carcinoma.

[0026] In another aspect, provided herein is a method of inhibiting angiogenesis in a subject comprising administering a therapeutically effective amount of the antibodies/AAs/BAAs/pharmaceutical compositions described above to a subject in need thereof. In some embodiments, the method comprises administering an additional agent. In some embodiments, the additional agent is a therapeutic agent.

[0027] In another aspect, provided herein is a method to reduce damage to healthy tissue caused by an antibody binding to its target on healthy tissue as well as on diseased tissue (*e.g.* cancerous tissue), the method comprising administering to a subject in need thereof an AA or BAA or a pharmaceutical composition comprising an AA or BAA, wherein said AA or BAA is an AA or BAA of any one of the embodiments provided herein.

[0028] In another aspect, provided herein is a method to improve tolerability of an antibody treatment comprising administering to a subject in need thereof (*e.g.* a subject suffering from cancer) an AA or BAA or a pharmaceutical composition comprising an AA or BAA, wherein said AA or BAA is an AA or BAA of any one of the embodiments provided herein.

[0029] In another aspect provided herein is a method to recruit T cells to tumor tissue comprising administering to a subject in need thereof an AA or BAA or a pharmaceutical composition

comprising an AA or BAA, wherein said AA or BAA is an AA or BAA of any one of the embodiments provided herein.

[0030] In another aspect provided herein is an antibody, AA, BAA, or pharmaceutical composition, of any one of the embodiments provided herein, for use as a medicament. The medicament may be for use in a method of reducing damage to healthy tissue caused by an antibody binding to its target on healthy tissue as well as on diseased tissue. The medicament may be for use in improving the tolerability of an antibody treatment.

[0031] In another aspect provided herein is an antibody, AA, BAA, or pharmaceutical composition, of any one of the embodiments provided herein, for use in a method of treating, alleviating a symptom of, or delaying the progression of a disorder or disease, wherein the disorder or disease comprises disease cells expressing EGFR.

[0032] In another aspect provided herein is an antibody, AA, BAA, or pharmaceutical composition, of any one of the embodiments provided herein, for use in a method of treating cancer; optionally wherein the cancer is anal cancer, basal cell carcinoma, brain cancer, bladder cancer, breast cancer, bone cancer, cervical cancer, cholangiocarcinoma, colorectal cancer, endometrial cancer, esophageal cancer, gall bladder cancer, gastric cancer, glioblastoma, head and neck cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, small bowel carcinoma, squamous cell cancer, skin cancer testicular cancer, thyroid cancer or uterine cancer. The use may comprise the recruitment of T cells to tumor tissue.

[0033] In another aspect provided herein is an antibody, AA, BAA, or pharmaceutical composition, of any one of the embodiments provided herein, for use in a method comprising inhibiting angiogenesis.

[0034] The antibody, AA, BAA, or pharmaceutical composition, of any one of the embodiments provided herein, may be for use in a method of treatment comprising administering an additional agent; optionally wherein the additional agent is a therapeutic agent.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] FIG. 1A demonstrates that incorporation of the h20GG CD3 ϵ masking peptide into the EGFR masked BAAs CI106 and CI107 significantly reduced binding to Jurkat cells relative to

CI011. A reduction in binding to EGFR+ HT29-luc2 cells was also evident for CI106 and CI107 relative to CI011 (FIG. 1B).

[0036] FIG. 2A demonstrates that killing of EGFR+ HT29-luc2 cells was further attenuated by CI106 and CI107 relative to CI011 and CI040. FIG. 2B shows that no detectable cytotoxicity was observed when cells were treated with CI127 and CI128 demonstrating the dependence of EGFR targeting for cell killing. FIG. 2B also depicts a more than 300,000 fold EC50 shift of the dually masked antibodies (i.e., BAAs masked at both the EGFR and CD3 target binding domains) CI106 and CI107 relative to the protease activated bispecific antibody (i.e., BAA activated by protease treatment) act-104 (interchangeably referred to as CI104). FIG. 2C depicts the EGFR receptor number on a panel of cell lines that includes HT29. The approximate EGFR receptor number on HT29 cells was 75,000, indicating that high antigen density was not required for potent cytotoxicity of the tested BAAs.

[0037] FIG. 3A demonstrates that activation of primary CD8+ T cells was attenuated by CI106 and CI107, relative to CI011 and CI040. FIG. 3B demonstrates that dually masked antibodies display a shifted dose response curve for T cell activation relative to protease activated bispecific antibody act-104 indicating that masking attenuates T cell activation.

[0038] FIG. 4, which plots tumor volume versus days post initial treatment dose, demonstrates a dose-dependent effect of CI106 and CI107 dually masked, bispecific, AAs on the growth of HT29-luc2 xenograft tumors.

[0039] FIG. 5, which plots tumor volume versus days post initial treatment dose, demonstrated a dose-dependent effect of CI106 and CI107 dually masked, bispecific, AAs on the growth of HCT116 xenograft tumors.

[0040] FIGS. 6A-6B demonstrate that the EC50s of the tested dually masked and protease activated bispecific antibodies are similar when either human (6A) or cyno (6B) effector cells are used. FIGS. 6C-6D demonstrate that binding of the protease-activated and dually masked antibodies to human (6C) and cyno (6D) T cells is similar.

[0041] FIGS. 7A-7C depict pre-dose, 48 h, and 7 days post-dose serum concentrations of ALT (7A), AST (7B), and total bilirubin (7C) in cynomolgus monkeys treated with CI106 or CI107.

[0042] FIGS. 8A-8C plot the increase in serum cytokine levels for IL-2 (8A), IL-6 (8B), and IFN- γ (8C) in cynomolgus monkeys treated with CI106 or CI107.

[0043] FIGS. 9A-9C depict T cell activation as measured by CD69 (9A), Ki67 (9B), and PD-1 (9B) expression on CD4⁺ T cells in cynomolgus monkeys treated with CI106 or CI107.

[0044] FIGS. 10A-E plot dose dependent increases in AST at 48h post dose (10A), ALT at 48h post dose (10B), IL-6 at 8h post dose (10C), IFN γ at 8h post dose (10D), and Ki67 at 72h post dose (10E) in cynomolgus monkeys treated with act-104, CI106 or CI107. The dose response curve for all parameters was shifted for the dually masked antibodies indicating improved tolerability and decreased pharmacodynamics effects relative to the protease activated bispecific antibody.

[0045] FIGS. 11A-11C compare the effects of EGFR-binding CI107 and non-EGFR-binding CI128 (RSV $\times$ CD3) on increases in total bilirubin (11A), IL-6 (11B), and PD-1 expressing CD4⁺ T cells (11C) in cynomolgus monkeys treated with CI107 or CI128.

[0046] FIG. 12A depicts the affinity measurements of v12, v16, and v19 CD3 antibodies relative to hSP34. FIG. 12B depicts the cytotoxicity of activated or dually masked, bispecific antibodies on HT29-luc2 cells.

[0047] FIG. 13 depicts the extended PK of the dually masked antibody, CI107, relative to the protease activated bispecific antibody, act-104.

[0048] FIG. 14A depicts efficacy in a HT29-luc2 tumor intervention model in PBMC engrafted NSG mice. Anti-tumor potency in this example correlates to protease sensitivity and substrate cleavability of the test articles, with the most efficacious test article being the fully protease activated CI048. FIG. 14B depicts staining of tumor sections for CD3 (dark staining) as a measure of the degree of T cell infiltration into tumors. Tumor T cell infiltration correlates with protease sensitivity and substrate cleavability of the test articles.

[0049] FIG. 15 and FIG. 16 are plots of binding isotherms for activatable anti-EGFR C225v5 antibodies of the disclosure, for activatable anti-EGFR antibody 3954-2001-C225v5 described herein, and for anti-EGFR antibody C225v5.

[0050] FIGS. 17-19 illustrate exemplary BAAs provided herein.

[0051] FIG. 20 depicts PK of the dually masked BAA CI107 following a single dose administration of 600, 2000, or 4000 ug/kg.

[0052] FIG. 21 demonstrates that the cytotoxicity of CI090 and CI091 was attenuated relative to CI011, on HT29-luc2 cells.

[0053] FIG. 22 demonstrates that activation of primary CD8+ T cells was attenuated by CI090 and CI091 relative to CI011.

[0054] FIG. 23 depicts efficacy in a HT29-luc2 tumor intervention model in PBMC engrafted NSG mice. Showing anti-tumor potency of CI091, CI090 and CI011.

[0055] FIG. 24 plots IL-6 levels in cynomolgus monkeys *in vivo* study at 8h post dose.

DETAILED DESCRIPTION OF THE INVENTION

[0056] Provided herein are antibodies, activatable antibodies (AAs), bispecific antibodies, and bispecific activatable antibodies (BAAs).

[0057] In some embodiments, provided herein are humanized antibodies that specifically bind to the epsilon chain of CD3 (CD3ε; referred to herein interchangeably as CD3).

[0058] In some embodiments, provided herein are IgG1 antibodies that specifically bind to Epidermal Growth Factor Receptor (EGFR), wherein the antibodies comprise point mutations in the Fc region, such that the antibody has reduced effector function.

[0059] In some embodiments provided herein are AAs, for example AAs that specifically bind to EGFR or CD3. These AAs are optimized for affinity, effector function, masking, and cleavability.

[0060] In some embodiments, provided herein are BAAs, for example BAAs that bind to a target antigen (e.g. tumor antigen, such as a target presented in Table 9) and a second antigen (e.g. immune effector antigen on an immune effector cell). In some embodiments, the immune effector cell is a leukocyte cell. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is a natural killer (NK) cell. In some embodiments, the immune effector cell is a macrophage. In some embodiments, the immune effector cell is a mononuclear cell, such as a myeloid mononuclear cell. In some embodiments, the BAAs are immune effector cell-engaging BAAs. In some embodiments, the BAAs are leukocyte cell-engaging BAAs. In some embodiments, the BAAs are T cell engaging bispecific (TCB) AAs, also referred to herein as TCBAAs. In some embodiments, the BAAs are NK cell-engaging BAAs. In some embodiments, the BAAs are macrophage cell-engaging BAAs. In some embodiments, the BAAs are mononuclear cell-engaging BAAs, such as myeloid mononuclear cell-engaging BAAs. In some embodiments, the bispecific antibodies bind EGFR and CD3. These BAAs are optimized for affinity, effector function, masking, and cleavability.

[0061] Also provided herein are methods of making and methods of use of these antibodies, AAs, and BAAs. AAs, including general production thereof and identification of masking moieties (MMs) and cleavable moieties (CMs) is described in International Publication Numbers WO 2009/025846 by Daugherty et al., published 26 February 2009, and WO 2010/081173 by Stagliano et al., published 15 July 2010, both of which are incorporated by reference in their entirety. BAAs, including general production thereof and identification of masking moieties (MMs) and cleavable moieties (CMs) is described in International Publication Numbers WO2015/013671 by Lowman et al., published 29 January 2015 and WO2016/014974 by Irving et al., published 28 January 2016, both of which are incorporated by reference in their entirety. Also incorporated by reference are International Publication WO2016/014974 by Irving et al., published 28 January 2016, and International Publication WO2016/118629 by Moore et al., published 28 July 2016 which provide AAs, general production, MMs, and CMs.

[0062] As used herein, unless specified otherwise, the term “antibody” includes an antibody or antigen-binding fragment thereof that specifically binds its target and is a monoclonal antibody, domain antibody, single chain, Fab fragment, a F(ab')₂ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody. In some embodiments, the antibody is an IgG antibody. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG4 antibody. In some embodiments, the antibody is a scFv antibody. In some embodiments, such an antibody or immunologically active fragment thereof that binds its target is a mouse, chimeric, humanized or fully human monoclonal antibody.

1. CD3 Antibodies

[0063] Provided herein is an antibody or antigen binding fragment thereof (AB) that specifically binds to the epsilon chain of CD3 (CD3ε, referred to herein throughout as CD3).

[0064] Exemplary amino acid sequences of CD3-binding antibodies of the disclosure (variable domains) are provided in Table 1. (Predicted CDR sequences are underlined). As provided below, L3 is a linker, linking the light and heavy chain variable domains, in the exemplary CD3-binding antibodies.

Table 1
<u>Anti-CD3 variant v12</u>
<i>Light Chain Variable Domain LV12</i>
QTVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIGGTNKRAPG VPDRFSGSILGNKAALTITGAQADDES DY C <u>ALWYSNLWVFGGGTKLTVL</u> (SEQ ID NO: 1)
<i>Heavy Chain Variable Domain HV12, wherein L3 is SEQ ID NO: 98</i>
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYW GQGLTVTVSS (SEQ ID NO: 2)
<i>LV12-L3-HV12</i>
QTVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIGGTNKRAPG VPDRFSGSILGNKAALTITGAQADDES DY C <u>ALWYSNLWVFGGGTKLTVL</u> [GGGGSGGG GSGGGGS]EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVAR IRSKYNNYATYYADSVKDRFTISRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYV SWFAYWGQGLTVTVSS (SEQ ID NO: 143)
<u>Anti-CD3 variant v16</u>
<i>Light Chain Variable Domain LV12</i>
Sequence provided above
<i>Heavy Chain Variable Domain HV20</i>
EVQLVESGGGLVQPGGSLKLSAASGFTFSTYAMNWVRQASGKGLEWVGRIRSKYNNY ATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTR HGNFGNSYVSWFAYW GQGLTVTVSS (SEQ ID NO: 3)
<i>LV12-L3-HV20, wherein L3 is SEQ ID NO: 98</i>
QTVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIGGTNKRAPG VPDRFSGSILGNKAALTITGAQADDES DY C <u>ALWYSNLWVFGGGTKLTVL</u> [GGGGSGGG GSGGGGS]EVQLVESGGGLVQPGGSLKLSAASGFTFSTYAMNWVRQASGKGLEWVGR IRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTR HGNFGNSY VSWFAYWGQGLTVTVSS (SEQ ID NO: 144)
<u>Anti-CD3 variant v19</u>
<i>Light Chain Variable Domain LV19</i>
QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPG TPARFSGSLIGGKAALTLGAQPEDEAEYYC <u>ALWYSNLWVFGGGTKLTVL</u> (SEQ ID NO: 4)

<i>Heavy Chain Variable Domain HV20</i>
Sequence provided above
<i>LV19-L3-HV20, wherein L3 is SEQ ID NO: 98</i>
QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPG TPARFSGSLIGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL[GGGGSGGG GSGGGGS]EVQLVESGGGLVQPGGSLKLSCAASGFTFSTYAMNWVRQASGKGLEWVGR IRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRHGNGNSY VSWFAYWGQGTLLTVSS (SEQ ID NO: 145)
<u>Anti-CD3 variant v26</u>
<i>Light Chain Variable Domain LV19</i>
Sequence provided above
<i>Heavy Chain Variable Domain HV12</i>
Sequence provided above
<i>LV19-L3-HV12, wherein L3 is SEQ ID NO: 98</i>
QAVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPG TPARFSGSLIGGKAALTLGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL[GGGGSGGG GSGGGGS]EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVARI RSKYNNYATYYADSVKDRFTISRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNGNSYV SWFAYWGQGTLLTVSS (SEQ ID NO: 150)

[0065] Exemplary scFv linkers (referred to herein as “L3” linking a VH and VL) are provided in Table 1-1.

Table 1-1

SEQ ID NO:	Linker Amino Acid Sequence
98	GGGGSGGGSGGGGS

[0066] Exemplary CDR sequences of CD3-binding antibodies are provided in Table 2.

Table 2

Name	CD3 Ab CDR Sequences	SEQ ID NO:
SP34L1	RSSTGAVTTSNYAN	SEQ ID NO: 149
SP34L2	GTNKRAP	SEQ ID NO: 5
SP34L3	ALWYSNLWV	SEQ ID NO: 6

Name	CD3 Ab CDR Sequences	SEQ ID NO:
SP34H1	TYAMN	SEQ ID NO: 7
SP34H2	RIRSKYNNYATYYADSVKD	SEQ ID NO: 8
SP34H3	HGNFGNSYVSWFAY	SEQ ID NO: 9

[0067] As provided herein, the CD3 antibody comprises at least one of the CDR sequences provided in Table 2.

[0068] In some embodiments, the CD3 antibody comprises heavy chain variable domain as set forth in SEQ ID NO: 2.

[0069] In some embodiments, the CD3 antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 3.

[0070] In some embodiments, the CD3 antibody comprises a light chain variable domain as set forth in SEQ ID NO: 1.

[0071] In some embodiments, the CD3 antibody comprises a light chain variable domain as set forth in SEQ ID NO: 4.

[0072] In some embodiments, the CD3 antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain variable domain as set forth in SEQ ID NO: 1.

[0073] In some embodiments, the CD3 antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 1.

[0074] In some embodiments, the CD3 antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 4.

[0075] In some embodiments, the CD3 antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain variable domain as set forth in SEQ ID NO: 4.

[0076] In some embodiments, the CD3 antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or comprises a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4.

[0077] In some embodiments, the CD3 antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 and comprises a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4.

[0078] In some embodiments, the CD3 antibody is a scFv antibody. In some embodiments, the variable domains comprise the following structure from N terminus to C terminus: LV—HV. In

some embodiments, the variable domains comprise the following structure from N terminus to C terminus: HV—LV.

[0079] In some embodiments, the CD3 antibody is a scFv antibody comprising a heavy chain variable region (VH) linked to a light chain variable region (VL), wherein the VH is linked to the VL by a linker comprising amino acid sequence SEQ ID NO: 98. Exemplary sequences with such a linker are provided in Table 1.

[0080] In exemplary embodiments, provided herein is an antibody that specifically binds to CD3 (AB), wherein the antibody is an IgG1 antibody or a scFv linked to an Fc domain, wherein the antibody comprises an Fc region comprising an amino acid substitution in at least one of amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the antibody has reduced effector function. In some embodiments, the amino acid substitution is any one or more of L234F, L235E, and P331S. In some embodiments, the antibody comprises amino acid substitutions in at least two of amino acid positions L234, L235, and P331. In some embodiments, the antibody comprises amino acid substitutions at amino acid positions L234, L235, and P331. In some embodiments, the antibody comprises L234F, L235E, and P331S amino acid substitutions. In some embodiments, the antibody comprises an Fc region comprising an amino acid substitution at N297. In some embodiments, the Fc region comprises an N297Q mutation. In some embodiments, the antibody comprises L234F, L235E, P331S, and N297Q amino acid substitutions. In some embodiments, the heavy chain variable domain of the antibody comprises any one of SEQ ID NO: 2 or SEQ ID NO: 3 or wherein the light chain variable domain of the AB comprises any one of SEQ ID NO: 1 or SEQ ID NO: 4.

2. Activatable CD3 Antibodies

[0081] In some embodiments, any one of the CD3 antibodies provided herein is in an activatable antibody (AA) format.

[0082] As generally provided herein, the AAs of the invention comprise MM-CM constructs, also referred to herein as a prodomain. Accordingly, as used herein, the term “prodomain” refers to a polypeptide comprising a masking moiety (MM) and a cleavable moiety (CM). In some embodiments, the MM and the CM are separated by a linker, referred to herein as L1. In some embodiments, the prodomain comprises a linker at the carboxyl terminus of the CM; this linker,

referred to herein as L2, links the CM of the prodomain to the AB. In some embodiments, the prodomain comprises a linker between MM and CM and a linker after CM. In some embodiments, the MM and the CM are not separated by a linker. In certain embodiments a prodomain comprises one of the following formulae (where the formula below represents an amino acid sequence in either N- to C-terminal direction or C- to N-terminal direction): (MM)-L1-(CM), (MM)-(CM)-L2, (MM)-L1-(CM)-L2, or (MM)-(CM). In exemplary embodiments, a prodomain comprises an EGFR MM and a CM cleavable by a matriptase or MMP; or a CD3 ϵ MM and a CM cleavable by a matriptase or MMP. In some embodiments, a prodomain comprises an EGFR MM and a CM that is cleavable by a matriptase and an MMP. In some embodiments, a prodomain comprises a CD3 ϵ MM and a CM that is cleavable by a matriptase and an MMP. Provided herein are activatable antibodies (AAs) comprising a prodomain. Also provided herein are nucleotides encoding a prodomain of the invention.

[0083] Accordingly, provided herein is a CD3 AA comprising: (a) an antibody or antigen binding fragment thereof (AB) that specifically binds to the epsilon chain of CD3 (CD3 ϵ), wherein the antibody comprises a heavy chain domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or comprises a light chain domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4; (b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the CD3 ϵ when the AA is in an uncleaved state; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. As described above, (b) and (c) together are part of the prodomain.

[0084] In some embodiments, the AB of the CD3 AA is any one of the CD3 antibodies described in the preceding section.

[0085] In some embodiments, the AB of the CD3 AA comprises a heavy chain variable domain as set forth in SEQ ID NO: 2.

[0086] In some embodiments, the AB of the CD3 AA comprises a heavy chain variable domain as set forth in SEQ ID NO: 3.

[0087] In some embodiments, the AB of the CD3 AA comprises a light chain variable domain as set forth in SEQ ID NO: 1.

[0088] In some embodiments, the AB of the CD3 AA comprises a light chain variable domain as set forth in SEQ ID NO: 4.

[0089] In some embodiments, the AB of the CD3 AA comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain domain as set forth in SEQ ID NO: 1.

[0090] In some embodiments, the AB of the CD3 AA comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain domain as set forth in SEQ ID NO: 1.

[0091] In some embodiments, the AB of the CD3 AA comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain domain as set forth in SEQ ID NO: 4.

[0092] In some embodiments, the AB is a scFv comprising a heavy chain variable region (VH) linked to a light chain variable region (VL), wherein the VH is linked to the VL by a linker L3 comprising amino acid sequence SEQ ID NO: 98. Exemplary sequences with such a linker are provided in Table 1.

[0093] In some embodiments, the MM of the CD3 AA comprises any one of the sequences set forth in Table 3.

[0094] Exemplary CD3 masking moieties (MMs) of the invention are provided in Table 3.

[0095] In some embodiments, the MM of the CD3 AA comprises the sequence set forth in SEQ ID NO: 12. In some embodiments, the MM of the CD3 AA is the sequence set forth in SEQ ID NO: 10. In some embodiments, the MM of the CD3 AA is the sequence set forth in SEQ ID NO: 11.

Table 3

MM	Name	AA sequence	SEQ ID NO:
CD3 MM	JF15865	MMYCGGNEVLCGPRV	SEQ ID NO: 10
CD3 MM	JF15003	GYRWGCEWNCGGITT	SEQ ID NO: 11
CD3 MM	h20GG	GYLWGCEWNCGGITT	SEQ ID NO: 12

[0096] In some embodiments, the CM of the CD3 AA comprises any one of the sequences set forth in Table 4. Exemplary cleavable moieties (CMs) of the invention are provided in Table 4.

[0097] In some embodiments, the CM of an AA of the disclosure comprises any one of the sequences set forth in Table 4-1.

Table 4

CM Name	AA sequence	SEQ ID NO:
0001	LSGRSDNH	SEQ ID NO: 13
0011	LSGRSDDH	SEQ ID NO: 14
2001	ISSGLLSGRSDNH	SEQ ID NO: 15
2008	ISSGLLSGRSDQH	SEQ ID NO: 16
2006	ISSGLLSGRSDDH	SEQ ID NO: 17

Table 4-1

CM Name	AA sequence	SEQ ID NO:
0001	LSGRSDNH	SEQ ID NO: 18
0002	LSGRSGNH	SEQ ID NO: 19
0003	TSTSGRSANPRG	SEQ ID NO: 20
1001	ISSGLLSS	SEQ ID NO: 21
1002	QNQALRMA	SEQ ID NO: 22
1003	VHMP LGFLGP	SEQ ID NO: 23
1004	AVGLLAPP	SEQ ID NO: 24
0011	LSGRSDDH	SEQ ID NO: 25
0021	LSGRSDIH	SEQ ID NO: 26
0031	LSGRSDQH	SEQ ID NO: 27
0041	LSGRSDTH	SEQ ID NO: 28
0051	LSGRSDYH	SEQ ID NO: 29
0061	LSGRSDNP	SEQ ID NO: 30
0071	LSGRSANP	SEQ ID NO: 31
0081	LSGRSANI	SEQ ID NO: 32
0091	LSGRSDNI	SEQ ID NO: 33
2001	ISSGLLSGRSDNH	SEQ ID NO: 34
2002	ISSGLLSGRSGNH	SEQ ID NO: 35
2003	ISSGLLSGRSANPRG	SEQ ID NO: 36
2005	AVGLLAPPSGRSANPRG	SEQ ID NO: 37
2006	ISSGLLSGRSDDH	SEQ ID NO: 38
2007	ISSGLLSGRSDIH	SEQ ID NO: 39
2008	ISSGLLSGRSDQH	SEQ ID NO: 40
2009	ISSGLLSGRSDTH	SEQ ID NO: 41
2010	ISSGLLSGRSDYH	SEQ ID NO: 42
2011	ISSGLLSGRSDNP	SEQ ID NO: 43
2012	ISSGLLSGRSANP	SEQ ID NO: 44
2013	ISSGLLSGRSANI	SEQ ID NO: 45

CM Name	AA sequence	SEQ ID NO:
2014	ISSGLLSGRSDNI	SEQ ID NO: 46
3001	AVGLLAPPGGLSGRSDNH	SEQ ID NO: 47
3006	AVGLLAPPGGLSGRSDDH	SEQ ID NO: 48
3007	AVGLLAPPGGLSGRSDIH	SEQ ID NO: 49
3008	AVGLLAPPGGLSGRSDQH	SEQ ID NO: 50
3009	AVGLLAPPGGLSGRSDTH	SEQ ID NO: 51
3010	AVGLLAPPGGLSGRSDYH	SEQ ID NO: 52
3011	AVGLLAPPGGLSGRSDNP	SEQ ID NO: 53
3012	AVGLLAPPGGLSGRSANP	SEQ ID NO: 54
3013	AVGLLAPPGGLSGRSANI	SEQ ID NO: 55
3014	AVGLLAPPGGLSGRSDNI	SEQ ID NO: 56

3. Antibodies with Fc Mutations

[0098] Provided herein are IgG1 antibodies that have Fc mutations or antibody fragments containing antigen-binding domains (e.g. scFv, Fab, F(ab')₂) linked to a Fc domain, wherein the Fc exhibits reduced effector function (referred to herein as Fc variants). Any of the BAAs, AAs, and antibodies described herein may comprise any Fc variants disclosed herein.

[0100] The antibodies that comprise these Fc mutations result in reduced effector function, while maintaining target binding affinity. Accordingly, provided herein are antibodies that bind to a target of interest, wherein the antibody is an IgG1 antibody or an antibody fragment linked to an Fc, wherein the Fc region comprises an amino acid substitution in at least one of amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the antibody has reduced effector function. In some embodiments, the amino acid substitution is any one or more of L234F, L235E, and P331S. In some embodiments, there is an additional mutation in N297. In some embodiments, the amino acid substitution is N297Q or N297A.

[0101] In some embodiments, the Fc is selected from the Fc sequences presented in Table 4-2. In some embodiments, the Fc is selected from SEQ ID NO: 154, SEQ ID NO: 156, SEQ ID NO: 158, and SEQ ID NO: 160, wherein the X is selected from the group consisting of any naturally occurring amino acid (e.g. alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline,

pyrrolysine, selenocysteine, serine, threonine, tryptophan, tyrosine, valine) or any non-naturally occurring amino acid (e.g. trans-3-methylproline, 2,4-methanoproline, cis-4-hydroxyproline, trans-4-hydroxyproline, N-methylglycine, allo-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, tert-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine).

Table 4-2

Name	AA Sequence
SEQ ID NO:	
Fc-N297X (SEQ ID NO: 154)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYXSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Fc-N297Q (SEQ ID NO: 155)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Fc-L234X (SEQ ID NO: 156)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Fc-L234F (SEQ ID NO: 157)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE

Name	AA Sequence
SEQ ID NO:	
	EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
Fc-L235X (SEQ ID NO: 158)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLLVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELXGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
Fc-L235E (SEQ ID NO: 159)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Fc-P331X (SEQ ID NO: 160)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAXIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Fc-P331S (SEQ ID NO: 161)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Name	AA Sequence
SEQ ID NO:	
Fc-Fcmt3 (SEQ ID NO: 162)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
C225v5Fcmt 4 HC (SEQ ID NO: 163)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0102] Antibodies, AAs, bispecific antibodies, and BAAs comprising these Fc mutations are provided herein.

[0103] In some embodiments, such Fc variant-containing AAs and BAAs can bind an immune effector cell. In some embodiments, they can bind a target selectively located on an immune effector cell. In some embodiments, they can bind CD3. In some embodiments, they can bind any target listed in Table 9. In some embodiments, they can bind EGFR.

[0104] Accordingly, in some embodiments, provided herein is an activatable antibody (AA) comprising:

- an antibody (AB) that specifically binds a target, wherein the antibody is an IgG1 antibody, and wherein the Fc region of the antibody comprises an amino acid substitution in at least one of amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the AA has reduced effector function;
- a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the target when the AA is in an uncleaved state; and
- a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

[0105] In some embodiments, the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, N297 and P331, as numbered by the EU index as set forth in Kabat, such that the AA has reduced effector function. In some embodiments, the target is selected from the group consisting of the targets presented in Table 9.

[0106] In some embodiments, provided herein is a bispecific activatable antibody (BAA) comprising:

- a) an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises:
 - i. two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and
 - ii. a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein
the MM1 inhibits the binding of the AB1 to its target; and
the CM1 is a polypeptide that functions as a substrate for a first protease,
- b) two scFvs (AB2) that each specifically binds to a second target wherein each AB2 comprises:
 - i. a heavy chain variable region linked to a light chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and
 - ii. a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of a MM2-CM2 construct is linked to the amino terminus of each AB2 wherein
the MM2 inhibits the binding of the AB2 to its target; and
the CM2 is a polypeptide that functions as a substrate for a second protease,

[0107] and wherein the AB1 comprises an Fc region comprises an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0108] In some embodiments provided herein, the BAAs provided herein comprise:

- a) A bispecific activatable antibody (BAA) comprising:

- i) an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and wherein the AB1 is linked to a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein
- the MM1 inhibits the binding of the AB1 to its target; and
 - the CM1 is a polypeptide that functions as a substrate for a first protease,
- ii) two scFvs (each an AB2) that each specifically binds to a second target wherein each AB2 comprises a heavy chain variable region linked to a light chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and wherein each AB2 is linked to a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of each MM2-CM2 construct is linked to the amino terminus of each AB2 wherein
- the MM2 inhibits the binding of the AB2 to its target; and
 - the CM2 is a polypeptide that functions as a substrate for a second protease,
- [0109] and wherein the AB1 comprises an Fc region comprises an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

4. EGFR Antibodies

[0110] Provided herein are antibodies or antigen binding fragments thereof (AB) that specifically bind to EGFR. Exemplary CDR sequences of EGFR-binding antibodies are provided in Table 5.

[0111] Provided herein are EGFR antibodies, bispecific antibodies with one arm targeting EGFR, AAs capable of binding EGFR upon activation, and BAAs capable of binding EGFR upon activation. In some embodiments, the EGFR antibody comprises the CDRs of Table 5.

[0112] In some embodiments, *e.g.* in a BAA format, provided herein are IgG1 antibodies that specifically bind to the Epidermal Growth Factor Receptor (EGFR) and impart reduced effector function. The antibodies comprise Fc mutations that result in reduced effector function, while maintaining EGFR binding affinity. Accordingly, provided herein are antibodies that bind to

EGFR, wherein the antibody is an IgG1 antibody, wherein the antibody comprises an Fc region comprising an amino acid substitution in at least one of amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the antibody has reduced effector function. In some embodiments, the amino acid substitution is any one or more of L234F, L235E, and P331S.

[0113] In some embodiments, the antibody comprises amino acid substitutions in at least two of amino acid positions L234, L235, and P331.

[0114] In some embodiments, the antibody comprises amino acid substitutions at amino acid positions L234, L235, and P331.

[0115] In some embodiments, the antibody comprises L234F, L235E, and P331S amino acid substitutions.

[0116] In some embodiments, the antibody comprises an Fc region comprising an amino acid substitution at N297 along with an amino acid substitution in at least one of amino acid positions L234, L235, and/or P331. In some embodiments, the Fc region comprises an N297Q mutation. In some embodiments, the Fc region comprises an N297A mutation.

[0117] In some embodiments, the antibody comprises L234F, L235E, P331S and N297Q substitutions. In some embodiments, the antibody comprises L234F, L235E, P331S and N297A substitutions.

[0118] Exemplary CDR sequences of EGFR-binding antibodies are provided in Table 5, set forth in Kabat.

Table 5

Name	Sequence	SEQ ID NO:
C225L1	RASQSIGTNIH	SEQ ID NO: 57
C225L2	YASESIS	SEQ ID NO: 58
C225L3	QQNNNWPTT	SEQ ID NO: 59
C225H1	NYGVH	SEQ ID NO: 60
C225H2	VIWSGGNTDYNTPTS	SEQ ID NO: 61
C225H3	ALTYDYEFAY	SEQ ID NO: 62

[0119] Exemplary amino acid sequences of EGFR-binding antibodies are provided in Table 6. (VL and VH denote the variable light and variable heavy chains, respectively; LC and HC denote the light and heavy chains, respectively).

[0120] In some embodiments, the EGFR antibodies comprise any one of the sequences provided in Table 6.

[0121] In some embodiments, the heavy chain of the EGFR antibody comprises any one of the sequences set forth in SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 76, as set forth in Table 6. In some embodiments, the heavy chain EGFR antibody comprises any one of the sequences set forth in SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, and SEQ ID NO: 73, wherein in X is selected from any naturally occurring amino acid (e.g. alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, pyrrolysine, selenocysteine, serine, threonine, tryptophan, tyrosine, valine) or any non-naturally occurring amino acid (e.g., , trans-3-methylproline, 2,4-methanoproline, cis-4-hydroxyproline, trans-4-hydroxyproline, N-methylglycine, allo-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, tert-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine). The notation Fcmt3 comprises a triple point mutation, wherein the Fc region of the heavy chain of the EGFR antibody comprises the following three point mutations: L234F, L235E, and P331S. Accordingly, in some embodiments, the EGFR antibody comprises a heavy chain with an amino acid sequence set forth in SEQ ID NO: C225v5Fcmt3 HC. In some embodiments, the Fc region of the heavy chain of the EGFR antibody comprises a fourth point mutation, N297Q. The notation Fcmt4 comprises the Fcmt3 triple point mutation and the fourth point mutation, N297Q. Accordingly, in such embodiments, the EGFR antibody comprises a heavy chain with an amino acid sequence set forth in SEQ ID NO: 76.

Table 6

Name	AA Sequence
SEQ ID NO:	
C225v5-VL (SEQ ID NO: 63)	QILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNGSPRLLIKYAS ESISGIPSRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGTKL ELK
C225v5-VH (SEQ ID NO: 64)	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLLTVS(S/A)
C225v5 LC (SEQ ID NO: 65)	QILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNGSPRLLIKYAS ESISGIPSRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGTKL ELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNA LQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSS PVTKSFNRGEC
C225v5 HC (SEQ ID NO: 66)	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLLTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
C225v5N297 X HC (SEQ ID NO: 67)	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLLTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYXSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
C225v5N297 Q HC	QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLLTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC

Name	AA Sequence
SEQ ID NO:	
(SEQ ID NO: 68)	LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
C225v5 L234X HC (SEQ ID NO: 69)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEXLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK
C225v5 L234F HC (SEQ ID NO: 70)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFLGGPSVFLFP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
C225v5 L235X HC (SEQ ID NO: 71)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELXGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQ KSLSLSPGK

Name	AA Sequence
SEQ ID NO:	
C225v5 L235E HC (SEQ ID NO: 72)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELEGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQ KSLSLSPGK
C225v5 P331X HC (SEQ ID NO: 73)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAXIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQ KSLSLSPGK
C225v5 P331S HC (SEQ ID NO: 74)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQ KSLSLSPGK
C225v5Fcm1 3 HC (SEQ ID NO: 75)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEGGPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY

Name	AA Sequence
SEQ ID NO:	
	KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
C225v5Fcmt 4 HC (SEQ ID NO: 76)	QVQLKQSGPGLVQPSSQLSITCTVSGFSLTNYGVHWVRQSPGKGLEWLG VIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARAL TYYDYEFAYWGQGTLLTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLG TQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPP KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQ PREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKS LSLSPGK
SynFcmt4 HC (SEQ ID NO: 77)	QVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMSVGVIRQPPGKALEWL ADIWDDKKDYNPSLKSRLTISKDTSKNQVVLKVTNMDPADTATYYCA RSMITNWFYFDVWGAGTTVTVS(S/A)ASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSS LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEGGPSVFL FPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK

5. Activatable EGFR Antibodies

[0122] In some embodiments, any one of the EGFR antibodies provided herein are in an AA format (EGFR AAs). As described above for the CD3 AAs, the EGFR AAs also comprise a prodomain.

[0123] Accordingly provided herein are AAs comprising antibodies or antigen binding fragments thereof (AB) that specifically bind to EGFR. Exemplary CDR sequences of EGFR-binding antibodies are provided in Table 5.

[0124] In some embodiments, the AA comprises: (a) any antibody or an antigen binding fragment thereof (AB) that specifically binds to Epidermal Growth Factor Receptor (EGFR); (b) and a prodomain, wherein the prodomain comprises (i) a masking moiety (MM) coupled to the AB,

wherein the MM reduces or inhibits the binding of the AB to the EGFR when the AA is in an uncleaved state, and wherein the MM comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7; and (ii) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

[0125] Exemplary EGFR masking moieties (MMs) of the invention are provided in Table 7 and Table 8.

Table 7

MM	Name	AA sequence	SEQ ID NO:
EGFR MM	CF41	LSCEGWAMNREQCRA	SEQ ID NO: 78
EGFR MM	CF08	PPLECNTKSMCSKHD	SEQ ID NO: 79
EGFR MM	CF13	DRDCRGRRARCCQEG	SEQ ID NO: 80
EGFR MM	CF19	FTCEGWAMNREQCRT	SEQ ID NO: 81
EGFR MM	CF22	GRCPPSRDIFCTYM	SEQ ID NO: 82
EGFR MM	CF46	FSCEGWAMNRSQCRT	SEQ ID NO: 83
EGFR MM	CF48	FTCEGWAMNRDQCRT	SEQ ID NO: 84

Table 8

MM	Name	AA sequence	SEQ ID NO:
EGFR MM	3954	CISPRGCPDGPYVMY	SEQ ID NO: 85
EGFR MM	3954a	CISPRGCPDGPYVM	SEQ ID NO: 86
EGFR MM	3960	CISPRGC	SEQ ID NO: 87

[0126] In some embodiments, the MM of the EGFR AA comprises the amino acid sequence of SEQ ID NO: 78. In some embodiments, the MM of the EGFR AA comprises the amino acid sequence of SEQ ID NO: 85.

[0127] In some embodiments, the CM of the EGFR AA comprises an amino acid sequence selected from the group consisting of sequences presented in Table 4. In some embodiments, the CM comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the CM comprises the amino acid sequence of SEQ ID NO: 16.

[0128] In some embodiments, provided herein is an activatable antibody (AA) comprising: (a) an antibody that specifically binds to Epidermal Growth Factor Receptor (EGFR), wherein the

antibody is an IgG1 antibody, and wherein the Fc region of the antibody comprises an amino acid substitution in at least one of amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the AA has reduced effector function; (b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the EGFR when the AA is in an uncleaved state; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. The EGFR IgG1 antibodies can be any of the IgG1 antibodies described in the immediately preceding section. In some embodiments, the MM comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7.

[0129] In an exemplary embodiment, provided herein is an activatable antibody (AA) comprising: (a) an antibody (AB) that specifically binds to Epidermal Growth Factor Receptor (EGFR), wherein the AB is an IgG1 antibody, and wherein the Fc region of the AB comprises an amino acid substitution in at least one of amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the AA has reduced effector function; (b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the EGFR when the AA is in an uncleaved state; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease. In some embodiments, the amino acid substitution is any one or more of L234F, L235E, and P331S. In some embodiments, the AB comprises amino acid substitutions in at least two of amino acid positions L234, L235, and P331. In some embodiments, the AB comprises amino acid substitutions at amino acid positions L234, L235, and P331. In some embodiments, the AB comprises L234F, L235E, and P331S amino acid substitutions. In some embodiments, the AB comprises an Fc region comprising an amino acid substitution at N297. In some embodiments, the Fc region comprises an N297Q mutation. In some embodiments, the AB comprises L234F, L235E, P331S, and N297Q amino acid substitutions. In some embodiments, the MM comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7 or Table 8. In some embodiments, the MM comprises the amino acid sequence of SEQ ID NO: 78. In some embodiments, the MM comprises the amino acid sequence of SEQ ID NO: 85. In some embodiments, the CM comprises an amino acid sequence selected from the group consisting of sequences presented in Table 4. In some embodiments, the CM comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the CM

comprises the amino acid sequence of SEQ ID NO: 16. In some embodiments, the AA is part of a BAA.

6. Bispecific Activatable Antibodies (BAAs)

[0130] Provided herein are BAAs (bispecific AAs, BAAs), wherein said BAA, when activated, specifically binds to two targets (*e.g.* binds two different targets, or binds two different epitopes on the same target) and can comprise one of the exemplary structures provided in FIGS. 17-19.

[0131] In some embodiments, the first target is selected from the group consisting of the targets presented in Table 9 and the second target is selected from the group consisting of the target presented in Table 9.

[0132] As generally provided herein, and as described above in the section describing AAs, the BAAs of the invention comprise MM-CM constructs, also referred to herein as a prodomain. Accordingly, as used herein, the term “prodomain” refers to a polypeptide comprising a masking moiety (MM) and a cleavable moiety (CM). In some embodiments, the MM and the CM are separated by a linker, referred to herein as L1. In some embodiments, the prodomain comprises a linker at the carboxyl terminus of the CM; this linker, referred to herein as L2, links the CM of the prodomain to the AB. In some embodiments, the prodomain comprises a linker between MM and CM and a linker after CM. In some embodiments, the MM and the CM are not separated by a linker. In certain embodiments a prodomain comprises one of the following formulae (where the formula below represents an amino acid sequence in either N- to C-terminal direction or C- to N-terminal direction): (MM)-L1-(CM), (MM)-(CM)-L2, (MM)-L1-(CM)-L2, or (MM)-(CM). In exemplary embodiments, a prodomain comprises an EGFR MM and a CM cleavable by a matriptase or MMP; or a CD3ε MM and a CM cleavable by a matriptase or MMP. In some embodiments, a prodomain comprises an EGFR MM and a CM that is cleavable by a matriptase and an MMP. In some embodiments, a prodomain comprises a CD3ε MM and a CM that is cleavable by a matriptase and an MMP. Provided herein are bispecific activatable antibodies (BAAs) comprising a prodomain. Also provided herein are nucleotides encoding a prodomain of the invention.

[0133] In some embodiments, provided herein is a BAA, wherein said BAA, when activated, specifically binds to two targets (*e.g.* two different targets; or two different epitopes on the same target), and wherein said BAA, when not activated, comprises the following structure:

- a) an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises:
 - i. two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and
 - ii. a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein
 1. the MM1 inhibits the binding of the AB1 to its target; and
 2. the CM1 is a polypeptide that functions as a substrate for a first protease,
 - b) two scFvs (AB2) that each specifically bind to a second target wherein each AB2 comprises:
 - i. a heavy chain variable region linked to a light chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and
 - ii. a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of a MM2-CM2 construct is linked to the amino terminus of each AB2 wherein
 - the MM2 inhibits the binding of the AB2 to its target; and
 - the CM2 is a polypeptide that functions as a substrate for a second protease,
- and wherein the BAA has at least one of the following characteristics:
- i. MM2 comprises amino acid sequence SEQ ID NO: 12;
 - ii. MM1 comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7;
 - iii. AB2 comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4; and
 - iv. AB1 comprises an Fc region comprising an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331 or L234, L235 and P331, as

numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0134] In some embodiments, the BAAs provided herein comprise:

1. A bispecific activatable antibody (BAA), wherein said BAA, when activated, specifically binds to two targets and comprises the following structure:
 - a. an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and wherein the AB1 is linked to a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein
 - the MM1 inhibits the binding of the AB1 to its target; and
 - the CM1 is a polypeptide that functions as a substrate for a first protease,
 - b. two scFvs (each an AB2) that each specifically binds to a second target wherein each AB2 comprises a light chain variable region linked to a heavy chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and wherein each AB2 is linked to a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of each MM2-CM2 construct is linked to the amino terminus of each AB2 wherein
 - the MM2 inhibits the binding of the AB2 to its target; and
 - the CM2 is a polypeptide that functions as a substrate for a second protease,and wherein the BAA has at least one of the following characteristics:
 - i. MM2 comprises amino acid sequence SEQ ID NO: 12;
 - ii. MM1 comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7;
 - iii. AB2 comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4; and

- iv. AB1 comprises an Fc region comprising an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0135] In some embodiments, the BAAs provided herein comprise:

- a) an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises:
 - a. two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and
 - b. a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein
 - 1. the MM1 inhibits the binding of the AB1 to its target; and
 - 2. the CM1 is a polypeptide that functions as a substrate for a first protease,
- b) two scFvs (AB2) that each specifically binds to a second target wherein each AB2 comprises:
 - a. a heavy chain variable region linked to a light chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and
 - b. a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of a MM2-CM2 construct is linked to the amino terminus of each AB2 wherein
 - 1. the MM2 inhibits the binding of the AB2 to its target; and
 - 2. the CM2 is a polypeptide that functions as a substrate for a second protease,

and wherein the AB1 comprises an Fc region comprising an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function. In some embodiments, the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, N297 and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0136] In some embodiments, the BAAs provided herein comprise:

(1) A bispecific activatable antibody (BAA) comprising:

(a) an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and wherein the AB1 is linked to a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein

the MM1 inhibits the binding of the AB1 to its target; and

the CM1 is a polypeptide that functions as a substrate for a first protease,

(b) two scFvs (each an AB2) that each specifically binds to a second target wherein each AB2 comprises a heavy chain variable region linked to a light chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and wherein each AB2 is linked to a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of each MM2-CM2 construct is linked to the amino terminus of each AB2 wherein

the MM2 inhibits the binding of the AB2 to its target; and

the CM2 is a polypeptide that functions as a substrate for a second protease,

and wherein the AB1 comprises an Fc region comprises an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0137] As provided above, the BAAs of the invention comprise two scFvs (AB2) that each specifically binds to a second target. The VL and VH of the scFvs can be in any order, either VL-VH or VH-VL.

[0138] In some embodiments, the Fc region of the AB1 comprises amino acid substitutions in at least amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function. In some embodiments, the first target is

selected from the group consisting of the targets presented in Table 9 and the second target is selected from the group consisting of the targets presented in Table 9.

[0139] In some embodiments, AB1 binds a target antigen, *e.g.* a tumor antigen, and the AB2 binds an immune effector target.

[0140] In some embodiments, AB2 binds a target antigen, *e.g.* a tumor antigen, and the AB1 binds an immune effector target.

[0141] In some embodiments, the AB1 binds EGFR and the AB2 binds CD3.

[0142] In some embodiments, the MM1 comprises SEQ ID NO: 78.

[0143] In some embodiments, the MM2 comprises the amino acid sequence SEQ ID NO: 12.

[0144] In some embodiments, the bispecific AA is CI106, as provided in Table 11, in Example 1.

[0145] In some embodiments, the BAA is CI107, as provided in Table 11, in Example 1.

[0146] In some embodiments, the BAA is CI011, as provided in Table 11, in Example 1.

[0147] In some embodiments, the BAA is CI020, as provided in Table 11, in Example 1.

[0148] In some embodiments, the BAA is CI040, as provided in Table 11, in Example 1.

[0149] In some embodiments, the BAA is CI079, as provided in Table 11, in Example 1.

[0150] In some embodiments, the BAA is CI090, as provided in Table 11, in Example 1.

[0151] In an exemplary embodiment, AB1 comprises the amino acid sequence of C225v5Fcmt3 HC or C225v5Fcmt4 HC.

[0152] In some embodiments, the first and second proteases are the same protease. In some embodiments, the first and second proteases are different proteases. In some embodiments, CM1 and CM2 comprise the same amino acid sequence. In some embodiments, CM1 and CM2 comprise different amino acid sequences. In some embodiments, CM1 and CM2 comprise different amino acid sequences that are cleavable by the same protease or proteases. In some embodiments, CM1 and CM2 are cleavable by more than one protease. In some embodiments, CM1 and/or CM2 is cleavable by a serine protease. In some embodiments, CM1 and/or CM2 is cleavable by a matrix metalloproteinase (MMP). In some embodiments, CM1 and/or CM2 is cleavable by a serine protease and an MMP.

[0153] Exemplary BAAs of the disclosure include, for example, those shown in the Examples provided herein, and variants thereof.

[0154] In some non-limiting embodiments, at least one of the AB in the BAA is specific for CD3 and at least one other AB is a binding partner for any target listed in Table 9.

[0155] In an exemplary embodiment, AB2 of the BAA is specific for CD3 and AB1 is a binding partner for any target listed in Table 9.

Table 9: Exemplary Targets

1-92-LFA-3	CD52	DL44	HVEM	LAG-3	STEAP1
Alpha-4 integrin	CD56	DLK1	Hyaluronidase	LIF-R	STEAP2
Alpha-V integrin	CD64	DLL4	ICOS	Lewis X	TAG-72
alpha4beta1 integrin	CD70	DPP-4	IFNalpha	LIGHT	TAPA1
alpha4beta7 integrin	CD71	DSG1	IFNbeta	LRP4	TGFbeta
AGR2	CD74	EGFR	IFNgamma	LRRC26	TIGIT
Anti-Lewis-Y		EGFRviii	IgE	MCSP	TIM-3
Apelin J receptor	CD80	Endothelin B receptor (ETBR)	IgE Receptor (FcεRI)	Mesothelin	TLR2
APRIL	CD81	ENPP3	IGF	MRP4	TLR4
B7-H4	CD86	EpCAM	IGF1R	MUC1	TLR6
BAFF	CD95	EPHA2	IL1B	Mucin-16 (MUC16, CA-125)	TLR7
BTLA	CD117	EPHB2	IL1R	Na/K ATPase	TLR8
C5 complement	CD125	ERBB3	IL2	Neutrophil elastase	TLR9
C-242	CD132 (IL-2RG)	F protein of RSV	IL11	NGF	TMEM31
CA9	CD133	FAP	IL12	Nicestrin	TNFalpha
CA19-9 (Lewis a)	CD137	FGF-2	IL12p40	Notch Receptors	TNFR
Carbonic anhydrase 9	CD138	FGF8	IL-12R, IL-12Rbeta1	Notch 1	TNFRS12 A
CD2	CD166	FGFR1	IL13	Notch 2	TRAIL-R1
CD3	CD172A	FGFR2	IL13R	Notch 3	TRAIL-R2
CD6	CD248	FGFR3	IL15	Notch 4	Transferrin

CD9	CDH6	FGFR4	IL17	NOV	Transferrin receptor
CD11a	CEACAM5 (CEA)	Folate receptor	IL18	OSM-R	TRK-A
CD19	CEACAM6 (NCA-90)	GAL3ST1	IL21	OX-40	TRK-B
CD20	CLAUDIN-3	G-CSF	IL23	PAR2	uPAR
CD22	CLAUDIN-4	G-CSFR	IL23R	PDGF-AA	VAP1
CD24	cMet	GD2	IL27/IL27R (wsx1)	PDGF-BB	VCAM-1
CD25	Collagen	GITR	IL29	PDGFRalpha	VEGF
CD27	Cripto	GLUT1	IL-31R	PDGFRbeta	VEGF-A
CD28	CSFR	GLUT4	IL31/IL31R	PD-1	VEGF-B
CD30	CSFR-1	GM-CSF	IL2R	PD-L1	VEGF-C
CD33	CTLA-4	GM-CSFR	IL4	PD-L2	VEGF-D
CD38	CTGF	GP IIb/IIIa receptors	IL4R	Phosphatidyl-serine	VEGFR1
CD40	CXCL10	Gp130	IL6, IL6R	P1GF	VEGFR2
CD40L	CXCL13	GPIIB/IIIA	Insulin Receptor	PSCA	VEGFR3
CD41	CXCR1	GPNMB	Jagged Ligands	PSMA	VISTA
CD44	CXCR2	GRP78	Jagged 1	RAAG12	WISP-1
CD44v6		HER2/neu	Jagged 2	RAGE	WISP-2
CD47	CXCR4	HGF		SLC44A4	WISP-3
CD51	CYR61	hGH		Sphingosine 1 Phosphate	

[0156] In some embodiments, the unmasked EGFR-CD3 bispecific antibody exhibits EGFR-dependent tumor cell killing, while the doubly-masked EGFR-CD3 BAA reduces target-dependent cytotoxicity by more than 100,000-fold. In established tumor models where tumor-resident proteases are expected to be active, it is shown that BAAs potently induce tumor regressions. In non-human primates, the maximum tolerated dose (MTD) of the EGFR-CD3 BAA is more than 60-fold higher than the MTD of the unmasked bispecific antibody, and the tolerated exposure (AUC) is more than 10,000-fold higher. Despite the 60-fold dose differential at the MTDs, transient serum cytokine and AST/ALT increases observed in non-human primates treated with the BAA are still lower than those induced by the bispecific antibody. By localizing activity to the tumor

microenvironment, BAAs have the potential to expand clinical opportunities for T cell-engaging bispecific therapies that are limited by on target toxicities, especially in solid tumors. Moreover, an EGFR-CD3 BAA has the potential to address EGFR-expressing tumors that are poorly responsive to existing EGFR-directed therapies.

7. Cleavable Moieties (CM)

[0157] Both the monospecific AAs and the BAAs of the disclosure comprise at least one CM, when masked and not activated.

[0158] In some embodiments, the cleavable moiety (CM) of the AA or BAA includes an amino acid sequence that can serve as a substrate for at least one protease, usually an extracellular protease. In the case of a BAA, the CM may be selected based on a protease that is co-localized in tissue with the desired target of at least one AB of the BAA or AA. A CM can serve as a substrate for multiple proteases, *e.g.* a substrate for a serine protease and a second different protease, *e.g.* an MMP. In some embodiments, a CM can serve as a substrate for more than one serine protease, *e.g.*, a matriptase and uPA. In some embodiments, a CM can serve as a substrate for more than one MMP, *e.g.*, an MMP9 and an MMP14.

[0159] A variety of different conditions are known in which a target of interest is co-localized with a protease, where the substrate of the protease is known in the art. In the example of cancer, the target tissue can be a cancerous tissue, particularly cancerous tissue of a solid tumor. There are reports in the literature of increased levels of proteases in a number of cancers, *e.g.*, liquid tumors or solid tumors. See, *e.g.*, La Rocca et al, (2004) British J. of Cancer 90(7): 1414-1421. Non-limiting examples of disease include: all types of cancers, (such as, but not limited to breast, lung, colorectal, gastric, glioblastoma, ovarian, endometrial, renal, sarcoma, skin cancer, cervical, liver, bladder, cholangiocarcinoma, prostate, melanomas, head and neck cancer (*e.g.* head and neck squamous cell cancer, pancreatic, etc.), rheumatoid arthritis, Crohn's disease, SLE, cardiovascular damage, ischemia, etc. For example, indications would include leukemias, including T-cell acute lymphoblastic leukemia (T-ALL), lymphoblastic diseases including multiple myeloma, and solid tumors, including lung, colorectal, prostate, pancreatic and breast, including triple negative breast cancer. For example, indications include bone disease or metastasis in cancer, regardless of primary tumor origin; breast cancer, including by way of non-limiting example, ER/PR+ breast cancer,

Her2+ breast cancer, triple-negative breast cancer; colorectal cancer; endometrial cancer; gastric cancer; glioblastoma; head and neck cancer, such as head and neck squamous cell cancer; esophageal cancer; lung cancer, such as by way of non-limiting example, non-small cell lung cancer; multiple myeloma ovarian cancer; pancreatic cancer; prostate cancer; sarcoma, such as osteosarcoma; renal cancer, such as by way of non-limiting example, renal cell carcinoma; and/or skin cancer, such as by way of non-limiting example, squamous cell cancer, basal cell carcinoma, or melanoma. In some embodiments, the cancer is a squamous cell cancer. In some embodiments, the cancer is a skin squamous cell carcinoma. In some embodiments, the cancer is an esophageal squamous cell carcinoma. In some embodiments, the cancer is a head and neck squamous cell carcinoma. In some embodiments, the cancer is a lung squamous cell carcinoma.

[0160] The CM is specifically cleaved by an enzyme at a rate of about $0.001\text{--}1500 \times 10^4 \text{ M}^{-1}\text{S}^{-1}$ or at least 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 50, 75, 100, 125, 150, 200, 250, 500, 750, 1000, 1250, or $1500 \times 10^4 \text{ M}^{-1}\text{S}^{-1}$.

[0161] For specific cleavage by an enzyme, contact between the enzyme and CM is made. When the AA or BAA comprises at least a first AB coupled to a MM and a CM, *e.g.*, the AA comprises an AB coupled to a MM via a CM, is in the presence of target and sufficient enzyme activity, the CM can be cleaved. Sufficient enzyme activity can refer to the ability of the enzyme to make contact with the CM and effect cleavage. It can readily be envisioned that an enzyme may be in the vicinity of the CM but is unable to cleave because of other cellular factors or protein modification of the enzyme.

[0162] Exemplary CMs of the disclosure are provided in Table 4 above. In some embodiments, the CM has a length of up to 15 amino acids, a length of up to 20 amino acids, a length of up to 25 amino acids, a length of up to 30 amino acids, a length of up to 35 amino acids, a length of up to 40 amino acids, a length of up to 45 amino acids, a length of up to 50 amino acids, a length of up to 60 amino acids, a length in the range of 10-60 amino acids, a length in the range of 15-60 amino acids, a length in the range of 20-60 amino acids, a length in the range of 25-60 amino acids, a length in the range of 30-60 amino acids, a length in the range of 35-60 amino acids, a length in the range of 40-50 amino acids, a length in the range of 45-60 amino acids, a length in the range of 10-40 amino acids, a length in the range of 15-40 amino acids, a length in the range of 20-40 amino acids, a length in the range of 25-40 amino acids, a length in the range of 30-40 amino acids, a length in the

range of 35-40 amino acids, a length in the range of 10-30 amino acids, a length in the range of 15-30 amino acids, a length in the range of 20-30 amino acids, a length in the range of 25-30 amino acids, a length in the range of 10-20 amino acids, or a length in the range of 10-15 amino acids.

8. Masking Moieties (MMs)

[0163] In both the activatable monospecific CD3 and EGFR AAs and the BAAs described above, the AAs/BAAs contain a MM. As described herein, the AAs and BAAs of the invention comprise a prodomain, which comprises a MM.

[0164] In some embodiments, the MM is selected for use with a specific antibody or antibody fragment.

[0165] In certain embodiments, the MM is not a natural binding partner of the AB. In some embodiments, the MM contains no or substantially no homology to any natural binding partner of the AB. In other embodiments the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% similar to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 25% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 20% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 10% identical to any natural binding partner of the AB.

[0166] Exemplary MMs of the disclosure can have a length of up to 15 amino acids, a length of up to 20 amino acids, a length of up to 25 amino acids, a length of up to 30 amino acids, a length of up to 35 amino acids, a length of up to 40 amino acids, a length of up to 45 amino acids, a length of up to 50 amino acids, a length of up to 60 amino acids, a length in the range of 10-60 amino acids, a length in the range of 15-60 amino acids, a length in the range of 20-60 amino acids, a length in the range of 25-60 amino acids, a length in the range of 30-60 amino acids, a length in the range of 35-60 amino acids, a length in the range of 40-50 amino acids, a length in the range of 45-60 amino acids, a length in the range of 10-40 amino acids, a length in the range of 15-40 amino acids, a length in the range of 20-40 amino acids, a length in the range of 25-40 amino acids, a length in the

range of 30-40 amino acids, a length in the range of 35-40 amino acids, a length in the range of 10-30 amino acids, a length in the range of 15-30 amino acids, a length in the range of 20-30 amino acids, a length in the range of 25-30 amino acids, a length in the range of 10-20 amino acids, a length in the range of 10-15 amino acids, or a length of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids.

[0167] As provided herein, the MM inhibits the binding of the AB to the target. The MM binds the antigen binding domain of the AB and inhibits binding of the AB to the target. The MM can sterically inhibit the binding of the AB to the target. The MM can allosterically inhibit the binding of the AB to its target. In these embodiments when the AB is modified by or coupled to a MM and in the presence of target there is no binding or substantially no binding of the AB to the target, or no more than 0.001%, 0.01%, 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, or 50% binding of the AB to the target, as compared to the binding of the AB not modified by or coupled to an MM, the parental AB, or the AB not coupled to an MM to the target, for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours, or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or longer when measured *in vivo* or in an *in vitro* assay.

[0168] When an AB is coupled to or modified with a MM, the MM ‘masks’ or reduces or otherwise inhibits the specific binding of the AB to the target. When an AB is coupled to or modified by a MM, such coupling or modification can effect a structural change that reduces or inhibits the ability of the AB to specifically bind its target.

[0169] Exemplary MMs of the disclosure are provided in Tables 3, 7, and 8, above.

[0170] In any of the AAs and BAAs provided herein, the masked AB has a lower binding affinity than unmasked AB.

9. Linkers

[0171] In many embodiments, it may be desirable to insert one or more linkers, *e.g.*, flexible linkers, into the AA/BAA constructs so as to provide for flexibility at one or more of the MM-CM junction, the CM-AB/CM-scFv junction, or both. For example, the AB, MM, and/or CM may not contain a sufficient number of residues (*e.g.*, Gly, Ser, Asp, Asn, especially Gly and Ser) to provide the desired flexibility. As such, the ability of such BAA constructs to remain intact (not activated)

or be activated as disclosed herein may benefit from introduction of one or more amino acids to provide for a flexible linker.

[0172] For example, in certain embodiments an AA comprises one of the following formulae (where the formula below represents an amino acid sequence in either N- to C-terminal direction or C- to N-terminal direction):

(MM1)-L1-(CM1)-(AB1)
 (MM1)-(CM1)-L2-(AB1)
 (MM1)-L1-(CM1)-L2-(AB1)
 (MM2)-L1-(CM2)-(AB2)
 (MM2)-(CM2)-L2-(AB2)
 (MM2)-L1-(CM2)-L2-(AB2)

wherein MM, CM, and AB are as defined above; wherein L1 and L2 are each independently and optionally present or absent, are the same or different flexible linkers that include at least 1 flexible amino acid (*e.g.*, Gly, Ser).

[0173] In some embodiments, the BAA comprises 2 heavy chains, each comprising the structural arrangement from N-terminus to C-terminus of MM2-CM2-AB2-AB1 HC and two light chains each comprising the structural arrangement from N-terminus to C-terminus of MM1-CM1-AB1 LC.

[0174] In some embodiments, the structure including with linkers is provided in FIG. 17.

[0175] In some embodiments, (MM2)-L1-(CM2)-L2-(AB2) is linked to the heavy chain of AB1 and AB2 is a scFv.

[0176] Linkers suitable for use in compositions described herein are generally ones that provide flexibility of the modified AB or the AAs to facilitate the inhibition of the binding of the AB to the target. Such linkers are generally referred to as flexible linkers. Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (*e.g.*, Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length. In some embodiments, a suitable linker can be from 4 to 25 amino acids in length. In some embodiments, a suitable linker can be from 5 to 25 amino

acids in length. In some embodiments, a suitable linker can be from 4 to 20 amino acids in length.

In some embodiments, a suitable linker can be from 5 to 20 amino acids in length.

[0177] Exemplary linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, (GSGGS)_n (SEQ ID NO: 88) and (GGGS)_n (SEQ ID NO: 89), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. In some embodiments, n is from about 1 to about 10, or from about 1 to about 9, or from about 1 to about 8, or from about 1 to about 7, or from about 1 to about 6, or from about 1 to about 5, or from about 1 to about 4, or from about 1 to about 3, or from about 1 to about 2.

Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, Rev. Computational Chem. 11173-142 (1992)). Exemplary linkers are provided in Table 9-1.

Table 9-1: Exemplary L1 and L2 Linkers

SEQ ID NO:	Linker Amino Acid Sequence
SEQ ID NO: 88	GSGGS
SEQ ID NO: 89	GGGS
SEQ ID NO: 90	GGSG
SEQ ID NO: 91	GGSGG
SEQ ID NO: 92	GSGSG
SEQ ID NO: 93	GSGGG
SEQ ID NO: 94	GGGSG
SEQ ID NO: 95	GSSSG
SEQ ID NO: 96	GSSGSGGSGG
SEQ ID NO: 97	GGGS
SEQ ID NO: 99	GGGGS
SEQ ID NO: 100	GSSGSGGSGGSG
SEQ ID NO: 101	GSSGSGGSGGGGSGGGSGGS
SEQ ID NO: 102	GSSGSGGSGGSGGGSGGGSGGS
SEQ ID NO: 103	GSSGT
SEQ ID NO: 104	GGGSSGGS

[0178] The ordinarily skilled artisan will recognize that design of an AA can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure to provide for a desired AA structure.

10. Conjugation

[0179] In some embodiments, any of the antibodies, or ABs of the AAs, and BAAs disclosed herein may be conjugated to an agent. In some embodiments, the agent is a therapeutic agent. In some embodiments, the agent is a detectable moiety. In some embodiments, the agent is an antineoplastic agent. In some embodiments, the agent is a toxin or fragment thereof. In some embodiments, the agent is conjugated to the AB via a linker. In some embodiments, the linker is a non-cleavable linker. In some embodiments, the agent is a microtubule inhibitor. In some embodiments, the agent is a nucleic acid damaging agent, such as a DNA alkylator or DNA intercalator, or other DNA damaging agent. In some embodiments, the linker is a cleavable linker. In some embodiments, the agent is an agent selected from the group listed in Table 10.

Table 10: Exemplary Pharmaceutical Agents for Conjugation

<u>CYTOTOXIC AGENTS</u>	
Auristatins	Turbostatin
Auristatin E	Phenstatins
Monomethyl auristatin D (MMAD)	Hydroxyphenstatin
Monomethyl auristatin E (MMAE)	Spongistatin 5
Desmethyl auristatin E (DMAE)	Spongistatin 7
Auristatin F	Halistatin 1
Monomethyl auristatin F (MMAF)	Halistatin 2
Desmethyl auristatin F (DMAF)	Halistatin 3
Auristatin derivatives, <i>e.g.</i> , amides thereof	Modified Bryostatins
Auristatin tyramine	Halocomstatins
Auristatin quinoline	Pyrrolobenzimidazoles
Dolastatins	Cibrostatin6
Dolastatin derivatives	Doxaliform
Dolastatin 16 DmJ	Anthracyclins analogues
Dolastatin 16 Dpv	

Maytansinoids, e.g. DM-1; DM-4	
Maytansinoid derivatives	Cemadotin analogue (CemCH ₂ -SH)
Duocarmycin	Pseudomonas toxin A (PE38) variant
Duocarmycin derivatives	Pseudomonas toxin A (ZZ-PE38) variant
Alpha-amanitin	ZJ-101
Anthracyclines	OSW-1
Doxorubicin	4-Nitrobenzyloxycarbonyl Derivatives of O6-Benzylguanine
	Topoisomerase inhibitors
Daunorubicin	Hemiasterlin
Bryostatins	Cephalotaxine
Camptothecin	Homoharringtonine
Camptothecin derivatives	Pyrrolobenzodiazepine dimers (PBDs)
7-substituted Camptothecin	Functionalized pyrrolobenzodiazepenes
10, 11- Difluoromethylenedioxcamptothecin	
Combretastatins	Calicheamicins
Debromoaplysiatoxin	Podophyllotoxins
Kahalalide-F	Taxanes
Discodermolide	Vinca alkaloids
Ecteinascidins	
	<u>CONJUGATABLE DETECTABLE MOIETIES</u>
<u>ANTIVIRALS</u>	Fluorescein and derivatives thereof
Acyclovir	Fluorescein isothiocyanate (FITC)
Vira A	
Symmetrel	<u>RADIOPHARMACEUTICALS</u>
	¹²⁵ I
<u>ANTIFUNGALS</u>	¹³¹ I
Nystatin	⁸⁹ Zr
	¹¹¹ In
<u>ADDITIONAL ANTI-NEOPLASTICS</u>	¹²³ I
Adriamycin	¹³¹ I
Cerubidine	^{99m} Tc
Bleomycin	²⁰¹ Tl
Alkeran	¹³³ Xe

Velban	¹¹ C
Oncovin	⁶² Cu
Fluorouracil	¹⁸ F
Methotrexate	⁶⁸ Ga
Thiotepa	¹³ N
Bisantrene	¹⁵ O
Novantrone	³⁸ K
Thioguanine	⁸² Rb
Procarabazine	^{99m} Tc (Technetium)
Cytarabine	
<u>ANTI-BACTERIALS</u>	<u>HEAVY METALS</u>
Aminoglycosides	Barium
Streptomycin	Gold
Neomycin	Platinum
Kanamycin	
Amikacin	<u>ANTI-MYCOPLASMA</u>
Gentamicin	Tylosine
Tobramycin	Spectinomycin
Streptomycin B	
Spectinomycin	
Ampicillin	
Sulfanilamide	
Polymyxin	
Chloramphenicol	

[0180] Those of ordinary skill in the art will recognize that a large variety of possible moieties can be coupled to the resultant antibodies, AAs, and BAAs of the disclosure. (*See, for example*, “Conjugate Vaccines”, Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr (eds), Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference).

[0181] In some embodiments, the antibody, AA or BAA comprises a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent.

[0182] In some embodiments, the antibody, AA or BAA contains one or more disulfide bonds. In some embodiments, the antibody, AA or BAA contains one or more lysines. In some embodiments, the antibody, AA or BAA can be engineered to include one or more disulfide bonds or can be otherwise engineered to enable site-specific conjugation.

11. Production

[0183] The disclosure also provides an isolated nucleic acid molecule encoding an antibody, AA or BAA described herein, as well as vectors that include these isolated nucleic acid sequences. The disclosure provides methods of producing an antibody, AA or BAA by culturing a cell under conditions that lead to expression of the antibody, AA or BAA, wherein the cell comprises such a nucleic acid molecule.

[0184] In some embodiments, the cell comprises such a vector. In some embodiments, the vector is pLW289. In some embodiments, the vector is pLW246. In some embodiments, the vector is pLW307. In some embodiments, the vector is pLW291. In some embodiments, the vector is pLW352. In some embodiments, the vector is pLW353. (these vectors and described and sequences provided below in Example 1)

12. Use of Antibodies, AAs, Bispecific Antibodies and BAAs

[0185] In some embodiments, the antibodies/bispecific antibodies/AAs/BAAs thereof may be used as therapeutic agents. Such agents will generally be employed to treat, alleviate, and/or prevent a disease or pathology in a subject. A therapeutic regimen is carried out by identifying a subject, *e.g.*, a human patient or other mammal suffering from (or at risk of developing) a disorder using standard methods.

[0186] Administration of the antibodies/bispecific antibodies/AAs/BAAs thereof may abrogate or inhibit or interfere with the signaling function of one or more of the targets.

[0187] It will be appreciated that administration of therapeutic entities in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA

(1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present disclosure, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci. 89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[0188] Generally, alleviation or treatment of a disease or disorder involves the lessening of one or more symptoms or medical problems associated with the disease or disorder. For example, in the case of cancer, the therapeutically effective amount of the drug can accomplish one or a combination of the following: reduce the number of cancer cells; reduce the tumor size; inhibit (*i.e.*, to decrease to some extent and/or stop) cancer cell infiltration into peripheral organs; inhibit tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. In some embodiments, a composition of this disclosure can be used to prevent the onset or reoccurrence of the disease or disorder in a subject, *e.g.*, a human or other mammal, such as a non-human primate, companion animal (*e.g.*, cat, dog, horse), farm animal, work animal, or zoo animal. The terms subject and patient are used interchangeably herein.

[0189] A therapeutically effective amount of antibodies/bispecific antibodies/AAs/BAAs thereof of the disclosure relates generally to the amount needed to achieve a therapeutic objective.

[0190] Common ranges for therapeutically effective dosing of an antibodies/bispecific antibodies/AAs/BAAs thereof of the disclosure may be, by way of nonlimiting example, from about

0.1 mg/kg body weight to about 50 mg/kg body weight. Common dosing frequencies may range, for example, from twice daily to once a week.

[0191] Efficaciousness of treatment is determined in association with any known method for diagnosing or treating the particular disorder. Methods for the screening antibodies/bispecific antibodies/AAs/BAAs that possess the desired specificity include, but are not limited to, enzyme linked immunosorbent assay (ELISA) and other immunologically mediated techniques known within the art.

[0192] Other contemplated uses involve diagnostics, imaging, prognostics, and detection uses. In some embodiments, antibodies/bispecific antibodies/AAs/BAAs are used in methods known within the art relating to the localization and/or quantitation of the target (*e.g.*, for use in measuring levels of one or more of the targets within appropriate physiological samples, for use in diagnostic methods, for use in imaging the protein, and the like).

[0193] In some embodiments, antibodies/bispecific antibodies/AAs/BAAs are used to isolate one or more of the targets by standard techniques, such as immunoaffinity, chromatography or immunoprecipitation. An antibody, an AA, a bispecific antibody or a BAA can be used diagnostically to monitor protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling (*i.e.*, physically linking) the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

[0194] In yet another embodiment, an antibody, bispecific antibody, AA, BAA directed two or more targets can be used as an agent for detecting the presence of one or more of the targets (or a fragment thereof) in a sample. In some embodiments, the antibody contains a detectable label. Antibodies are polyclonal, or in some embodiments, monoclonal. An intact antibody, or a fragment

thereof (*e.g.*, Fab, scFv, or F(ab')₂) is used. The term “labeled”, with regard to the probe or antibody, is intended to encompass direct labeling of the probe or antibody by coupling (*i.e.*, physically linking) a detectable substance to the probe or antibody, as well as indirect labeling of the probe or antibody by reactivity with another reagent that is directly labeled. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of an antibody with biotin such that it can be detected with fluorescently-labeled streptavidin. The term “biological sample” is intended to include tissues, cells and biological fluids isolated from a subject, as well as tissues, cells and fluids present within a subject. Included within the usage of the term “biological sample”, therefore, is blood and a fraction or component of blood including blood serum, blood plasma, or lymph. That is, the detection method of the disclosure can be used to detect a protein in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of an analyte protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, and immunofluorescence. Procedures for conducting immunoassays are described, for example in “ELISA: Theory and Practice: Methods in Molecular Biology”, Vol. 42, J. R. Crowther (Ed.) Human Press, Totowa, NJ, 1995; “Immunoassay”, E. Diamandis and T. Christopoulos, Academic Press, Inc., San Diego, CA, 1996; and “Practice and Theory of Enzyme Immunoassays”, P. Tijssen, Elsevier Science Publishers, Amsterdam, 1985. Furthermore, *in vivo* techniques for detection of an analyte protein include introducing into a subject a labeled anti-analyte protein antibody. For example, the antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard imaging techniques.

[0195] The antibodies, bispecific antibodies, AAs, and bispecific antibodies of the disclosure are also useful in a variety of diagnostic and prophylactic formulations. In one embodiment, an antibody, AA, bispecific antibody, BAA is administered to patients that are at risk of developing one or more of the aforementioned disorders. A patient's or organ's predisposition to one or more of the disorders can be determined using genotypic, serological or biochemical markers.

[0196] In another embodiment of the disclosure, an antibody, AA, bispecific antibody, BAA is administered to human individuals diagnosed with a clinical indication associated with one or more of the aforementioned disorders. Upon diagnosis, an antibody, AA, bispecific antibody, BAA is administered to mitigate or reverse the effects of the clinical indication.

[0197] Antibodies, bispecific antibodies, AAs, and bispecific antibodies are also useful in the detection of one or more targets in patient samples and accordingly are useful as diagnostics. For example, the antibodies, bispecific antibodies, AAs, and bispecific antibodies of the disclosure are used in *in vitro* assays, *e.g.*, ELISA, to detect one or more target levels in a patient sample.

[0198] In one embodiment, an antibody, AA, bispecific antibody, BAA is immobilized on a solid support (*e.g.*, the well(s) of a microtiter plate). The immobilized antibody and/or AA serves as a capture antibody for any target(s) that may be present in a test sample. Prior to contacting the immobilized antibody/AA with a patient sample, the solid support is rinsed and treated with a blocking agent such as milk protein or albumin to prevent nonspecific adsorption of the analyte.

[0199] Subsequently the wells are treated with a test sample suspected of containing the antigen, or with a solution containing a standard amount of the antigen. Such a sample is, *e.g.*, a serum sample from a subject suspected of having levels of circulating antigen considered to be diagnostic of a pathology. After rinsing away the test sample or standard, the solid support is treated with a second antibody that is detectably labeled. The labeled second antibody serves as a detecting antibody. The level of detectable label is measured, and the concentration of target antigen(s) in the test sample is determined by comparison with a standard curve developed from the standard samples.

[0200] It will be appreciated that based on the results obtained using the antibody, AA, bispecific antibody, BAA in an *in vitro* diagnostic assay, it is possible to stage a disease in a subject based on expression levels of the target antigen(s). For a given disease, samples of blood are taken from subjects diagnosed as being at various stages in the progression of the disease, and/or at various points in the therapeutic treatment of the disease. Using a population of samples that provides statistically significant results for each stage of progression or therapy, a range of concentrations of the antigen that may be considered characteristic of each stage is designated.

[0201] Antibodies, bispecific antibodies, AAs, and BAAs can also be used in diagnostic and/or imaging methods. In some embodiments, such methods are *in vitro* methods. In some embodiments, such methods are *in vivo* methods. In some embodiments, such methods are *in situ* methods. In some embodiments, such methods are *ex vivo* methods. For example, AAs, and bispecific antibodies having an enzymatically cleavable CM can be used to detect the presence or absence of an enzyme that is capable of cleaving the CM. Such AAs, and bispecific antibodies can

be used in diagnostics, which can include *in vivo* detection (*e.g.*, qualitative or quantitative) of enzyme activity (or, in some embodiments, an environment of increased reduction potential such as that which can provide for reduction of a disulfide bond) through measured accumulation of activated or bispecific activated antibodies (*i.e.*, antibodies or bispecific antibodies resulting from cleavage of an AA or a BAA) in a given cell or tissue of a given host organism. Such accumulation of activated bispecific antibodies indicates not only that the tissue expresses enzymatic activity (or an increased reduction potential depending on the nature of the CM) but also that the tissue expresses at least one target to which the activated bispecific antibody binds.

[0202] For example, the CM can be selected to be a protease substrate for a protease found at the site of a tumor, at the site of a viral or bacterial infection at a biologically confined site (*e.g.*, such as in an abscess, in an organ, and the like), and the like. At least one of the AB can be one that binds a target antigen. Using methods familiar to one skilled in the art, a detectable label (*e.g.*, a fluorescent label or radioactive label or radiotracer) can be conjugated to an AB or other region of an antibody, AA, bispecific antibody, BAA. Suitable detectable labels are discussed in the context of the above screening methods and additional specific examples are provided below. Using at least one AB specific to a protein or peptide of the disease state, along with a protease whose activity is elevated in the disease tissue of interest, AAs will exhibit an increased rate of binding to disease tissue relative to tissues where the CM specific enzyme is not present at a detectable level or is present at a lower level than in disease tissue or is inactive (*e.g.*, in zymogen form or in complex with an inhibitor). Since small proteins and peptides are rapidly cleared from the blood by the renal filtration system, and because the enzyme specific for the CM is not present at a detectable level (or is present at lower levels in non-disease tissues or is present in inactive conformation), accumulation of activated bispecific antibodies in the disease tissue is enhanced relative to non-disease tissues.

[0203] In another example, antibodies, antibodies/bispecific antibodies/AAs/BAAs of the present disclosure can be used to detect the presence or absence of a cleaving agent in a sample. For example, where the antibodies/bispecific antibodies/AAs/BAAs contain a CM susceptible to cleavage by an enzyme, the BAAs can be used to detect (either qualitatively or quantitatively) the presence of an enzyme in the sample. In another example, where the antibodies/bispecific antibodies/AAs/BAAs contain a CM susceptible to cleavage by reducing agent, the antibodies/bispecific antibodies/AAs/BAAs can be used to detect (either qualitatively or

quantitatively) the presence of reducing conditions in a sample. To facilitate analysis in these methods, the antibodies/bispecific antibodies/AAs/BAAAs can be detectably labeled, and can be bound to a support (*e.g.*, a solid support, such as a slide or bead). The detectable label can be positioned on a portion of the antibodies/bispecific antibodies/AAs/BAAAs that is not released following cleavage, for example, the detectable label can be a quenched fluorescent label or other label that is not detectable until cleavage has occurred. The assay can be conducted by, for example, contacting the immobilized, detectably labeled antibodies/bispecific antibodies/AAs/BAAAs with a sample suspected of containing an enzyme and/or reducing agent for a time sufficient for cleavage to occur, then washing to remove excess sample and contaminants. The presence or absence of the cleaving agent (*e.g.*, enzyme or reducing agent) in the sample is then assessed by a change in detectable signal of the antibodies/bispecific antibodies/AAs/BAAAs prior to contacting with the sample *e.g.*, the presence of and/or an increase in detectable signal due to cleavage of the antibodies/bispecific antibodies/AAs/BAAAs by the cleaving agent in the sample.

[0204] Such detection methods can be adapted to also provide for detection of the presence or absence of a target that is capable of binding at least one AB of the antibodies/bispecific antibodies/AAs/BAAAs of the present disclosure. Thus, the assays can be adapted to assess the presence or absence of a cleaving agent and the presence or absence of a target of interest. The presence or absence of the cleaving agent can be detected by the presence of and/or an increase in detectable label of the antibodies/bispecific antibodies/AAs/BAAAs as described above, and the presence or absence of the target can be detected by detection of a target-AB complex *e.g.*, by use of a detectably labeled anti-target antibody.

[0205] AAs/BAAAs of the present disclosure are also useful in *in situ* imaging for the validation of AA activation, *e.g.*, by protease cleavage, and binding to a particular target. *In situ* imaging is a technique that enables localization of proteolytic activity and target in biological samples such as cell cultures or tissue sections. Using this technique, it is possible to confirm both binding to a given target and proteolytic activity based on the presence of a detectable label (*e.g.*, a fluorescent label).

[0206] These techniques are useful with any frozen cells or tissue derived from a disease site (*e.g.* tumor tissue) or healthy tissues. These techniques are also useful with fresh cell or tissue samples.

[0207] In these techniques, an AA/BAA is labeled with a detectable label. The detectable label may be a fluorescent dye, (e.g. a fluorophore, Fluorescein Isothiocyanate (FITC), Rhodamine Isothiocyanate (TRITC), an Alexa Fluor® label), a near infrared (NIR) dye (e.g., Qdot® nanocrystals), a colloidal metal, a hapten, a radioactive marker, biotin and an amplification reagent such as streptavidin, or an enzyme (e.g. horseradish peroxidase or alkaline phosphatase).

[0208] Detection of the label in a sample that has been incubated with the labeled, AA or BAA indicates that the sample contains the target and contains a protease that is specific for the CM of the AAs or BAAs of the present disclosure. In some embodiments, the presence of the protease can be confirmed using broad spectrum protease inhibitors such as those described herein, and/or by using an agent that is specific for the protease, for example, an antibody such as A11, which is specific for the protease matriptase (MT-SP1) and inhibits the proteolytic activity of MT-SP1; see e.g., International Publication Number WO 2010/129609, published 11 November 2010. The same approach of using broad spectrum protease inhibitors such as those described herein, and/or by using a more selective inhibitory agent can be used to identify a protease or class of proteases specific for the CM of the AAs or BAAs of the present disclosure. In some embodiments, the presence of the target can be confirmed using an agent that is specific for the target or the detectable label can be competed with unlabeled target. In some embodiments, unlabeled AA could be used, with detection by a labeled secondary antibody or more complex detection system.

[0209] Similar techniques are also useful for *in vivo* imaging where detection of the fluorescent signal in a subject, e.g., a mammal, including a human, indicates that the disease site contains the target and contains a protease that is specific for the CM of the AAs or BAAs of the present disclosure.

[0210] These techniques are also useful in kits and/or as reagents for the detection, identification or characterization of protease activity in a variety of cells, tissues, and organisms based on the protease-specific CM in the AAs or BAAs of the present disclosure.

13. Therapeutic Administration

[0211] It will be appreciated that administration of therapeutic entities in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A

multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present disclosure, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. See also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci. 89(8):967-78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[0212] In some embodiments, the antibodies, bispecific antibodies, AAs, or BAAs (or conjugated compositions thereof) are administered in conjunction with one or more additional agents, or with a combination of additional agents. Suitable additional agents include current pharmaceutical and/or surgical therapies for an intended application. For example, they can be used in conjunction with an additional chemotherapeutic or anti-neoplastic agent.

[0213] In some embodiments, the antibodies, bispecific antibodies, AAs, or BAAs (or conjugated compositions thereof) of the present disclosure are administered in conjunction with one or more additional agents selected from the group consisting of antibodies, conjugated antibodies, AAs, conjugated AAs, bispecific antibodies, conjugated bispecific antibodies, BAAs, or conjugated BAAs. In some embodiments, the antibody portion of any of the above-referenced additional agents is directed against a target such as one or more of the targets disclosed in Table 9. It is appreciated that in some embodiments the antibody portion of antibodies, bispecific antibodies, AAs, or BAAs (or conjugated compositions thereof) of the present disclosure and the antibody

portion of the additional agent is directed against the same target (*e.g.* both may target EGFR). In some embodiments, they are directed against the same target, but target different epitopes. In some embodiments, they are directed against different targets entirely (*e.g.*, an activatable antibody of the present disclosure that targets EGFR may be administered in conjunction with an AA targeting a different target; likewise *e.g.* a BAA of the present disclosure that targets EGFR and CD3 may be administered in conjunction with an AA targeting a different target.

[0214] In some embodiments, antibodies, bispecific antibodies, AAs or BAAs (or conjugated compositions thereof) of the disclosure are administered in conjunction with an immunotherapeutic agent. In some embodiments, antibodies, bispecific antibodies, AAs or BAAs (or conjugated compositions thereof) of the disclosure are administered in conjunction with a chemotherapeutic agent. In some embodiments, antibodies, bispecific antibodies, AAs or BAAs (or conjugated compositions thereof) of the disclosure are administered in conjunction with both an immunotherapeutic agent and a chemotherapeutic agent. In some embodiments, one or more additional agents is administered with any of these combination embodiments.

[0215] In some embodiments, they are formulated into a single therapeutic composition, and the antibodies/bispecific antibodies/AAs/BAAs thereof and the additional agent are administered simultaneously. Alternatively, the antibodies/bispecific antibodies/AAs/BAAs thereof are administered separate from each other, *e.g.*, each is formulated into a separate therapeutic composition, and the antibodies/bispecific antibodies/AAs/BAAs thereof and the additional agent are administered simultaneously, or the antibodies/bispecific antibodies/AAs/BAAs thereof and the additional agent are administered at different times during a treatment regimen. The antibodies/bispecific antibodies/AAs/BAAs thereof and the additional agent can be administered in multiple doses.

[0216] The antibodies/bispecific antibodies/AAs/BAAs thereof can be incorporated into pharmaceutical compositions suitable for administration. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are provided, for example, in Remington's Pharmaceutical Sciences: The Science And Practice Of Pharmacy 19th ed. (Alfonso R. Gennaro, et al., editors) Mack Pub. Co., Easton, Pa. : 1995; Drug Absorption Enhancement : Concepts, Possibilities, Limitations, And Trends, Harwood Academic Publishers,

Langhorne, Pa., 1994; and Peptide And Protein Drug Delivery (Advances In Parenteral Sciences, Vol. 4), 1991, M. Dekker, New York.

[0217] Such compositions typically comprise the antibodies/bispecific antibodies/AAs/BAAAs thereof and a pharmaceutically acceptable carrier.

[0218] As used herein, the term “pharmaceutically acceptable carrier” is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Suitable carriers are described in the most recent edition of Remington’s Pharmaceutical Sciences, a standard reference text in the field, which is incorporated herein by reference. Suitable examples of such carriers or diluents include, but are not limited to, water, saline, ringer’s solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated.

[0219] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[0220] A pharmaceutical composition of the disclosure is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.*, topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0221] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of

sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be suitable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

[0222] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0223] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can

contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

[0224] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from pressured container or dispenser that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[0225] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

[0226] The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0227] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as sustained/controlled release formulations, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art.

[0228] For example, the active ingredients can be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

[0229] Sustained-release preparations can be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the

antibody, 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 DEPOTTM (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

[0230] The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) and can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[0231] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[0232] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[0233] The formulation can also contain more than one active compound as necessary for the particular indication being treated, for example, those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition can comprise an agent that enhances its function, such as, for example, a cytotoxic agent, cytokine, chemotherapeutic agent, or growth-inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0234] In one embodiment, the active compounds are administered in combination therapy, *i.e.*, combined with other agents, *e.g.*, therapeutic agents, that are useful for treating pathological conditions or disorders, such as autoimmune disorders and inflammatory diseases. The term “in combination” in this context means that the agents are given substantially contemporaneously, either simultaneously or sequentially. If given sequentially, at the onset of administration of the second compound, the first of the two compounds is still detectable at effective concentrations at the site of treatment.

[0235] For example, the combination therapy can include one or more antibodies/bispecific antibodies/AAs/BAAAs thereof of the disclosure coformulated with, and/or coadministered with, one or more additional therapeutic agents, *e.g.*, one or more cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents, as described in more detail below. Furthermore, one or more antibodies/bispecific antibodies/AAs/BAAAs thereof described herein may be used in combination with two or more of the therapeutic agents described herein (*e.g.* one BAA administered with another BAA or AA of the disclosure, and the like). Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

[0236] In other embodiments, one or more antibodies of the disclosure can be coformulated with, and/or coadministered with, one or more anti-inflammatory drugs, immunosuppressants, or metabolic or enzymatic inhibitors. Nonlimiting examples of the drugs or inhibitors that can be used in combination with the antibodies described herein, include, but are not limited to, one or more of: nonsteroidal anti-inflammatory drug(s) (NSAIDs), *e.g.*, ibuprofen, tenidap, naproxen, meloxicam, piroxicam, diclofenac, and indomethacin; sulfasalazine; corticosteroids such as prednisolone; cytokine suppressive anti-inflammatory drug(s) (CSAIDs); inhibitors of nucleotide biosynthesis, *e.g.*, inhibitors of purine biosynthesis, folate antagonists (*e.g.*, methotrexate (N-[4-[(2,4-diamino-6-pteridiny)methyl] methylamino] benzoyl]-L-glutamic acid); and inhibitors of pyrimidine biosynthesis, *e.g.*, dihydroorotate dehydrogenase (DHODH) inhibitors. Suitable therapeutic agents for use in combination with the antibodies of the disclosure include NSAIDs, CSAIDs, (DHODH) inhibitors (*e.g.*, leflunomide), and folate antagonists (*e.g.*, methotrexate).

[0237] Examples of additional inhibitors include one or more of: corticosteroids (oral, inhaled and local injection); immunosuppressants, *e.g.*, cyclosporin, tacrolimus (FK-506); and mTOR inhibitors, *e.g.*, sirolimus (rapamycin - RAPAMUNE™ or rapamycin derivatives, *e.g.*, soluble rapamycin derivatives (*e.g.*, ester rapamycin derivatives, *e.g.*, CCI-779); agents that interfere with signaling by proinflammatory cytokines such as TNF α or IL-1 (*e.g.* IRAK, NIK, IKK, p38 or MAP kinase inhibitors); COX2 inhibitors, *e.g.*, celecoxib, rofecoxib, and variants thereof; phosphodiesterase inhibitors, *e.g.*, R973401 (phosphodiesterase Type IV inhibitor); phospholipase inhibitors, *e.g.*, inhibitors of cytosolic phospholipase 2 (cPLA2) (*e.g.*, trifluoromethyl ketone analogs); inhibitors of vascular endothelial cell growth factor or growth factor receptor, *e.g.*, VEGF inhibitor and/or VEGF-R inhibitor; and inhibitors of angiogenesis. Suitable therapeutic agents for use in combination with the antibodies of the disclosure are immunosuppressants, *e.g.*, cyclosporin, tacrolimus (FK-506); mTOR inhibitors, *e.g.*, sirolimus (rapamycin) or rapamycin derivatives, *e.g.*, soluble rapamycin derivatives (*e.g.*, ester rapamycin derivatives, *e.g.*, CCI-779); COX2 inhibitors, *e.g.*, celecoxib and variants thereof; and phospholipase inhibitors, *e.g.*, inhibitors of cytosolic phospholipase 2 (cPLA2), *e.g.*, trifluoromethyl ketone analogs.

[0238] Additional examples of therapeutic agents that can be combined with an antibody of the disclosure include one or more of: 6-mercaptopurines (6-MP); azathioprine sulphasalazine; mesalazine; olsalazine; chloroquine/ hydroxychloroquine (PLAQUENIL®); pencillamine; aurothiornalate (intramuscular and oral); azathioprine; coichicine; beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral); xanthines (theophylline, aminophylline); cromoglycate; nedocromil; ketotifen; ipratropium and oxitropium; mycophenolate mofetil; adenosine agonists; antithrombotic agents; complement inhibitors; and adrenergic agents.

[0239] In some embodiments, antibodies/bispecific antibodies/AAs/BAAAs thereof of the disclosure can be combined with one or more antibodies/bispecific antibodies/AAs/BAAAs thereof.

14. Kits and Articles of Manufacture

[0240] Provided herein are kits and articles of manufacture comprising any one or more of the antibodies, AAs, bispecific antibodies, and BAAAs provided herein

[0241] The kits and articles of manufacture may comprise any one or more of the antibodies, AAs, bispecific antibodies, and BAAAs provided herein in a format suitable for storage or shipping.

[0242] The kits and articles of manufacture may comprise at least a second component.

[0243] The kits and articles of manufacture may comprise a vessel, a diluent, a solvent, a second composition, or any component useful for converting a composition in a format for storage into a composition suitable for use in a method disclosed herein, if such a conversion is required. The method may be, for instance, a therapeutic method disclosed herein. The kit may comprise instructions for use.

[0244] The kits and articles of manufacture may comprise an agent as disclosed herein, for instance a cytotoxic agent or a detectable label, in a format suitable for conjugation to the antibodies, AAs, bispecific antibodies, and BAAs provided herein.

[0245] The following examples are included for illustrative purposes and are not intended to limit the scope of the invention.

ENUMERATED EMBODIMENTS

[0246] The invention may be defined by reference to the following enumerated, illustrative embodiments.

[0247] 1. A bispecific activatable antibody (BAA), wherein said BAA, when activated, specifically binds to two targets and comprises the following structure:

a. an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and wherein the AB1 is linked to a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein

the MM1 inhibits the binding of the AB1 to its target; and

the CM1 is a polypeptide that functions as a substrate for a first protease,

b. two scFvs (each an AB2) that each specifically binds to a second target wherein each AB2 comprises a light chain variable region linked to a heavy chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and wherein each AB2 is linked to a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of each MM2-CM2 construct is linked to the amino terminus of each AB2 wherein

the MM2 inhibits the binding of the AB2 to its target; and

the CM2 is a polypeptide that functions as a substrate for a second protease,
and wherein the BAA has at least one of the following characteristics:

- i. MM2 comprises amino acid sequence SEQ ID NO: 12;
- ii. MM1 comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7;
- iii. AB2 comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4; and
- iv. AB1 comprises an Fc region comprising an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0248] 2. The BAA of embodiment 1, wherein AB2 comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4.

[0249] 3. The BAA of embodiment 1, wherein the AB1 binds a tumor target and the AB2 binds an immune effector target.

[0250] 4. The BAA of any one of embodiments 1 to 3, wherein the BAA is a T cell-engaging bispecific (TCB) AA (TCBAA).

[0251] 5. The BAA of any one of embodiments 1 to 4, wherein the AB1 binds EGFR and the AB2 binds CD3ε.

[0252] 6. The BAA of any one of embodiments 1 to 5, wherein the MM1 comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7.

[0253] 7. The BAA of any one of embodiments 1 to 5, wherein the MM1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 85 and SEQ ID NO: 78.

[0254] 8. The BAA of any one of embodiments 1 to 5, wherein the MM1 comprises SEQ ID NO: 78.

[0255] 9. The BAA of any one of embodiments 1 to 8, wherein the MM2 comprises the amino acid sequence SEQ ID NO: 12.

- [0256] 10. The BAA of any one of embodiments 1 to 9, wherein the CM comprises the amino acid sequence of SEQ ID NO: 14.
- [0257] 11. The BAA of any one of embodiments 1 to 9, wherein the CM comprises the amino acid sequence of SEQ ID NO: 17.
- [0258] 12. The BAA of any one of embodiments 1 to 9, wherein the CM the CM comprises the amino acid sequence of SEQ ID NO: 16.
- [0259] 13. The BAA of any one of embodiments 1 to 9, wherein CM1 comprises an amino acid sequence selected from the group comprising SEQ ID NO: 14 and SEQ ID NO: 16.
- [0260] 14. The BAA of any one of embodiments 1 to 9, wherein CM2 comprises an amino acid sequence selected from the group comprising SEQ ID NO: 14 and SEQ ID NO: 17.
- [0261] 15. The BAA of any one of embodiments 1 to 14, wherein AB1 comprises amino acid substitutions in at least two of amino acid positions L234, L235, and P331.
- [0262] 16. The BAA of embodiment 15, wherein AB1 comprises amino acid substitutions at amino acid positions L234, L235, and P331.
- [0263] 17. The BAA of embodiment 15, wherein AB1 comprises L234F, L235E, and P331S amino acid substitutions.
- [0264] 18. The BAA of embodiment 15, wherein the AB1 comprises an Fc region comprising an amino acid substitution at N297.
- [0265] 19. The BAA of any one of embodiments 1 to 14, wherein AB1 comprises amino acid substitutions at amino acid positions L234F, L235E, P331S, and N297Q.
- [0266] 20. The BAA of embodiment 1, wherein the heavy chain of the AB1 comprises any one of SEQ ID NO: 69, SEQ ID NO: 70, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 76, as set forth in Table 6.
- [0267] 21. The BAA CI106, comprising the layout and sequence as provided in Table 11 and Example 1.
- [0268] 22. The BAA CI107, comprising the layout and sequence as provided in Table 11 and Example 1.
- [0269] 23. The BAA CI079, comprising the layout and sequence as provided in Table 11 and Example 1.

- [0270] 24. The BAA CI090, comprising the layout and sequence as provided in Table 11 and Example 1.
- [0271] 25. An activatable antibody (AA) comprising:
- a) an antibody or antigen binding fragment thereof (AB) that specifically binds to the epsilon chain of CD3 (CD3 ϵ);
 - b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the CD3 ϵ when the AA is in an uncleaved state, wherein the MM comprises amino acid sequence SEQ ID NO: 12; and
 - c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.
- [0272] 26. The AA of embodiment 25, wherein the CM comprises any one of the sequences set forth in Table 4.
- [0273] 27. The AA of embodiment 25, wherein the CM comprises a substrate cleavable by a serine protease or an MMP.
- [0274] 28. The AA of embodiment 25, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18-56.
- [0275] 29. The AA of embodiment 25, wherein the protease is an MMP.
- [0276] 30. The AA of embodiment 25, wherein the protease is a serine protease.
- [0277] 31. The AA of embodiment 25, wherein the AB that specifically binds to CD3 is the antibody of any one of embodiments 38-47.
- [0278] 32. An activatable antibody (AA) comprising:
- a. an antibody or an antigen binding fragment thereof (AB) that specifically binds to Epidermal Growth Factor Receptor (EGFR);
 - b. a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the EGFR when the AA is in an uncleaved state, and wherein the MM comprises an amino acid sequence selected from the group consisting of sequences presented in Table 7; and
 - c. a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

- [0279] 33. The AA of embodiment 32, wherein the MM comprises the amino acid sequence of SEQ ID NO: 78.
- [0280] 34. The AA of any one of embodiments 32 to 33, wherein the CM comprises a substrate cleavable by a serine protease or an MMP.
- [0281] 35. The AA of any one of embodiments 32 to 33, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 18-56.
- [0282] 36. The AA of embodiment 32, wherein the CM comprises the amino acid sequence of SEQ ID NO: 14.
- [0283] 37. The AA of embodiment 32, wherein the CM comprises the amino acid sequence of SEQ ID NO: 16.
- [0284] 38. An antibody or antigen binding fragment thereof (AB) that specifically binds to the epsilon chain of CD3 (CD3 ϵ), wherein the antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4.
- [0285] 39. The AB of embodiment 38, wherein, the antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4.
- [0286] 40. The AB of embodiment 38, comprising a heavy chain variable domain as set forth in SEQ ID NO: 2.
- [0287] 41. The AB of embodiment 38, comprising a heavy chain variable domain as set forth in SEQ ID NO: 3.
- [0288] 42. The AB of embodiment 38, wherein the antibody comprises a light chain variable domain as set forth in SEQ ID NO: 1.
- [0289] 43. The AB of embodiment 38, comprising a light chain variable domain as set forth in SEQ ID NO: 4.
- [0290] 44. The AB of embodiment 38, comprising a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain variable domain as set forth in SEQ ID NO: 1.
- [0291] 45. The AB of embodiment 38, comprising a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 1.

- [0292] 46. The AB of embodiment 38, comprising a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 4.
- [0293] 47. The AB of embodiment 38, comprising a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain variable domain as set forth in SEQ ID NO: 4.
- [0294] 48. The AB of any one of embodiments 32 to 47, wherein the AB is a bispecific AB.
- [0295] 49. The AA of any one of embodiments 32 to 47, wherein the antibody is a scFv.
- [0296] 50. The AA of any one of embodiments 32 to 47, wherein the antibody is an IgG1 antibody.
- [0297] 51. An activatable antibody (AA) comprising:
- a) an antibody or antigen binding fragment thereof (AB) that specifically binds to the epsilon chain of CD3 (CD3 ϵ), wherein the antibody comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 or SEQ ID NO: 3 or comprises a light chain variable domain as set forth in SEQ ID NO: 1 or SEQ ID NO: 4;
 - b) a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the CD3 ϵ when the AA is in an uncleaved state; and
 - c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.
- [0298] 52. The AA of embodiment 51, wherein the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 2.
- [0299] 53. The AA of embodiment 51, wherein the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 3.
- [0300] 54. The AA of embodiment 51, wherein the AB comprises a light chain variable domain as set forth in SEQ ID NO: 1.
- [0301] 55. The AA of embodiment 51, wherein the AB comprises a light chain variable domain as set forth in SEQ ID NO: 4.
- [0302] 56. The AA of embodiment 51, wherein the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain variable domain as set forth in SEQ ID NO: 1.

- [0303] 57. The AA of embodiment 51, wherein the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 1.
- [0304] 58. The AA of embodiment 51, wherein the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 2 and a light chain variable domain as set forth in SEQ ID NO: 4.
- [0305] 59. The AA of embodiment 51, wherein the AB comprises a heavy chain variable domain as set forth in SEQ ID NO: 3 and a light chain variable domain as set forth in SEQ ID NO: 4.
- [0306] 60. The AA of any one of embodiments 51 to 59, wherein the MM comprises any one of the sequences set forth in Table 3.
- [0307] 61. The AA of any one of embodiments 51 to 59, wherein the CM comprises any one of the sequences set forth in Table 4.
- [0308] 62. A bispecific activatable antibody (BAA) comprising any one of the AAs of embodiments 51 to 61.
- [0309] 63. An activatable antibody (AA) comprising:
- a. an antibody (AB) that specifically binds a target, wherein the antibody is an IgG1 antibody, and wherein the Fc region of the antibody comprises an amino acid substitution in amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the AA has reduced effector function;
 - b. a masking moiety (MM) coupled to the AB, wherein the MM reduces or inhibits the binding of the AB to the target when the AA is in an uncleaved state; and
 - c. a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.
- [0310] 64. The AA of embodiment 63, wherein the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, N297 and P331, as numbered by the EU index as set forth in Kabat, such that the AA has reduced effector function.
- [0311] 65. The AA of embodiment 63 or 64, wherein the target is selected from the group consisting of the targets presented in Table 9.
- [0312] 66. A bispecific activatable antibody (BAA) comprising:

a. an IgG antibody (AB1) that specifically binds to a first target wherein the AB1 comprises two heavy chains (AB1 HCs) and two light chains (AB1 LCs); and wherein the AB1 is linked to a first masking moiety (MM1) linked to a first cleavable moiety (CM1) to form a MM1-CM1 construct, wherein the carboxyl terminus of a MM1-CM1 construct is linked to each amino terminus of each light chain of the AB1, wherein

the MM1 inhibits the binding of the AB1 to its target; and

the CM1 is a polypeptide that functions as a substrate for a first protease,

b. two scFvs (each an AB2) that each specifically binds to a second target wherein each AB2 comprises a heavy chain variable region linked to a light chain variable region, wherein the carboxyl terminus of each AB2 is linked to the amino terminus each of the AB1 heavy chains; and wherein each AB2 is linked to a second masking moiety (MM2) linked to a second cleavable moiety (CM2) to form a MM2-CM2 construct, wherein the carboxyl terminus of each MM2-CM2 construct is linked to the amino terminus of each AB2 wherein

the MM2 inhibits the binding of the AB2 to its target; and

the CM2 is a polypeptide that functions as a substrate for a second protease,

and wherein the AB1 comprises an Fc region comprises an amino acid substitution in at least one of amino acid positions L234, L235, N297, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0313] 67. The BAA of embodiment 66, wherein the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, N297 and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0314] 68. The BAA of embodiment 66, wherein the Fc region comprises amino acid substitutions in at least amino acid positions L234, L235, and P331, as numbered by the EU index as set forth in Kabat, such that the BAA has reduced effector function.

[0315] 69. The BAA of any one of embodiments 66 to 68, wherein the first target is selected from the group consisting of the targets presented in Table 9 and the second target is selected from the group consisting of the targets presented in Table 9.

[0316] 70. The AA or BAA of any one of the above embodiments, wherein the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

- [0317] 71. The AA or BAA of any one of the above embodiments wherein the antibody is a rodent antibody, a chimeric antibody, a humanized antibody, or a fully human monoclonal antibody.
- [0318] 72. The AA of any one of embodiments 32-37 and 51-71, wherein the AA is a BAA.
- [0319] 73. A pharmaceutical composition comprising the antibody, AA, or BAA of any one of embodiments 1-72 and optionally a carrier.
- [0320] 74. The pharmaceutical composition of embodiment 73 comprising an additional agent.
- [0321] 75. The pharmaceutical composition of embodiment 74, wherein the additional agent is a therapeutic agent.
- [0322] 76. An isolated nucleic acid molecule encoding the antibody, AA, or BAA of any one of embodiments 1-72.
- [0323] 77. A vector comprising the isolated nucleic acid molecule of embodiment 76.
- [0324] 78. A vector comprising the nucleic acid sequence of pLW289.
- [0325] 79. A vector comprising the nucleic acid sequence of pLW246.
- [0326] 80. A vector comprising the nucleic acid sequence of pLW307.
- [0327] 81. A vector comprising the nucleic acid sequence of pLW291.
- [0328] 82. A cell comprising any one of the vectors of embodiments 77-81.
- [0329] 83. A cell comprising pLW289 and pLW246.
- [0330] 84. A cell comprising pLW307 and pLW291.
- [0331] 85. A method of producing the antibody, AA, or BAA of any one of embodiments 1-72 by culturing a cell under conditions that lead to expression of the antibody, AA, or BAA, wherein the cell comprises the nucleic acid molecule of embodiment 76 or the vector of any one of embodiments 78-81.
- [0332] 86. A method of treating, alleviating a symptom of, or delaying the progression of a disorder or disease comprising administering a therapeutically effective amount of the antibody, AA, or BAA of any one of embodiments 1-72, or the pharmaceutical composition of any one of embodiments 73-75 to a subject in need thereof.
- [0333] 87. The method of embodiment 86, wherein the disorder or disease comprises disease cells expressing EGFR.
- [0334] 88. The method of embodiments 86 or 87, wherein the disorder or disease is cancer.

[0335] 89. The method of embodiment 88, wherein the cancer is anal cancer, basal cell carcinoma, brain cancer, bladder cancer, breast cancer, bone cancer, cervical cancer, cholangiocarcinoma, colorectal cancer, endometrial cancer, esophageal cancer, gall bladder cancer, gastric cancer, glioblastoma, head and neck cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, small bowel carcinoma, squamous cell cancer, skin cancer testicular cancer, thyroid cancer or uterine cancer.

[0336] 90. A method of inhibiting angiogenesis in a subject comprising administering a therapeutically effective amount of the antibody, AA, or BAA of any one of embodiments 1-72, or the pharmaceutical composition of any one of embodiments 73-75 to a subject in need thereof.

[0337] 91. The method of any one of embodiments 86-90, wherein the method comprises administering an additional agent.

[0338] 92. The method of embodiment 91 wherein the additional agent is a therapeutic agent.

[0339] 93. A method of reducing damage to healthy tissue caused by an antibody binding to its target on healthy tissue as well as on diseased tissue, the method comprising administering to a subject in need thereof an AA or BAA or a pharmaceutical composition comprising an AA or BAA, wherein said AA or BAA is an AA or BAA of any one of the embodiments provided herein.

[0340] 94. A method to improve tolerability of an antibody treatment comprising administering to a subject in need thereof an AA or BAA or a pharmaceutical composition comprising an AA or BAA, wherein said AA or BAA is an AA or BAA of any one of the embodiments provided herein.

[0341] 95. A method to recruit T cells to tumor tissue comprising administering to a subject in need thereof an AA or BAA or a pharmaceutical composition comprising an AA or BAA, wherein said AA or BAA is an AA or BAA of any one of the embodiments provided herein.

[0342] 96. An antibody, AA, or BAA of any one of embodiments 1-72, or the pharmaceutical composition of any one of embodiments 73-75, for use as a medicament.

[0343] 97. An antibody, AA, or BAA of any one of embodiments 1-72, or the pharmaceutical composition of any one of embodiments 73-75, for use in a method of treating, alleviating a symptom of, or delaying the progression of a disorder or disease, wherein the disorder or disease comprises disease cells expressing EGFR.

[0344] 98. An antibody, AA, or BAA of any one of embodiments 1-72, or the pharmaceutical composition of any one of embodiments 73-75, for use in a method of treating cancer; optionally

wherein the cancer is anal cancer, basal cell carcinoma, brain cancer, bladder cancer, breast cancer, bone cancer, cervical cancer, cholangiocarcinoma, colorectal cancer, endometrial cancer, esophageal cancer, gall bladder cancer, gastric cancer, glioblastoma, head and neck cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, small bowel carcinoma, squamous cell cancer, skin cancer testicular cancer, thyroid cancer or uterine cancer.

[0345] 99. An antibody, AA, or BAA of any one of embodiments 1-72, or the pharmaceutical composition of any one of embodiments 73-75, for use in a method of treatment, wherein the method comprises inhibiting angiogenesis.

[0346] 100. The antibody, AA, or BAA, or the pharmaceutical composition, for use according to any of embodiments 96 to 99, wherein the use comprises administering an additional agent, optionally wherein the additional agent is a therapeutic agent.

EXAMPLES

EXAMPLE 1. Sequences, Vector Construction and Expression of Antibodies, BAAs and Activated BAAs

Antibodies of Interest

[0347] The molecules as provided in Table 11 below were constructed and tested. As indicated, activated molecules were produced as masked and proteolytically cleaved to produce the activated forms.

Table 11

Molecule Name	Molecule Component Parts	Heavy Chain Vector	Light Chain Vector
CI011	3954-0001-C225v5N297Q-JF15865-0001-CD3LvHv-H-N	pLW023	OPP022
CI020	3954-Nsub-C225v5N297Q-JF15865-Nsub-hSP34LvHv-H-N	pLW073	pLW071
CI040	3954-2001-C225v5N297Q-JF15865-2001-hSP34LvHv-H-N	pLW101	CTX122
CI048	Activated CI011: 3954-0001-C225v5N297Q-JF15865-0001-CD3LvHv-H-N	pLW023	OPP022
CI079	3954-0001-C225v5Fcmt3-h20GG-0001-v16sc-H-N	pLW225	OPP022
CI090	3954-0001-C225v5Fcmt4-h20GG-0001-v16sc-H-N	pLW233	OPP022
Activated CI090	Activated 3954-0001-C225v5Fcmt4-h20GG-0001-v16sc-H-N	pLW233	OPP022

Molecule Name	Molecule Component Parts	Heavy Chain Vector	Light Chain Vector
Activated CI104	Activated 3954-0011-C225v5Fcmt4-h20GG-0011-v16sc-H-N	pLW289	pLW291
CI106	CF41-2008-C225v5Fcmt4-h20GG-0011-v16sc-H-N	pLW289	pLW246
CI107	3954-0011-C225v5Fcmt4-h20GG-2006-v16sc-H-N	pLW307	pLW291
CI127	SynFcmt4-h20GG-0011-v16sc-H-N	pLW334	pLW139
CI128	SynFcmt4-h20GG-2006-v16sc-H-N	pLW335	pLW139
CI135	CF41-2008-C225v5Fcmt4-h20GG-0011-v12sc-H-N	pLW352	pLW246
CI136	CF41-2008-C225v5Fcmt4-h20GG-0011-v19sc-H-N	pLW353	pLW246
CI091	3954-1490DQH-C225v5Fcmt4-h20GG-2008-v16sc-H-N	pLW242	CX320
CI064	SynN297Q-JF15865-0001-hSP34LvHv-H-N	pLW138	pLW139
v12	Anti-CD3 variant	HV12	LV12
v16	Anti-CD3 variant	HV20	LV12
v19	Anti-CD3 variant	HV20	LV19
v26	Anti-CD3 variant	HV12	LV19

[0348] The sequences of the molecules and vectors are provided below. Brackets denote some of the component parts of the molecules presented. In some sequences, linkers are provided. Underlined amino acids denote predicted CDR sequences.

CI011: 3954-0001-C225v5N297Q-JF15865-0001-CD3LvHv-H-N

pLW023: HC JF15865-0001-CD3LvHv-C225v5N297Q (H-N)

Nucleotide Sequence

[spacer SEQ ID NO:176][pLW023 without spacer SEQ ID NO:177]

CAAGGCCAGTCTGGCCAAATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGC
GGGTTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAA
TCATGGCGGCGGTTCTCAGACCGTGGTCAACAGGAGCCCTCACTGACAGTGAGCCCT
GGCGGGACCGTCACACTGACTTGTCGCAGTTCAACTGGCGCCGTGACTACCAGCAATT
ACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACACGAGGACTGATCGGAGGAA
CTAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGAAA
AGCCGCTCTGACACTGAGCGGCGTGCAGCCTGAGGACGAAGCTGAGTACTATTGCGCA
CTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAATAAGCTGACCGTCCTGGGAG
GAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGACAGCTGGTC
GAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCA
GTGGCTTCACCTTCAACACTTACGCAATGAACTGGGTGCGGCAGGCACCTGGGAAGGG
ACTGGAATGGGTGCGCCGGATCAGATCTAAATACAATAACTATGCCACCTACTATGCTG

ACAGTGTGAAGGATAGGTTACCATTTACGCGACGATAGCAAAAACACAGCTTATCT
GCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGC
AATTTGCGGAACCTTACGTGAGTTGGTTTGCCTATTGGGGACAGGGGACACTGGTCAC
CGTCTCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGCGGCCCCGGGCCTG
GTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCA
ACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGT
GATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATT
AACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAAATGAACAGCCTGCAAAGCCAGG
ATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTGCGTATT
GGGGCCAGGGCACCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCCATCGGTCTT
CCCCCTGGCACCTCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG
GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAAGTCAAGGCGCCCTGACCA
GCGGCGTGACACCTTCCCGGTGTCCTACAGTCTCAGGACTCTACTCCCTCAGCAGC
GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATC
ACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAA
CTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGT
GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTAC
CGTGTGGTGACGCTCCTCACCGTCTGACACGAGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAAGC
CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATG
ACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCG
CCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACTACAAGACCACGCCTCCCG
TGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTACCGTGGACAAGAGCAGG
TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTA
CACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 105)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW023 without spacer SEQ ID NO:179]

QQQSGQ[MMYCGGNEVLCGPRV][GSSGGSGSGG][LSGRSDNH][GGGS]QTVVTQEPLT
VSPGGTVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGG
KAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL[GGGGSGGGSGGGGS]EVQLV
ESGGGLVQPGSLKLSAASGFTFNTYAMNWVRQAPGKLEWVARIRSKYNNYATYYAD
SVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLVTV
SS[GGGS]QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKLEWLGVWS
GGNTDYNTPTFSLRSLINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGT
LVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 106)

OPP022: LC 3954-0001-C225v5*Nucleotide Sequence*

[spacer SEQ ID NO:180][OPP022 without spacer SEQ ID NO:181]

TCCGATAATCATGGCAGTAGCGGTACCCAGATCTTGCTGACCCAGAGCCCGGTGATTCT
 GAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGCATTGGC
 ACCAACATTTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAAT
 ATGCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCAC
 CGATTTTACCCTGAGCATTAAACAGCGTGGAAAGCGAAGATATTGCGGATTATTATTGCC
 AGCAGAACAACTGGCCGACCACCTTTGGCGCGGGCACCAAACTGGAAGTAAACG
 TACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG
 GAACTGCCTCTGTTGTGTGCTGCTGAATAACTTCTATCCAGAGAGGCCAAAGTACAG
 TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGG
 ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTA
 CGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
 ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO: 107)

Amino Acid Sequence

[spacer SEQ ID NO:178][OPP022 without spacer SEQ ID NO:182]

[SDNH][GSSGT][QILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIK
 YAS
 ESISGIPSRFSGSGSTDFTLINSVSEDIADYYCQNNNWPTTFGAGTKLELKRTVAAPSV
 FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLS
 STLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 108)

CI020: 3954-Nsub-C225v5N297Q-JF15865-Nsub-hSP34LvHv-H-N**pLW073: HC C225v5N297Q-JF15865-Nsub-hSP34LvHv (H-N)***Nucleotide Sequence*

[spacer SEQ ID NO:176][pLW073 without spacer SEQ ID NO:183]

CAAGGCCAGTCTGGCCAAATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGC
 GGGTTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTGGTGGAGGCTCGGGCGGTGG
 GAGCGGCGGCGGTTCTCAGACCGTGGTCACACAGGAGCCCTCACTGACAGTGAGCCCT
 GGCGGGACCGTCACACTGACTTGTGCGAGTTCAACTGGCGCCGTGACTACCAGCAATT
 ACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGGAGGAA
 CTAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGAAA
 AGCCGCTCTGACACTGAGCGGCGTGCAGCCTGAGGACGAAGCTGAGTACTATTGCGCA
 CTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAATAAGCTGACCGTCCTGGGAG
 GAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCAGCTGGTC
 GAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCA
 GTGGCTTCACCTTCAACACTTACGCAATGAACTGGGTGCGGCAGGCACCTGGGAAGGG
 ACTGGAATGGGTGCGCCGATCAGATCTAAATAACAATACTATGCCACCTACTATGCTG
 ACAGTGTGAAGGATAGGTTACCATTTACGCGACGATAGCAAAAACACAGCTTATCT
 GCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGC
 AATTCGGGAACCTTACGTGAGTTGGTTTGCCTATTGGGGACAGGGGACACTGGTCAC

CGTCTCCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGCGGCCCCGGGCCTG
 GTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCA
 ACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGT
 GATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATT
 AACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAAATGAACAGCCTGCAAAGCCAGG
 ATACCGCGATTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTGCGTATT
 GGGGCCAGGGCACCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCCATCGGTCTT
 CCCCCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG
 GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCA
 GCGGCGTGACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC
 GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATC
 ACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAA
 CTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
 TTCCCCCAAACCCAAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGT
 GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
 GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTAC
 CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
 AGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGC
 CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATG
 ACCAAGAACAGGTGAGCCTGAGCCTGCCTGGTCAAAAGGCTTCTATCCCAGCGACATG
 CCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAGCAACTACAAGACCACGCCTCCCG
 TGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTACCGTGGACAAGAGCAGG
 TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTA
 CACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 109)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW073 without spacer SEQ ID NO:184]

QQQSGQ[MMYCGGNEVLCGPRV][GSSGGSGSGGGGGSGGGSGGGGS]QTVVTQEPSLTVS
 PGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKA
 ALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL[GGGGSGGGSGGGGS]EVQLVES
 GGGLVQPGSLKLSAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSV
 KDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLLTVSS
 [GGGGS]QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG
 GNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGTLL
 VTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
 LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
 GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIKAKGQPREPQVYTLPPSRE
 EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 110)

pLW071: LC 3954-Nsub-C225v5

Nucleotide Sequence

[spacer SEQ ID NO:180][pLW071 without spacer SEQ ID NO:185]

CAAGGCCAGTCTGGCCAGTGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCAT
GTACGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGCTCAGGTGGAGGCTCGGGCGGT
GGGAGCGGCGGTTCTGATATCTTGCTGACCCAGAGCCCGGTGATTCTGAGCGTGAGCC
CGGGCGAACGTGTGAGCTTTAGCTGCCGCGCAGCCAGAGCATTGGCACCAACATTCA
TTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATATGCGAGCGAA
AGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCGATTTTACCCCT
GAGCATTAACAGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAGCAGAACAAAC
AACTGGCCGACCACCTTTGGCGCGGGCACCAAACTGGAACGTACGGTGGCTG
CACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACGTGCTCT
GTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG
ATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGA
CAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACAC
AAAGTCTACGCTGCGAAGTCACCCATCAGGGCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGT (SEQ ID NO: 111)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW071 without spacer SEQ ID NO:186]
QGQSGQ[CISPRGCPDGPYVMY][GSSGSGSGSGSGSGSGSGSGS]DILLTQSPVILSVSPG
ERVSFSCRASQSIGTNIHWYQRTNGSPRLLIK YASESISGIPSRFSGSGSGTDFTLINSVESE
DIADYYCQNNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNFFYPR
EAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLKADYEEKHKVYACEVTHQGLS
SPVTKSFNRGEC (SEQ ID NO: 112)

CI040: 3954-2001-C225v5N297Q-JF15865-2001-hSP34LvHv-H-N

pLW101: HC JF15865-2001-CD3LvHv-C225v5N297Q (H-N)

Nucleotide Sequence

[spacer SEQ ID NO:176][pLW101 without spacer SEQ ID NO:187]
CAAGGCCAGTCTGGCCAAATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGC
GGGTTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATCTCTTCCGGACTGCTGTCC
GGCAGATCCGACAATCACGGCGGCGGTTCTCAGACCGTGGTCACACAGGAGCCCTCAC
TGACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTCGCAGTTCAACTGGCGCCGT
GACTACCAGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGG
ACTGATCGGAGGAACTAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCT
CTGCTGGGGGAAAAGCCGCTCTGACACTGAGCGGCGTGCAGCCTGAGGACGAAGCTG
AGTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAATAAGCTG
ACCGTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGA
AGTGCAGCTGGTCGAGAGCGGAGGAGGAGTGGTGCAGCCAGGAGGATCCCTGAAGCT
GTCTTGTGCAGCCAGTGGCTTACCTTCAACACTTACGCAATGAACTGGGTGCGGCAGG
CACCTGGGAAGGGACTGGAATGGGTGCGCCCGGATCAGATCTAAATACAATAACTATGC
CACCTACTATGCTGACAGTGTGAAGGATAGGTTACCATTTACGCGACGATAGCAAA
AACACAGCTTATCTGCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATT
GCGTCAGACACGGCAATTTCCGGAACTCTTACGTGAGTTGGTTTGCCTATTGGGGACAG
GGGACACTGGTCACCGTCTCCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGA

GCGGCCCCGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGG
 CTTTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTG
 GAATGGCTGGGCGTGATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCA
 GCCGCCTGAGCATTAACAAAGATAACAGCAAAAAGCCAGGTGTTTTTAAATGAACAG
 CCTGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATT
 ATGAATTTGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAA
 GGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGG
 CCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCA
 GGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTA
 CTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCT
 GCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCCAAT
 CTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGGGGGGACC
 GTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTG
 AGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTG
 GTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA
 CCAGAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAAT
 GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA
 CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCATC
 CCGGAGGAGATGACCAAGAACCAGGTGACGCTGACCTGCCTGGTCAAAGGCTTCTAT
 CCCAGCGACATGCCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAGAACTACAAG
 ACCAGCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGT
 GGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCT
 CTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO:
 113)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW101 without spacer SEQ ID NO:188]

QQQSGQ[MMYCGGNEVLCGPRV][GSSGGSGSGG][ISSGLLSGRSDNH][GGGS]QTVVTQE
 PSLTVSPGGTVTLTCSRSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSL
 LGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL[GGGGSGGGSGGGGS]EV
 QLVESGGGLVQPGGSLKLSAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATY
 YADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGT
 LVTVSS[GGGG]QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWL
 GVIWSGGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAY
 WGQGTLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
 HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK
 PREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
 PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
 DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 114)

CTX122: LC 3954-2001-C225v5

Nucleotide Sequence

[spacer SEQ ID NO:180][CTX122 without spacer SEQ ID NO:189]

CAAGGCCAGTCTGGCCAGTGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCAT
GTACGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGATCCGGTATTAGCAGTGGTCTG
TTAAGCGGTCTGAGCGATAATCATGGCAGTAGCGGTACCCAGATCTTGCTGACCCAGA
GCCCCGTGATTCTGAGCGTGAGCCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAG
CCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCCGCGC
CTGCTGATTAAATATGCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCA
GCGGCAGCGGCACCGATTTTACCCTGAGCATTAAACAGCGTGGAAGCGAAGATATTGC
GGATTATTATTGCCAGCAGAACAACTGGCCGACCACCTTTGGCGCGGGCACCAAA
CTGGAACCTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGA
GCAGTTGAAATCTGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAG
AGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAG
TGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTG
AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO: 115)

Amino Acid Sequence

[spacer SEQ ID NO:178][CTX122 without spacer SEQ ID NO:190]

QGQSGQ[CISPRGCPDGPYVMY][GSSGSGSGSGSG][ISSGLLSGRSDNH][GSSGT]QILLTG
SPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLIKYASESISGIPSRFSGSGSGTDF
TLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVV
CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 116)

CI048: Activated CI011: 3954-0001-C225v5N297Q-JF15865-0001-CD3LvHv-H-N

pLW023 and OPP022 sequences encoding corresponding masked antibody components are provided herein as “pLW023” and “OPP022, respectively and are summarized in Table 11.

Activated pLW023: HC JF15865-0001-CD3LvHv-C225v5N297Q (H-N)

Nucleotide

SequenceTCCGATAATCATGGCGGCGGTTCTCAGACCGTGGTCACACAGGAGCCCTCACT
GACAGTGAGCCCTGGCGGGACCGTCACACTGACTTGTCGCAGTTCAACTGGCGCCGTG
ACTACCAGCAATTACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGAC
TGATCGGAGGAATAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCTCT
GCTGGGGGGAAAAGCCGCTCTGACACTGAGCGGCGTGCAGCCTGAGGACGAAGCTGA
GTACTATTGCGCACTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGAACTAAGCTGA
CCGTCCTGGGAGGAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAA
GTGCAGCTGGTCGAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGT
CTTGTGCAGCCAGTGGCTTACCTTCAACACTTACGCAATGAACTGGGTGCGGCAGGCA
CCTGGGAAGGGACTGGAATGGGTGCGCCCGGATCAGATCTAAATAACAATAACTATGCCA
CCTACTATGCTGACAGTGTGAAGGATAGGTTACCATTTACGCGACGATAGCAAAAA
CACAGCTTATCTGCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGC
GTCAGACACGGCAATTCGGGAACCTTTACGTGAGTTGGTTTGCCTATTGGGGACAGGG
GACACTGGTCACCGTCTCCTCAGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGC

GGCCCGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCT
 TTAGCCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGA
 ATGGCTGGGCGTGATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGC
 CGCCTGAGCATTAAACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAAATGAACAGCC
 TGCAAAGCCAGGATACCGCGATTTATTATTGCGCGCGCGCTGACCTATTATGATTAT
 GAATTTGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGG
 GCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCC
 CTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAG
 GCGCCCTGACCAGCGGCGTGACACCTTCCCGGTGTCCTACAGTCCTCAGGACTCTAC
 TCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTG
 CAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCCAAATCT
 TGTGACAAAACCTACACATGCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGT
 CAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAG
 GTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGT
 ACGTGGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACC
 AGAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGG
 CAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACC
 ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCC
 GGGAGGAGATGACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATCC
 CAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACTACAAGAC
 CACGCTCCCGTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGG
 ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT
 GCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 117)

Amino Acid Sequence

[SDNH][GGGS]QTVVTQEPSLTVSPGGTVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLI
 GGTNKRAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVL[
 GGGGSGGGGSGGGGS]EVQLVESGGGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGK
 GLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHG
 NFGNSYVSWFAYWGQGLVTVSS[GGGGS]QVQLKQSGPGLVQPSQSLITCTVSGFSLTNY
 GVHWVRQSPGKGLEWLGVIWSGGNTDYNTPFTSRLSINKDNSKSKVFFKMNSLQSQDTAI
 YYCARALTYDYEFAYWGQGLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYF
 PEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD
 KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK
 FNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE
 KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
 PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID
 NO: 118)

Activated OPP022: LC 3954-0001-C225v5

Nucleotide

SequenceTCCGATAATCATGGCAGTAGCGGTACCCAGATCTTGCTGACCCAGAGCCCGGT
 GATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGC
 ATTGGCACCAACATTCAATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGA

TTAAATATGCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAG
CGGCACCGATTTTACCCTGAGCATTAAACAGCGTGGAAGCGAAGATATTGCGGATTAT
TATTGCCAGCAGAACAACAACCTGGCCGACCACCTTTGGCGCGGGCACCAAACCTGGAAC
TGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTG
AAATCTGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAA
AGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACACA
GAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTGAGCAAA
GCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCT
CGCCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO: 119)

Amino Acid Sequence

[SDNH][GSSGT]QILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASE
SISGIPSRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 120)

CI079: 3954-0001-C225v5Fcm3-h20GG-0001-v16sc-H-N

pLW225: HC h20GG-0001-v16sc-C225v5Fcm3 (H-N)

Nucleotide Sequence

[spacer SEQ ID NO:191][pLW225_without spacer SEQ ID NO:192]
CAAGGCCAGTCTGGATCCGGTTATCTGTGGGGTTGCGAGTGGAATTGCGGAGGGATCA
CTACAGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAA
TCATGGCGGCGGTTCTCAAACCTGTAGTAACTCAAGAACCAAGCTTCTCCGTCTCCCCTG
GGGGAACAGTCACTTACCTGCCGAAGTAGTACAGGTGCTGTTACGACCAGTAACTA
TGCCAATTGGGTACAACAAACGCCTGGTCAGGCTCCGCGCGGATTGATAGGAGGCACG
AATAAACGGGCACCCGGTGTCCCGGACAGATTCAGCGGAAGCATACTCGGTAAATAAGG
CAGCTCTTACTATCACTGGGGCCCAAGCTGATGATGAAAGTGATTATTATTGTGCGCTC
TGGTACAGCAACCTCTGGGTGTTTGGGGGTGGCACGAACTTACTGTCTTGGGCGGCG
GCGGATCAGGGGGAGGTGGCTCTGGAGGAGGAGGCTCAGAAGTCCAACCTGGTTCGAAT
CCGGGGGAGGGCTCGTACAGCCGGGTGGGTCCCTCAAACCTCTTGTGCGGCCTCAGG
GTTTACCTTCAGTACATACGCGATGAATTGGGTCCGGCAGGCCAGTGGGAAAGGGCTC
GAATGGGTAGGACGAATCCGATCAAAATACAACAACCTACGCTACTTATTACGCTGATT
CCGTGAAGGACAGATTCACAATATCCCGCGACGATAGCAAGAATACGGCATATCTTCA
GATGAATTCTCTTAAACCTGAGGATACCGCTGTGTATTACTGCACAAGACATGGTAATT
TTGGAAACTCATATGTCTCTTGGTTTCGCTTATTGGGGACAGGGCACGTTGGTTACCGTG
TCTAGCGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGC
AGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCAACTA
TGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATT
TGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAAACA
AAGATAACAGCAAAAGCCAGGTGTTTTTAAATGAACAGCCTGCAAAGCCAGGATAC
CGCGATTTATTATTGCGCGCGCGCTGACCTATTATGATTATGAATTTGCGTATTGGG
GCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCC
CCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC

AAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCG
 GCGTGACACCTTCCCGGTGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG
 GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACA
 AGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCA
 CACATGCCCCACCGTGCCAGCACCTGAATTTGAAGGGGGACCGTCAGTCTTCTCTTCC
 CCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGT
 GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG
 GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAATAGCACGTACCGT
 GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGT
 GCAAGGTCTCCAACAAAGCCCTCCCAGCCTCAATCGAGAAAACCATCTCCAAGCCAA
 AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC
 AAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCGCCG
 TGGAGTGGGAGAGCAATGGGCAGCCGAGAACTACAAGACCACGCCTCCCGTGTCT
 GGACTCCGACGGCTCCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
 CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACAC
 GCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 121)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW225_without spacer SEQ ID NO:193]

QGQSGS[GYLWGCEWNCGGITT][GSSGSGSGSGG][LSGRSDNH][GGGS]QTVVTQEPSFSV
 SPGGTVTLTCRSDSTSNYANWVQTPGQAPRGLIGGTNKRAPGVDPDRFSGSILGNKA
 ALTITGAQADDESYYCALWYSNLWVFGGKTCLTVL[GGGSGGGSGGGGS]EVQLVES
 GGGLVQPGGSLKLSAASGFTFSTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYADSV
 KDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRHGNFGNSYVSWFAYWGQGLTVTVSS[
 GGGGS]QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWWSG
 NTDYNTPTFSLRSLKNDNSKQVFFKMNSLQSQDIAIYYCARALTYDYEFAYWGQGLV
 TVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
 QSSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEG
 GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSREE
 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 122)

OPP022: LC 3954-0001-C225v5

Sequences provided above

CI090: 3954-0001-C225v5Fcm4-h20GG-0001-v16sc-H-N

pLW233: HC h20GG-0001-v16sc-C225v5Fcm4 (H-N)

Nucleotide Sequence

[spacer SEQ ID NO:191][pLW233_without spacer SEQ ID NO:194]

CAAGGCCAGTCTGGATCCGGTTATCTGTGGGGTTGCGAGTGGAATTGCGGAGGGATCA
 CTACAGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAA

TCATGGCGGCGGTTCTCAAACCTGTAGTAACTCAAGAACCAAGCTTCTCCGTCTCCCCTG
 GGGGAACAGTCACACTTACCTGCCGAAGTAGTACAGGTGCTGTTACGACCAGTAACTA
 TGCCAATTGGGTACAACAAACGCCTGGTCAGGCTCCGCGCGGATTGATAGGAGGCACG
 AATAAACGGGCACCCGGTGTCCCGGACAGATTCAGCGGAAGCATACTCGGTAATAAGG
 CAGCTCTTACTATCACTGGGGCCCAAGCTGATGATGAAAGTGATTATTATTGTGCGCTC
 TGGTACAGCAACCTCTGGGTGTTTGGGGGTGGCACGAACTTACTGTCTTGGGCGGCG
 GCGGATCAGGGGGAGGTGGCTCTGGAGGAGGAGGCTCAGAAGTCCAACTGGTTCGAAT
 CCGGGGGAGGGCTCGTACAGCCGGGTGGGTCCCTCAAACCTCTCTTGTGCGGCCTCAGG
 GTTTACCTTCAGTACATACGCGATGAATTGGGTCCGGCAGGCCAGTGGGAAAGGGCTC
 GAATGGGTAGGACGAATCCGATCAAAATACAACAACCTACGCTACTTATTACGCTGATT
 CCGTGAAGGACAGATTCACAATATCCCGCGACGATAGCAAGAATACGGCATATCTTCA
 GATGAATTCTCTTAAACTGAGGATACCGCTGTGTATTACTGCACAAGACATGGTAATT
 TTGGAAACTCATATGTCTCTTGGTTTCGCTTATTGGGGACAGGGCACGTTGGTTACCGTG
 TCTAGCGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGC
 AGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCAACTA
 TGGCGTGCAATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATT
 TGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCTGAGCATTAAACA
 AAGATAACAGCAAAAGCCAGGTGTTTTTAAATGAACAGCCTGCAAAGCCAGGATAC
 CGCGATTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTGCGTATTGGG
 GCCAGGGCACCTTGGTGACCGTGAGCGCGGTACACCAAGGGCCCCATCGGTCTTCCC
 CCTGGCACCTCTCTCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC
 AAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCG
 GCGTGACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG
 GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACA
 AGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCA
 CACATGCCCACCGTGCCCAGCACCTGAATTTGAAGGGGGACCGTCAGTCTTCCTCTTCC
 CCCCCAAAACCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGT
 GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG
 GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGT
 GTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGT
 GCAAGGTCTCCAACAAAGCCCTCCCAGCCTCAATCGAGAAAACCATCTCCAAGCCAA
 AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC
 AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCG
 TGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCT
 GGACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGG
 CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACAC
 GCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGA (SEQ ID NO: 123)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW233_without spacer SEQ ID NO:195]

QQQSGS[G Y L W G C E W N C G G I T T][G S S G G S G G S G G][L S G R S D N H][G G G S]Q T V V T Q E P S F S V
 S P G G T V T L T C R S S T G A V T T S N Y A N W V Q Q T P G Q A P R G L I G G T N K R A P G V P D R F S G S I L G N K A
 A L T I T G A Q A D D E S D Y Y C A L W Y S N L W V F G G G T K L T V L[G G G G S G G G S G G G S]E V Q L V E S
 G G G L V Q P G G S L K L S C A A S G F T F S T Y A M N W V R Q A S G K L E W V G R I R S K Y N N Y A T Y Y A D S V
 K D R F T I S R D D S K N T A Y L Q M N S L K T E D T A V Y Y C T R H G N F G N S Y V S W F A Y W G Q G T L V T V S S[

GGGGS]QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSG
 NTDYNTPTFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLV
 TVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
 QSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEG
 GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 QSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSREE
 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSCSVMEALHNHYTQKSLSLSPGK (SEQ ID NO: 124)

OPP022: LC 3954-0001-C225v5

Sequences provided above

Activated CI090: Activated- 3954-0001-C225v5Fcmt4-h20GG--0001-v16sc-H-N

Activated pLW233: HC C225v5Fcmt4-h20GG-0001-v16sc (H-N)

Nucleic Acid Sequence

TCCGATAATCATGGCGCGGTTCTCAAACCTGTAGTAACCTCAAGAACCAAGCTTCTCCGT
 CTCCCCTGGGGGAACAGTCACACCTTACCTGCCGAAGTAGTACAGGTGCTGTTACGACC
 AGTAACATAGCCAATTGGGTACAACAACCGCTGGTCAGGCTCCGCGCGGATTGATAG
 GAGGCACGAATAAACGGGCACCCGGTGTCCCGGACAGATTCAGCGGAAGCATACTCGG
 TAATAAGGCAGCTCTTACTATCACTGGGGCCCAAGCTGATGATGAAAGTGATTATTATT
 GTGCGCTCTGGTACAGCAACCTCTGGGTGTTTGGGGGTGGCAGCAAACCTTACTGTCTTG
 GGCGGCGGCGGATCAGGGGGAGGTGGCTCTGGAGGAGGAGGCTCAGAAGTCCAACCTG
 GTCGAATCCGGGGGAGGGCTCGTACAGCCGGGTGGGTCCCTCAAACCTCTCTTGTGCGG
 CCTCAGGGTTTACCTTCAGTACATACGCGATGAATTGGGTCCGGCAGGCCAGTGGGAA
 AGGGCTCGAATGGGTAGGACGAATCCGATCAAAATACAACAACCTACGCTACTTATTAC
 GCTGATTCCGTGAAGGACAGATTCACAATATCCCGCGACGATAGCAAGAATACGGCAT
 ATCTTCAGATGAATTCTCTTAAACTGAGGATACCGCTGTGTATTACTGCACAAGACAT
 GGTAATTTTGGAAACTCATATGTCTCTTGGTTTCGCTTATTGGGGACAGGGCACGTTGGT
 TACCGTGTCTAGCGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGCGGCCCGGGC
 CTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGA
 CCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGG
 CGTGATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGC
 ATTAACAAAGATAACAGCAAAAAGCCAGGTGTTTTTTAAAATGAACAGCCTGCAAAGCC
 AGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTGCG
 TATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGG
 TCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGC
 CTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGA
 CCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGC
 AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGA
 ATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAA
 AACTCACACATGCCCACCGTGCCCAGCACCTGAATTTGAAGGGGGACCGTCAGTCTTCC
 TCTTCCCCCAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGC
 GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG

GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGT
 ACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTA
 CAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCTCAATCGAGAAAACCATCTCCAAA
 GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCATCCCGGGAGGAGA
 TGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACAT
 CGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACTACAAGACCACGCCTCC
 CGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCA
 GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCA
 CTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 164)

Amino Acid Sequence

[SDNH]GGGSQTVVTQEFSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIGG
 TNKRAPGVPRDFSGSILGNKAALTITGAQADDESYYCALWYSNLWVFGGGTKLTVLGGG
 GSGGGSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFSTYAMNWVRQASGKGLEW
 VGRIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRHNFGNS
 YVSWFAYWGQGLTVTVSSGGGGSQVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWV
 RQSPGKGLEWLGVWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARA
 LTYDYEFAYWGQGLTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS
 WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
 SCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
 GVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKNTISKYK
 GQPREPQVYITLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSD
 GSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 165)

Activated OPP022: 3954-0001-C225v5

Nucleic Acid Sequence

TCCGATAATCATGGCAGTAGCGGTACCCAGATCTTGCTGACCCAGAGCCCGGTGATTCT
 GAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGCATTGGC
 ACCAACATTTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAAT
 ATGCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCAC
 CGATTTTACCCTGAGCATTAAACAGCGTGGAAGCGAAGATATTGCGGATTATTATTGCC
 AGCAGAACAACTGGCCGACCACCTTTGGCGCGGGCACCAAACTGGAAGTAAACG
 TACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG
 GAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAG
 TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGG
 ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTA
 CGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
 ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO: 166)

Amino Acid Sequence

[SDNH]GSSGTQILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRNNGSPRLLIKYASES
 ISGIPSRFSGSGSGTDFTLINSVESEDIADYYCQNNNWPTTFGAGTKLELKRTVAAPSVFIF

PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTL
 TLSKADYEHKHYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 167)

CI104: 3954-0011-C225v5Fcm4-h20GG-0011-v16sc-H-N

pLW289 and pLW291 sequences encoding corresponding masked antibody components are provided herein as “pLW289” and “pLW291”, respectively and are summarized in Table 11.

Activated CI104: 3954-0011-C225v5Fcm4-h20GG-0011-v16sc-H-N

Activated pLW289: HC h20GG-0011-v16sc-C225v5Fcm4 (H-N)

Nucleotide Sequence

TCCGATGATCATGGCGGCGGTTCTCAAACCTGTAGTAAGTCAAGAACCAAGCTTCTCCGT
 CTCCCCTGGGGGAACAGTCACACTTACCTGCCGAAGTAGTACAGGTGCTGTACGACC
 AGTAACTATGCCAATTGGGTACAACAAACGCCTGGTCAGGCTCCGCGCGGATTGATAG
 GAGGCACGAATAAACGGGCACCCGGTGTCCCGGACAGATTACAGCGGAAGCATACTCGG
 TAATAAGGCAGCTCTTACTATCACTGGGGCCCAAGCTGATGATGAAAGTGATTATTATT
 GTGCGCTCTGGTACAGCAACCTCTGGGTGTTTGGGGGTGGCAGCAAAGTACTGCTTG
 GCGCGGCGGAGTACAGGGGGAGGTGGCTCTGGAGGAGGAGGCTCAGAAGTCCAATG
 GTCAATCCGGGGGAGGGCTCGTACAGCCGGGTGGGTCCCTCAAACCTCTCTTGTGCGG
 CCTCAGGGTTTACCTTCAGTACATACGCGATGAATTGGGTCCGGCAGGCCAGTGGGAA
 AGGGCTCGAATGGGTAGGACGAATCCGATCAAAATACAACAAGTACGCTACTTATTAC
 GCTGATTCCGTGAAGGACAGATTACAAATATCCCGCGACGATAGCAAGAATACGGCAT
 ATCTTCAGATGAATTCTCTTAAACTGAGGATACCGCTGTGTATTACTGCACAAGACAT
 GGTAATTTTGGAAACTCATATGTCTCTTGGTTCGCTTATTGGGGACAGGGCACGTTGGT
 TACCGTGTCTAGCGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGCGGCCCCGGGC
 CTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGA
 CCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGGCTGGG
 CGTGATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGC
 ATTAACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAAATGAACAGCCTGCAAAGCC
 AGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTGCG
 TATTGGGGCCAGGGCACCTGCTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGG
 TCTTCCCCCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGC
 CTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAAGTACAGGCGCCCTGA
 CCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCTCAGC
 AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGA
 ATCACAAGCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAA
 AACTCACACATGCCACCGTGCCAGCACCTGAATTTGAAGGGGGACCGTCAGTCTTCC
 TCTTCCCCCCTGAGGACACCTCATGATCTCCCGGACCCCTGAGGTCACATGC
 GTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACG
 GCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGT
 ACCGTGTGGTCAGCGTCTCACCCTGTCACAGGACTGGCTGAATGGCAAGGAGTA
 CAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCTCAATCGAGAAAACCATCTCCAAA

GCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGA
 TGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACAT
 CGCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAACTACAAGACCACGCCTCC
 CGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCA
 GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCA
 CTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 125)

Amino Acid Sequence

[SDDH][GGGS]QTVVTQEPSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIG
 GTNKRAPGVPRFSGSILGNKAALTITGAQADDESYYCALWYSNLWVFGGGTKLTVL][G
 GGGSGGGSGGGGS]EVQLVESGGGLVQPGGSLKLSCAASGFTFSTYAMNWVRQASGKGL
 EWVGRIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRHNFG
 GNSYVSWFAYWGQGLVTVSS[GGGS]QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYG
 VHWVRQSPGKGLEWLGVIWSSGNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIY
 YCARALTYDYEFAYWGQGLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
 EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD
 KKVEPKSCDKTHTCPPCPAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
 FNWYVDGVEVHNAKTKPREEQYQSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPASIE
 KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT
 PPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID
 NO: 126)

Activated pLW291: LC 3954-0011-C225v5

Nucleotide Sequence

TCCGATGATCATGGCAGTAGCGGTACCCAGATCTTGCTGACCCAGAGCCCGGTGATTCT
 GAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGCATTGGC
 ACCAACATTTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAAT
 ATGCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCAC
 CGATTTTACCCTGAGCATTAAACAGCGTGGAAGCGAAGATATTGCGGATTATTATTGCC
 AGCAGAAACAACAACTGGCCGACCACCTTTGGCGCGGGCACCAAACTGGAAGTAAACG
 TACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG
 GAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAG
 TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGG
 ACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTA
 CGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTC
 ACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO: 127)

Amino Acid Sequence

[SDDH][GSSGT]QILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRRTNGSPRLLIKYASE
 SISGIPSRFSGSGSGTDFTLINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFI
 FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSST
 LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 128)

CI106: CF41-2008-C225v5Fcmt4-h20GG-0011-v16sc-H-N**pLW289: HC h20GG-0011-v16sc-C225v5Fcmt4 (H-N)***Nucleotide Sequence*

[spacer SEQ ID NO:191][pLW289 without spacer SEQ ID NO:196]

CAAGGCCAGTCTGGATCCGGTTATCTGTGGGGTTGCGAGTGGAATTGCGGAGGGATCA
CTACAGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATGA
TCATGGCGGCGGTTCTCAAACCTGTAGTAACTCAAGAACCAAGCTTCTCCGTCTCCCCTG
GGGGAACAGTCACACTTACCTGCCGAAGTAGTACAGGTGCTGTTACGACCAGTAACTA
TGCCAATTGGGTACAACAAACGCCTGGTCAGGCTCCGCGCGGATTGATAGGAGGCACG
AATAAACGGGCACCCGGTGTCCCGGACAGATTCAGCGGAAGCATACTCGGTAATAAGG
CAGCTCTTACTATCACTGGGGCCCAAGCTGATGATGAAAGTGATTATTATTGTGCGCTC
TGGTACAGCAACCTCTGGGTGTTTGGGGGTGGCACGAACTTACTGTCTTGGGCGGCG
GCGGATCAGGGGGAGGTGGCTCTGGAGGAGGAGGCTCAGAAGTCCAACCTGGTCAAT
CCGGGGGAGGGCTCGTACAGCCGGGTGGGTCCCTCAAACCTCTTGTGCGGCCTCAGG
GTTTACCTTCAGTACATACGCGATGAATTGGGTCCGGCAGGCCAGTGGGAAAGGGCTC
GAATGGGTAGGACGAATCCGATCAAAATACAACAACCTACGCTACTTATTACGCTGATT
CCGTGAAGGACAGATTACAAATATCCCGCGACGATAGCAAGAATACGGCATATCTTCA
GATGAATTCTCTTAAACCTGAGGATACCGCTGTGTATTACTGCACAAGACATGGTAATT
TTGGAAACTCATATGTCTCTTGGTTTCGCTTATTGGGGACAGGGCACGTTGGTTACCGTG
TCTAGCGGAGGTGGTGGATCCAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTG
AGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCAACTA
TGGCGTGCATTGGGTGCGCCAGAGCCCAGGCAAAGGCCTGGAATGGCTGGGCGTGATT
TGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAAACA
AAGATAACAGCAAAAGCCAGGTGTTTTTTTAAATGAACAGCCTGCAAAGCCAGGATAC
CGCGATTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTGCGTATTGGG
GCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCC
CCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTC
AAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTGACCAGCG
GCGTGACACCTTCCCGGTGTCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTG
GTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACA
AGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCA
CACATGCCCACCGTGCCCAGCACCTGAATTTGAAGGGGGACCGTCAGTCTTCTCTTCC
CCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTG
GAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGT
GTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGT
GCAAGGTCTCCAACAAAGCCCTCCCAGCCTCAATCGAGAAAACCATCTCCAAGCCAA
AGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACC
AAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCG
TGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCGTGCT
GGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGAACAAGAGCAGGTGG
CAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACAC
GCAGAAGAGCCTCTCCCTGTCTCCGGGTAAG (SEQ ID NO: 129)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW289 without spacer SEQ ID NO:197]

QQQSGS[GYLWGCEWNCGGITT][GSSGGSGGSGG][LSGRSDDH][GGGS]QTVVTQEPSFSV
 SPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIGGTNKRAPGVPDFSGSILGNKA
 ALTITGAQADDES DYICALWYSNLWVFGGGTKLTVL[GGGGSGGGSGGGGS]EVQLVES
 GGGLVQPGGSLKLSCAASGFTFSTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYADSV
 KDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRHGNFGNSYVSWFAYWGQGTLVTVSS[
 GGGGS]QVQLKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSSG
 NTDYNTPFTRSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGTLV
 TVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
 QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEG
 GPSVFLFPPKPKD TLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 QSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSREE
 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 130)

pLW246: LC CF41-2008-C225v5*Nucleotide Sequence*

[spacer SEQ ID NO:176][pLW246 without spacer SEQ ID NO:198]

CAAGGCCAGTCTGGCCAAGGTCCTAGTTGTGAAGGTTGGGCGATGAATAGAGAACAAAT
 GTTCGAGCCGGAGGTGGCTCGAGCGGCGGCTCTATCTCTTCCGACTGCTGTCCGGCAG
 ATCCGACCAGCACGGCGGAGGATCCCAAATCCTGCTGACACAGTCTCCTGTCATACTG
 AGTGTCTCCCCCGCGAGAGAGTCTCTTTCTCATGTCTGGGCCAGTCAGTCTATTGGGAC
 TAACATACTACTGGTACCAGCAACGCACCAACGGAAGCCCGCGCCTGCTGATTAAATAT
 GCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCG
 ATTTTACCCTGAGCATTAAACAGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAG
 CAGAACAACTGGCCGACCACCTTTGGCGCGGGCACCAAACTGGAAGTAAACGTA
 CGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA
 ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG
 GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACACAGAGCAGGAC
 AGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACG
 AGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCAC
 AAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO: 131)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW246 without spacer SEQ ID NO:199]

QQQSGQG[LSCEGWAMNREQCRA][GGGSSGGGS][ISSGLLSGRSDQH][GGGS]QILLTQSPVI
 LSVSPGERVSFSCRASQSIGTNIHWYQQR TNGSPRL LIKYASESISGIPSRFSGSGSGTDFTLI
 NSVESEDIADYYCQNNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVCLL
 NNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEV
 THQGLSPVTKSFNRGEC (SEQ ID NO: 132)

CI107: 3954-0011-C225v5Fcm4-h20GG-2006-v16sc-H-N**pLW307: HC h20GG-2006-v16sc-C225v5Fcm4 (H-N)***Nucleotide Sequence*

[spacer SEQ ID NO:191][pLW307 without spacer SEQ ID NO:200]

CAAGGCCAGTCTGGATCCGGTTATCTGTGGGGTTGCGAGTGGAATTGCGGAGGGATCA
CTACAGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATATCGAGTGGATTGCTGTCT
GGCAGATCTGACGATCACGGCGGCGGTCTCAAACCTGTAGTAACTCAAGAACCAAGCT
TCTCCGTCTCCCCTGGGGGAACAGTCACACTTACCTGCCGAAGTAGTACAGGTGCTGTT
ACGACCAGTAACTATGCCAATTGGGTACAACAAACGCCTGGTCAGGCTCCGCGCGGAT
TGATAGGAGGCACGAATAAACGGGCACCCGGTGTCCCGGACAGATTACAGCGGAAGCAT
ACTCGGTAATAAGGCAGCTCTTACTATCACTGGGGCCCAAGCTGATGATGAAAGTGAT
TATTATTGTGCGCTCTGGTACAGCAACCTCTGGGTGTTTGGGGGTGGCACGAACTTAC
TGTCTTGGGCGGCGGCGGATCAGGGGGAGGTGGCTCTGGAGGAGGAGGCTCAGAAGTC
CAACTGGTCGAATCCGGGGGAGGGCTCGTACAGCCGGGTGGGTCCCTCAAACCTCTCTT
GTGCGGCCTCAGGGTTTACCTTCAGTACATACGCGATGAATTGGGTCCGGCAGGCCAGT
GGGAAAGGGCTCGAATGGGTAGGACGAATCCGATCAAAATACAACAACACTACGCTACTT
ATTACGCTGATTCCGTGAAGGACGATTACAAATATCCCGCGACGATAGCAAGAATAC
GGCATATCTTCAGATGAATTCTCTTAAACCTGAGGATACCGCTGTGTATTACTGCACAA
GACATGGTAATTTTGGAACTCATATGTCTCTTGGTTTCGCTTATTGGGGACAGGGCACG
TTGGTTACCGTGTCTAGCGGAGGTGGTGGATCCAGGTGCAGCTGAAACAGAGCGGCC
CGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAG
CCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCAGGCAAGGCCTGGAATGG
CTGGGCGTGATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCT
GAGCATTAACAAAGATAACAGCAAAAGCCAGGTGTTTTTTAAATGAACAGCCTGCAA
AGCCAGGATACCGGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATT
TGCGTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCA
TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGG
CTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACCTCAGGCGCC
CTGACCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCCTCAGGACTCTACTCCCT
CAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
ACAAAACCTCACACATGCCCACCGTGCCAGCACCTGAATTTGAAGGGGGACCGTCAGT
CTTCCTCTTCCCCCAAAACCCAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCA
CATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGT
GGACGGCGTGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAG
CACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAG
GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCTCAATCGAGAAAACCATCT
CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGGA
GGAGATGACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACG
CCTCCCGTGCTGGACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTCACCGTGGACAA
GAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCAC
AACCCTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 133)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW307 without spacer SEQ ID NO:201]

QQQSGS[GYLWGCEWNCGGITT][GSSGGSGGSGG][ISSGLLSGRSDDH][GGGS]QTVVTQE
 PSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIGGTNKRAPGVPDRFSGSIL
 GNKAALTITGAQADDESDYYCALWYSNLWVFGGGTKLTVL[GGGGSGGGSGGGGS]EV
 QLVESGGGLVQPGGSLKLSCAASGFTFSTYAMNWVRQASGKGLEWVGRIRSKYNNYATY
 YADSVKDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRHGNFGNSYVSWFAYWQGGL
 VTVSS[GGGS]QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLG
 VIWSGGNTDYNTPTFSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYW
 GQGLTVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
 TFPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
 APEFEGGSPVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
 REEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTL
 PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVD
 KSRWQQGNVDFSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 134)

pLW291: LC 3954-0011-C225v5*Nucleotide Sequence*

[spacer SEQ ID NO:180][pLW291 without spacer SEQ ID NO:202]

CAAGGCCAGTCTGGCCAGTGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCAT
 GTACGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGATCCGGTCTGAGCGGCCGTTCC
 GATGATCATGGCAGTAGCGGTACCCAGATCTTGCTGACCCAGAGCCCGGTGATTCTGA
 GCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAGCCAGAGCATTGGCAC
 CAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCGCGCCTGCTGATTAAATAT
 GCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCAGCGGCAGCGGCACCG
 ATTTTACCCTGAGCATTAAACAGCGTGGAAGCGAAGATATTGCGGATTATTATTGCCAG
 CAGAACAACTGGCCGACCACCTTTGGCGCGGGCACCAAACTGGAAGTAAACGTA
 CGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA
 ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTG
 GAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC
 AGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACG
 AGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCAC
 AAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO: 135)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW291 without spacer SEQ ID NO:203]

QQQSGQ[CISPRGCPDGPYVMY][GSSGGSGGSGGSG][LSGRSDDH][GSSGT]QILLTQSPVIL
 SVSPGERVVSFCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRFSGSGSGTDFTLIN
 SVESEDIADYYCQNNNWPTTFGAGTKLELKRVAAPSVFIFPPSDEQLKSGTASVVCLLN
 NFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVT
 HQGLSSPVTKSFNRGEC (SEQ ID NO: 136)

CI127: SynFcmt4-h20GG-0011-v16sc-H-N**pLW334: HC h20GG-0011-v16sc-Synagis®Fcmt4 (H-N)***Nucleotide Sequence*

[spacer SEQ ID NO:191][pLW334 without spacer SEQ ID NO:204]

CAAGGCCAGTCTGGATCCGGTTATCTGTGGGGTTGCGAGTGGAATTGCGGAGGGATCA
CTACAGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATGA
TCATGGCGGCGGTTCTCAAACCTGTAGTAACTCAAGAACCAAGCTTCTCCGTCTCCCCTG
GGGGAACAGTCACTTACCTGCCGAAGTAGTACAGGTGCTGTTACGACCAGTAACTA
TGCCAATTGGGTACAACAAACGCCTGGTCAGGCTCCGCGCGGATTGATAGGAGGCACG
AATAAACGGGCACCCGGTGTCCCGGACAGATTCAGCGGAAGCATACTCGGTAATAAGG
CAGCTCTTACTATCACTGGGGCCCAAGCTGATGATGAAAGTGATTATTATTGTGCGCTC
TGGTACAGCAACCTCTGGGTGTTTGGGGGTGGCACGAACTTACTGTCTTGGGCGGCG
GCGGATCAGGGGGAGGTGGCTCTGGAGGAGGAGGCTCAGAAGTCCAACCTGGTCAAT
CCGGGGGAGGGCTCGTACAGCCGGGTGGGTCCCTCAAACCTCTTGTGCGGCCTCAGG
GTTTACCTTCAGTACATACGCGATGAATTGGGTCCGGCAGGCCAGTGGGAAAGGGCTC
GAATGGGTAGGACGAATCCGATCAAAATACAACAACCTACGCTACTTATTACGCTGATT
CCGTGAAGGACAGATTACAAATATCCCGCGACGATAGCAAGAATACGGCATATCTTCA
GATGAATTCTCTTAAACCTGAGGATACCGCTGTGTATTACTGCACAAGACATGTTAATT
TTGAAACTCATATGTCTCTTGGTTTCGCTTATTGGGGACAGGGCACGTTGGTTACCGTG
TCTAGCGGAGGTGGTGGATCCCAAGTGACCCTGAGAGAGTCTGGCCCTGCCCTCGTGA
AGCCTACCCAGACCCTGACACTGACCTGCACCTTCAGCGGCTTCAGCCTGAGCACCAGC
GGCATGTCTGTGGGCTGGATCAGACAGCCTCCTGGCAAGGCCCTGGAATGGCTGGCCG
ACATTTGGTGGGACGACAAGAAGGACTACAACCCAGCCTGAAGTCCCGGCTGACCAT
CAGCAAGGACACCAGCAAGAACCAGGTGGTGGTGAAGTGACCAACATGGACCCCGC
CGACACCGCCACCTACTACTGCGCCAGATCCATGATCACCACCTGGTACTTCGACGTGT
GGGGAGCCGGCACCACCGTGACAGTGTCTGCTAGCACCAAGGGCCCATCGGTCTT
CCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTG
GTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCA
GCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGC
GTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATC
ACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAA
CTCACACATGCCACCGTGCCACGACCTGAATTTGAAGGGGGACCGTCAGTCTTCCTC
TTCCCCCAGAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGT
GGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC
GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTAC
CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACA
AGTGCAAGGTCTCCAACAAAGCCCTCCAGCCTCAATCGAGAAAACCATCTCCAAAGC
CAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATG
ACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATCCAGCGACATCG
CCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCCCG
TGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGG
TGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTA
CACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAG (SEQ ID NO: 137)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW334 without spacer SEQ ID NO:205]

QGQSGS[GYLWGCEWNCGGITT][GSSGGSGGSGG][LSGRSDDH][GGGS]QTVVTQEPSFSV
 SPGGTVTLTCRSSSTGAVTTSNYANWVQQTPGQAPRGLIGGTNKRAPGVPDRFSGSILGNKA
 ALTITGAQADDES DYICALWYSNLWVFGGGTKLTVL[GGGGSGGGSGGGGS]EVQLVES
 GGGLVQPGGSLKLSCAASGFTFSTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYADSV
 KDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRHGNFGNSYVSWFAYWGQGLTVTVSS[
 GGGGS]QVTLRESGPALVKPTQTLTLCTFSGFSLSSTSGMSVGVIRQPPGKALEWLADIWW
 DDKKDYNPSLKSRLTISKDTSKNQVVLKVTNMDPADTATYYCARSMITNWFYFDVWGAGT
 TVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
 LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFE
 GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSRE
 EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 138)

pLW139: LC Synagis®*Nucleotide Sequence*

GACATCCAGATGACCCAGAGCCCCAGCACACTGAGCGCCAGCGTGGGCGACAGAGTG
 ACCATCACATGCAAGTGCCAGCTGAGCGTGGGCTACATGCACTGGTATCAGCAGAAGC
 CCGGCAAGGCCCCCAAGCTGCTGATCTACGACACCAGCAAGCTGGCCTCCGGCGTGCC
 CAGCAGATTTTCTGGCAGCGGCTCCGGCACCGAGTTCACCCTGACAATCAGCAGCCTGC
 AGCCCGACGACTTCGCCACCTACTACTGTTTTCAAGGCTCCGGCTACCCCTTCACCTTC
 GGCGGAGGCACCAAGCTGGAAATCAAGCGGACGGTGGCTGCACCATCTGTCTTCATCT
 TCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAAT
 AACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGG
 GTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCA
 GCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGA
 AGTCAACCATCAGGGCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
 (SEQ ID NO: 139)

Amino Acid

*Sequence*DIQMTQSPSTLSASVGDRVITITCKCQLSVGYMHWYQQKPGKAPKLLIYDTSKLAS
 GVPSRFSGSGSGTEFTLTISSLQPDFFATYYCFQSGYPFTFGGGTKLEIKRTVAAPSVFIFPP
 SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
 LSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC (SEQ ID NO: 140)

CI128: SynFcmt4-h20GG-2006-v16sc-H-N**pLW335: HC h20GG-2006-v16sc-Synagis®Fcmt4 (H-N)***Nucleotide Sequence*

[spacer SEQ ID NO:191][pLW335 without spacer SEQ ID NO:206]

CAAGGCCAGTCTGGATCCGGTTATCTGTGGGGTTGCGAGTGGAATTGCGGAGGGATCA
CTACAGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATATCGAGTGGATTGCTGTCT
GGCAGATCTGACGATCACGGCGGCGGTCTCAAACCTGTAGTAACCAAGAACCAAGCT
TCTCCGTCTCCCCTGGGGGAACAGTCACACTTACCTGCCGAAGTAGTACAGGTGCTGTT
ACGACCAGTAACATATGCCAATTGGGTACAACAAACGCCTGGTCAGGCTCCGCGCGGAT
TGATAGGAGGCACGAATAAACGGGCACCCGGTGTCCCGGACAGATTACAGCGGAAGCAT
ACTCGGTAATAAGGCAGCTCTTACTATCACTGGGGCCCAAGCTGATGATGAAAGTGAT
TATTATTGTGCGCTCTGGTACAGCAACCTCTGGGTGTTTGGGGGTGGCACGAACTTAC
TGTCTTGGGCGGCGGCGGATCAGGGGGAGGTGGCTCTGGAGGAGGAGGCTCAGAAGTC
CAACTGGTCGAATCCGGGGGAGGGCTCGTACAGCCGGGTGGGTCCCTCAAACCTCTCTT
GTGCGGCCTCAGGGTTTACCTTCAGTACATACGCGATGAATTGGGTCCGGCAGGCCAGT
GGGAAAGGGCTCGAATGGGTAGGACGAATCCGATCAAAATACAACAACCTACGCTACTT
ATTACGCTGATTCCGTGAAGGACGATTACAAATATCCCGCGACGATAGCAAGAATAAC
GGCATCTTTCAGATGAATTCTCTTAAACCTGAGGATACCGCTGTATTACTGCACAA
GACATGGTAATTTTGGAACTCATATGTCTCTTGGTTCGCTTATTGGGGACAGGGCACG
TTGGTTACCGTGTCTAGCGGAGGTGGTGGATCCCAAGTGACCCTGAGAGAGTCTGGCC
CTGCCCTCGTGAAGCCTACCCAGACCCTGACACTGACCTGCACCTTCAGCGGCTTCAGC
CTGAGCACCAGCGGCATGTCTGTGGGCTGGATCAGACAGCCTCCTGGCAAGGCCCTGG
AATGGCTGGCCGACATTTGGTGGGACGACAAGAAGGACTACAACCCCAGCCTGAAGTC
CCGGCTGACCATCAGCAAGGACACCAGCAAGAACCAGGTGGTGGTGAAGTGACCAA
CATGGACCCCGCCGACACCGCCACCTACTACTGCGCCAGATCCATGATCACCAACTGGT
ACTTCGACGTGTGGGGAGCCGGCACCACCGTGACAGTGTCTGCTAGCACCAAGGG
CCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCC
TGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGG
CGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACT
CCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGC
AACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTT
GTGACAAAACCTCACACATGCCCACCGTGCCAGCACCTGAATTTGAAGGGGGACCGTC
AGTCTTCCTCTTCCCCCAAACCCAAAGGACACCCTCATGATCTCCCGGACCCCTGAGG
TCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTA
CGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCA
GAGCACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGC
AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCAGCCTCAATCGAGAAAACCA
TCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCG
GGAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCC
AGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACC
ACGCTCCCGTGCTGGACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTACCGTGGA

CAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTG
CACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 141)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW335 without spacer SEQ ID NO:207]

QQQSGS[GYLWGCEWNCGGITT][GSSGGSGGSGG][ISSGLLSGRSDDH][GGGS]QTVVTQE
PSFSVSPGGTVTLTCRSSTGAVTTSNYANWVQQTPGQAPRGLIGGTNKRAPGVDRFSGSIL
GNKAALTITGAQADDESYYCALWYSNLWVFGGGTKLTVL[GGGGSGGGSGGGGS]EV
QLVESGGGLVQPGGSLKLSAASGFTFSTYAMNWVRQASGKGLEWVGRIRSKYNNYATY
YADSVKDRFTISRDDSNTAYLQMNLSKLTEDTAVYYCTRHNFGNSYVSWFAYWGQGT
LTVSS[GGGG]QVTLRESGPALVKPTQTLTLCTFSGFSLSTSGMSVGVIRPPGKALEWL
ADIWDDDKDYNPSLKSRLTISKDTSKNQVVLKVTNMDPADTATYYCARSMITNWFYFDV
WGAGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
PAPEFEGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTV
DKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 142)

pLW139: LC Synagis®

Sequences provided above

CI135: CF41-2008-C225v5Fcmt4-h20GG-0011-v12sc-H-N

pLW352: HC h20GG-0011-v12sc-C225v5Fcmt4 (H-N)

Nucleotide Sequence

[spacer SEQ ID NO:208][pLW352 without spacer SEQ ID NO:209]

CAAGGCCAGTCTGGTTCTGGTTATCTGTGGGGTTGCGAGTGGAATTGCGGAGGGATCA
CTACAGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATGA
TCATGGCGGCGGATCCCAGACGGTAGTGACTCAGGAGCCATCATTTTCTGTCTCTCCTG
GAGGTACTGTGACACTCACATGTAGAAGCTCAACTGGTGCAGTCACCACTTCAAATTAC
GCGAATTGGGTCCAGCAGACCCCTGGGCAGGCTCCGAGAGGGTTGATTGGAGGTACTA
ACAAACGGGCACCGGGAGTGCCTGATAGGTTTTCCGGTTCTATTCTCGGAAACAAGGC
GGCTCTCACGATCACGGGTGCGCAGGCCGACGATGAATCAGACTATTACTGCGCTTTGT
GGTACTCAAACCTGTGGGTATTCGGAGGGGGCACCAAGCTGACGGTGTGGGTGGGGG
GGGCTCTGGGGGAGGGGGAAGCGGAGGTGGGGGCAGCGAGGTTGAGCTTGTGAAAG
TGGTGGCGGACTCGTACAACCGGGTGGAAGTCTTAGACTCTCATGTGCAGCATCTGGAT
TTACTTTTTCTACTTATGCTATGAAGTGGGTAAGACAGGCACCGGGGAAAGGGCTGGA
ATGGGTGTCACGCATTGATCTAAATACAATAACTATGCTACATACTACGCCGATAGTG
TTAAGGATCGATTCACTATATCTCGGGACGACAGTAAGAACTCACTTTACCTGCAGATG
AATTCCTGAAAACCTGAGGACACGGCCGTTTATTATTGTGTACGGCACGGGAATTCGG
CAATTCTTACGTTTCTGTTTCGCCTATTGGGGGCAAGGTACGCTGGTACGGTGTCTA
GCGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGCAGCC
GAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCAACTATGGC

GTGCATTGGGTGCGCCAGAGCCCGGGCAAAGGCCTGGAATGGCTGGGCGTGATTTGGA
 GCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCTGAGCATTAACAAAGA
 TAACAGCAAAAGCCAGGTGTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCGCG
 ATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATTTGCGTATTGGGGCCA
 GGGCACCTTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTG
 GCACCTCTCTCAAGAGCACCTCTGGGGGACAGCGGCCCTGGGCTGCCTGGTCAAGG
 ACTACTTCCCCGAACCGGTGACGGTGTCGTGGAATCAGGCGCCCTGACCAGCGGCGT
 GCACACCTTCCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGA
 CCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCC
 CAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACACA
 TGCCCACCGTGCCCAGCACCTGAATTTGAAGGGGGACCGTCAGTCTTCCTCTTCCCCC
 AAAACCCAAGGACACCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTG
 GACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGG
 TGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGTGGT
 CAGCGTCCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAG
 GTCTCCAACAAAGCCCTCCAGCCTCAATCGAGAAAACCATCTCCAAGCCAAAGGGC
 AGCCCCGAGAACCACAGGTGTACACCTGCCCCCATCCCGGGAGGAGATGACCAAGAA
 CCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGT
 GGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGTGCTGGACTC
 CGACGGCTCCTTCTTCTCTACAGCAAGCTCACCCTGGACAAGAGCAGGTGGCAGCAG
 GGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGA
 AGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 151)

Amino Acid Sequence

[spacer SEQ ID NO:176][pLW352 without spacer SEQ ID NO:210]

QQQSGS[GYLWGCEWNCGGITT][GSSGSGSGSGG][LSGRSDDH][GGGS]QTVVTQEPSFSV
 SPGGTVTLTCRSSTGAVTTSNYANWVQQTGQAPRGLIGGTNKRAPGVPDRFSGSILGNKA
 ALTITGAQADDES DYICALWYSNLWVFGGGTKLTVL[GGGSGGGSGGGGS]EVQLVES
 GGGLVQPGSLRLS CAASGFTFSTYAMNWVRQAPGKLEWVARIRSKYNNYATYYADSV
 KDRFTISRDDSKNSLYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTVSS[
 GGGGS]QVQLKQSGPGLVQPSQSL SITCTVSGFSLTNYGVHWVRQSPGKLEWLGVWWSG
 NTDYNTPTFSTRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTV
 TVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
 QSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEG
 GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 QSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSREE
 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 146)

pLW246: LC CF41-2008-C225v5

Nucleotide Sequence

Sequences provided above

*Amino Acid Sequence**Sequences provided above***CI136: CF41-2008-C225v5Fcmt4-h20GG-0011-v19sc-H-N****pLW353: HC h20GG-0011-v19sc-C225v5Fcmt4 (H-N)***Nucleotide Sequence*

[spacer SEQ ID NO:191][pLW353 without spacer SEQ ID NO:211]

CAAGGCCAGTCTGGATCCGGTTATCTGTGGGGTTGCGAGTGGAATTGCGGAGGGATCA
CTACAGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATGA
TCATGGCGGCGGTTCTCAGGCCGTTGTTACACAAGAGCCTTCACTTACTGTGTCTCCAG
GAGGCACTGTGACACTTACGTGCCGATCCTCTACGGGTGCCGTGACCACAAGCAACTA
TGCCAACTGGGTCCAGCAGAAGCCAGGTCAAGCGCCTCGAGGTCTGATCGGGGGCACG
AATAAACGAGCTCCTGGAACCTCCGGCCAGATTTTCTGGGAGTCTTATTGGTGGCAAGGC
GGCGTTGACCCTGAGTGGAGCCCAACCGGAAGACGAGGCCGAGTACTACTGCGCCTTG
TGGTATTCCAATTTGTGGGTCTTCGGAGGCGGAACAAAGCTCACAGTACTGGGAGGTG
GAGGTAGCGGGGGCGGAGGCTCCGGGGGAGGTGGTTCGGAAGTCCAGCTTGTGAATC
AGGTGGGGGCTTGGTACAACAGGTGGTTCCTGAAAGTTGCTGTGACGCTCCGGA
TTTACATTTAGTACGTATGCTATGAAGTGGGTGAGGCAGGCCAGTGTAAAGGTCTCGA
ATGGGTTGGCCGGATAAGGTCAAAGTACAATAATTACGCAACCTACTACGCGGATTCC
GTGAAAGACAGGTTCACTATTTACGAGATGATAGCAAAAATACTGCGTATCTCCAAA
TGAATAGTCTTAAAACTGAAGACACTGCCGTATATTATTGCACTAGGCACGGCAACTTT
GGTAACTCTTATGTTTCTTGGTTCGCATACTGGGGACAAGGAACCTTTGGTCACTGTCTC
ATCTGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGCGGCCCGGGCCTGGTGCAG
CCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAGCCTGACCAACTATG
GCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAGGCCTGGAATGGCTGGGCGTGATTTG
GAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCTGAGCATTAAACAAA
GATAACAGCAAAAGCCAGGTGTTTTTTAAATGAACAGCCTGCAAAGCCAGGATACCG
CGATTTATTATTGCGCGCGCGCTGACCTATTATGATTATGAATTTGCGTATTGGGGC
CAGGGCACCTTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCATCGGTCTTCCCCC
TGGCACCTCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAA
GGACTACTTCCCCGAACCGGTGACGGTGTCTGTGAACTCAGGCGCCCTGACCAGCGGC
GTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGT
GACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAG
CCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACCTCACA
CATGCCCACCGTGCCAGCACCTGAATTTGAAGGGGGACCGTCAGTCTTCTCTTCCCC
CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGG
TGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGA
GGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTACCGTGT
GGTCAGCGTCTCACCCTGCTGACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC
AAGGTCTCCAACAAAGCCCTCCAGCCTCAATCGAGAAAACCATCTCCAAAGCCAAAG
GGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGAGGAGATGACCAA
GAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTG

GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTG
 GACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGC
 AGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACAC
 GCAGAAGAGCCTCTCCCTGTCTCCGGGTAAG (SEQ ID NO: 152)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW353 without spacer SEQ ID NO:212]
 QGQSGS[GylWGCEWNCGGITT][GSSGGSGGSGG][LSGRSDDH][GGGS]QAVVTQEPSLTV
 SPGGTVTLTCRSSTGAVTTSNYANWVQQKPGQAPRGLIGGTNKRAPGTPARFSGSLIGGKA
 ALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLTVL[GGGSGGGGSGGGGS]EVQLVES
 GGGLVQPGGSLKLSAASGFTFSTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYADSV
 KDRFTISRDDSKNTAYLQMNSLKTEDTAVYYCTRHNFGNSYVSWFAYWGQGLTVTVSS[
 GGGGS]QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSG
 NTDYNTPTFSTRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTV
 TVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
 QSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFEG
 GPSVFLFPPKPKDITLMISRTPETCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
 QSTYRVVSVLTIVLHQDWLNGKEYKCKVSNKALPASIETISKAKGQPREPQVYTLPPSREE
 MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW
 QQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 148)

pLW246: LC CF41-2008-C225v5

Sequences provided above

CI091: 3954-1490DQH-C225v5Fcmt4-h20GG-2008-v16sc-H-N

pLW242: HC C225v5Fcmt4-h20GG-2008-v16sc (H-N)

Nucleic Acid Sequence

[spacer SEQ ID NO:191][pLW242 without spacer SEQ ID NO:213]
 CAAGGCCAGTCTGGATCCGGTTATCTGTGGGGTTGCGAGTGGAATTGCGGAGGGATCA
 CTACAGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTATATCGAGTGGATTGCTGTCT
 GGCAGATCTGACCAACACGGCGGCGGTCTCAAACCTGTAGTAACCTCAAGAACCAAGCT
 TCTCCGTCTCCCCTGGGGGAACAGTCACACTTACCTGCCGAAGTAGTACAGGTGCTGTT
 ACGACCAGTAACCTATGCCAATTGGGTACAACAAACGCCTGGTCAGGCTCCGCGCGGAT
 TGATAGGAGGCACGAATAAACGGGCACCCGGTGTCCCGGACAGATTACAGCGGAAGCAT
 ACTCGGTAATAAGGCAGCTCTTACTATCACTGGGGCCCAAGCTGATGATGAAAGTGAT
 TATTATTGTGCGCTCTGGTACAGCAACCTCTGGGTGTTTGGGGGTGGCACGAACTTAC
 TGTCTTGGGCGGCGGCGGATCAGGGGGAGGTGGCTCTGGAGGAGGAGGCTCAGAAGTC
 CAACTGGTTCGAATCCGGGGGAGGGCTCGTACAGCCGGGTGGGTCCCTCAAACCTCTCTT
 GTGCGGCCTCAGGGTTTACCTTCAGTACATACGCGATGAATTGGGTCCGGCAGGCCAGT
 GGGAAAGGGCTCGAATGGGTAGGACGAATCCGATCAAAATACAACAACCTACGCTACTT
 ATTACGCTGATTCCGTGAAGGACAGATTACAAATATCCCGCGACGATAGCAAGAATAC
 GGCATATCTTCAGATGAATTCTCTTAAACCTGAGGATACCGCTGTGTATTACTGCACAA
 GACATGGTAATTTTGGAACTCATATGTCTCTTGGTTTCGCTTATTGGGGACAGGGCACG
 TTGGTTACCGTGTCTAGCGGAGGTGGTGGATCCCAGGTGCAGCTGAAACAGAGCGGCC

CGGGCCTGGTGCAGCCGAGCCAGAGCCTGAGCATTACCTGCACCGTGAGCGGCTTTAG
 CCTGACCAACTATGGCGTGCATTGGGTGCGCCAGAGCCCCGGGCAAAGGCCTGGAATGG
 CTGGGCGTGATTTGGAGCGGCGGCAACACCGATTATAACACCCCGTTTACCAGCCGCCT
 GAGCATTAACAAAGATAACAGCAAAAAGCCAGGTGTTTTTAAATGAACAGCCTGCAA
 AGCCAGGATACCGCGATTTATTATTGCGCGCGCGCGCTGACCTATTATGATTATGAATT
 TGCCTATTGGGGCCAGGGCACCCCTGGTGACCGTGAGCGCGGCTAGCACCAAGGGCCCA
 TCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGG
 CTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACCGGTGTCGTGGAACCTCAGGCGCC
 CTGACCAGCGGCGTGACACCTTCCCGGTGTCCTACAGTCCTCAGGACTCTACTCCCT
 CAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAAC
 GTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTG
 ACAAACCTCACACATGCCCACCGTGCCAGCACCTGAATTTGAAGGGGGACCGTCAGT
 CTTCCTCTTCCCCCAAACCCAAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTCA
 CATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGT
 GGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAG
 CACGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGCAAG
 GAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCTCAATCGAGAAAACCATCT
 CCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGA
 GGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAGC
 GACATCGCCGTGGAGTGAGGAGCAATGGGCAGCCGAGAGAACAACATAAGACCACG
 CCTCCCGTGTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAA
 GAGCAGGTGGCAGCGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCAC
 AACCCTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 168)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW242 without spacer SEQ ID NO:214]

QQQSGS[GYLWGCEWNCGGITT]GSSGGSGSGG[ISSGLLSGRSDQH]GGGSQTVVTQEPSF
 SVSPGGTVTLTCSRSTGAVTTSNYANWVQQTGQAPRGLIGGTNKRAPGVPDRFSGSILGN
 KAALTITGAQADDESYYCALWYSNLWVFGGGTKLTVLGGGSGGGGSGGGGSEVQLVE
 SGGGLVQPGGSLKLSCAASGFTFSTYAMNWVRQASGKGLEWVGRIRSKYNNYATYYADS
 VKDRFTISRDDSKNTAYLQMNLSKTEDTAVYYCTRHNFGNSYVSWFAYWGQGLTVTS
 SGGGGSQVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSG
 GNTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSDTAIYYCARALTYDYEFAYWGQGLT
 VTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
 LQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEFE
 GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPASIEKTISKAKGQPREPQVYTLPPSRE
 EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSR
 WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 169)

CX320: 3954-C225v5-2008

Nucleic Acid Sequence

[spacer SEQ ID NO:180][CX320 without spacer SEQ ID NO:215]

CAAGGCCAGTCTGGCCAGTGCATCTCACCTCGTGGTTGTCCGGACGGCCCATACGTCAT
GTACGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGATCCGGTATATCGAGTGGATTG
CTGTCTGGCAGATCTGACCAACACGGCAGTAGCGGTACCCAGATCTTGCTGACCCAGA
GCCCCGTGATTCTGAGCGTGAGCCCGGGCGAACGTGTGAGCTTTAGCTGCCGCGCGAG
CCAGAGCATTGGCACCAACATTCATTGGTATCAGCAGCGCACCAACGGCAGCCCCGCGC
CTGCTGATTAAATATGCGAGCGAAAGCATTAGCGGCATTCCGAGCCGCTTTAGCGGCA
GCGGCAGCGGCACCGATTTTACCCTGAGCATTAAACAGCGTGGAAAGCGAAGATATTGC
GGATTATTATTGCCAGCAGAACAACTGGCCGACCACCTTTGGCGCGGGCACCAAA
CTGGAACCTGAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGA
GCAGTTGAAATCTGGAACCTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAG
AGGCCAAAGTACAGTGGAAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAG
TGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCCTGACGCTG
AGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
TGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT (SEQ ID NO: 170)

Amino Acid Sequence

[spacer SEQ ID NO:178][CX320 without spacer SEQ ID NO:216]
QGQSGQ[CISPRGCPDGPYVMY]GSSGSGSGSGSG[ISSGLLSGRSDQH]GSSGTQILLTQSP
VILSVSPGERVSFSCRASQSIGTNIHWYQQRRTNGSPRLIKYASESISGIPSRFSGSGSGTDFTL
SINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVVC
LNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 171)

CI064: SynN297Q-JF15865-0001-hSP34LvHv-H-N

pLW138: HC SynN297Q-JF15865-0001-hSP34LvHv-H-N

Nucleic Acid Sequence

[spacer SEQ ID NO:176][pLW138 without spacer SEQ ID NO:147]
CAAGGCCAGTCTGGCCAAATGATGTATTGCGGTGGGAATGAGGTGTTGTGCGGGCCGC
GGGTTGGCTCGAGCGGTGGCAGCGGTGGCTCTGGTGGTCTGAGCGGCCGTTCCGATAA
TCATGGCGGCGGTTCTCAGACCGTGGTCACACAGGAGCCCTCACTGACAGTGAGCCCT
GGCGGGACCGTCACACTGACTTGTTCGAGTTCAACTGGCGCCGTGACTACCAGCAATT
ACGCTAACTGGGTCCAGCAGAAACCAGGACAGGCACCACGAGGACTGATCGGAGGAA
CTAATAAGAGAGCACCAGGAACCCCTGCAAGGTTCTCCGGATCTCTGCTGGGGGGA
AGCCGCTCTGACACTGAGCGGCGTGCAGCCTGAGGACGAAGCTGAGTACTATTGCGCA
CTGTGGTACTCCAACCTGTGGGTGTTTGGCGGGGGAATAAGCTGACCGTCCTGGGAG
GAGGAGGAAGCGGAGGAGGAGGGAGCGGAGGAGGAGGATCCGAAGTGCAGCTGGTC
GAGAGCGGAGGAGGACTGGTGCAGCCAGGAGGATCCCTGAAGCTGTCTTGTGCAGCCA
GTGGCTTCACCTTCAACACTTACGCAATGAACTGGGTGCGGCAGGCACCTGGGAAGGG
ACTGGAATGGGTGCGCCGGATCAGATCTAAATAACAATAACTATGCCACCTACTATGCTG
ACAGTGTGAAGGATAGGTTTACCATTTCACGCGACGATAGCAAAAACACAGCTTATCT
GCAGATGAATAACCTGAAGACCGAGGATACAGCAGTGTACTATTGCGTCAGACACGGC
AATTTGCGGAACTCTTACGTGAGTTGGTTTGCCTATTGGGGACAGGGGACACTGGTCAC
CGTCTCCTCAGGAGGTGGTGGATCCCAAGTGACCCTGAGAGAGTCTGGCCCTGCCCTCG
TGAAGCCTACCCAGACCCTGACACTGACCTGCACCTTCAGCGGCTTCAGCCTGAGCACC

AGCGGCATGTCTGTGGGCTGGATCAGACAGCCTCCTGGCAAGGCCCTGGAATGGCTGG
 CCGACATTTGGTGGGACGACAAGAAGGACTACAACCCAGCCTGAAGTCCCGGCTGAC
 CATCAGCAAGGACACCAGCAAGAACCAGGTGGTGTGAAAGTGACCAACATGGACCC
 CGCCGACACCGCCACCTACTACTGCGCCAGATCCATGATCACCAACTGGTACTTCGACG
 TGTGGGGAGCCGGCACCACCGTGACAGTGTCTGCTAGCACCAAGGGCCCATCGGT
 CTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCC
 TGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGAC
 CAGCGGCGTGACACCTTCCCGGTGTCTACAGTCTCAGGACTCTACTCCCTCAGCA
 GCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAA
 TCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAA
 ACTCACACATGCCCACCGTGCCAGCACCTGAACCTCTGGGGGGACCGTCAGTCTTCT
 CTCCCCCCTGGAACCCAAAGGACACCCCTCATGATCTCCCGGACCCCTGAGGTACATGCG
 TGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGG
 CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACCAGAGCACGTA
 CCGTGTGGTCAGCGTCTCACCCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC
 AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAAG
 CCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCATCCCGGGAGGAGAT
 GACCAAGAACCAGGTGACCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATC
 GCCGTGGAGTGGGAGAGCAATGGGCAGCCGAGAGAACAACACTACAAGACCACGCCTCCC
 GTGCTGGACTCCGACGGCTCCTTCTCTCTACAGCAAGCTCACCGTGGACAAGAGCAG
 GTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT
 ACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA (SEQ ID NO: 172)

Amino Acid Sequence

[spacer SEQ ID NO:178][pLW138 without spacer SEQ ID NO:153]

QQQSGQ[MMYC GGNEVLC GPRV]GSSGSGSGSGG[LSGRSDNH]GGGSQTVVTQEPSLTVS
 PGGTVTLTCRSSTGAVTTSNYANWVQKPGQAPRGLIGGTNKRAPGTPARFSGSLLGGKA
 ALTLSGVQPEDEAEYYCALWYSNLWVFGGGTKLTVLGGGSGGGGSGGGGSEVQLVESG
 GGLVQPGGSLKLSCAASGFTFNTYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVK
 DRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYVSWFAYWGQGLTVTVSSG
 GGGSQVTLRESGPALVKPTQTLTLTCTFSGFSLSTSGMSVGVIRQPPGKALEWLADIWDD
 KKDYNPSLKSRLTISKDTSKNQVVLKVTNMDPADTATYYCARSMITNWFYFDVWGAGTTV
 TVSSASTKGPSVFPLAPSSKSTSGGTAALGLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
 SSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
 PSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQS
 TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT
 KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQ
 GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 173)

pLW139: LC Syn kappa

Nucleic Acid Sequence

GACATCCAGATGACCCAGAGCCCCAGCACACTGAGCGCCAGCGTGGGCGACAGAGTG
 ACCATCACATGCAAGTGCCAGCTGAGCGTGGGCTACATGCACTGGTATCAGCAGAAGC
 CCGGCAAGGCCCCCAAGCTGCTGATCTACGACACCAGCAAGCTGGCCTCCGGCGTGCC
 CAGCAGATTTTCTGGCAGCGGCTCCGGCACCGAGTTCACCTGACAATCAGCAGCCTGC

AGCCCGACGACTTCGCCACCTACTACTGTTTTCAAGGCTCCGGCTACCCCTTCACCTTC
 GGCGGAGGCACCAAGCTGGAAATCAAGCGGACGGTGGCTGCACCATCTGTCTTCATCT
 TCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAAT
 AACTTCTATCCCAGAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGG
 GTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCA
 GCAGCACCTTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGA
 AGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT
 (SEQ ID NO: 174)

Amino Acid Sequence

DIQMTQSPSTLSASVGDRTITCKCQLSVGYMHWYQQKPGKAPKLLIYDTSKLASGVPSRF
 SGSGSGTEFTLTISLQPDDEFATYYCFQGSQYPFTFGGGTKLEIKRTVAAPSVFIFPPSDEQLK
 SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADY
 EHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 175)

Anti-CD3 scFv variant v12

Light chain LV12

Heavy chain HV12

Sequences provided above

Anti-CD3 scFv variant v16

Light chain LV12

Heavy chain HV20

Sequences provided above

Anti-CD3 scFv variant v19

Light chain LV19

Heavy chain HV20

Sequences provided above

Anti-CD3 scFv variant v26

Light chain LV19

Heavy chain HV12

Sequences provided above

Vector Construction

[0349] The heavy and light chains were cloned separately into a mammalian expression vector using standard molecular biology techniques. Briefly, DNA fragments encoding the region of interest were amplified with primers binding to the terminal ends. Overlapping fragments were combined and amplified with flanking primers as needed to build the entire desired region. DNA fragments were subsequently cloned into the expression vector using a commercially available

homologous recombination kit (MCLabs, South San Francisco, CA). The mammalian expression vector is a modified version of cDNATM3.1(+) from Invitrogen with selection marker of G418 or hygromycin. Mutations were introduced using the QuikChange Kit (Agilent, Santa Clara, CA).

Expression of AAs and Dually Masked BAAs (BAAs)

[0350] AAs and BAAs were expressed in mammalian cells using a standard transfection kit (Life Technologies, Grand Island, NY). Briefly, 293 cells were transfected with nucleic acids using a lipid-based system, following the manufacturer's recommended protocol. AAs and dually masked BAAs were purified from cell-free supernatant using Protein A beads (GE, Piscataway, NJ) and concentrated using standard buffer exchange columns (Millipore, Temecula, CA).

EXAMPLE 2. Binding of dually masked, bispecific, AAs to EGFR+ HT-29 cells and CD3ε+ Jurkat cells

[0351] To determine if the described EGFR and CD3ε masking peptides and protease substrates could inhibit binding in the context of a dually masked, bispecific, AA, a flow cytometry-based binding assay was performed.

[0352] HT-29-luc2 (Caliper) and Jurkat (Clone E6-1, ATCC, TIB-152) cells were cultured in RPMI-1640+glutamax (Life Technologies, Catalog 72400-047), 10% Heat Inactivated-Fetal Bovine Serum (HI-FBS, Life Technologies, Catalog 10438-026), 100 U/ml penicillin, and 100 µg/ml streptomycin (Life Technologies, Catalog 15140-122) according to manufacturer guidelines. The following bispecific, activated antibodies CI048 and CI104 (act-104), and dually masked, bispecific, AAs CI011, CI106, and CI107 were tested. Two versions of SP34 scFv were utilized, namely the scFv in CI011 and CI048 versus the scFv in CI104, CI106, and CI107. Two versions of the EGFR mask were utilized, namely the EGFR mask in CI011 and CI107 versus the EGFR mask in CI106. Two versions of the CD3 mask were utilized, namely the CD3 mask in CI011 versus the CD3 mask in CI106 and CI107.

[0353] HT29-luc2 cells were detached with VerseneTM (Life Technologies, Catalog 15040-066), washed, plated in 96 well plates at 150,000 cells/well, and re-suspended in 50 µL of primary antibody. Titrations started at the concentrations indicated in FIGS. 1A-1B followed by 3-fold serial dilutions in FACS Stain Buffer + 2% FBS (BD Pharmingen, Catalog 554656). Cells were incubated

at 4°C with shaking for about 1 hour, harvested, and washed with 2x200 µL of FACS Stain Buffer. Cells were resuspended in 50 µL of Alexa Fluor 647 conjugated anti-Human IgG Fc (10 µg/ml, Jackson ImmunoResearch, Product 109-606-008) and incubated at 4°C with shaking for about 1 hour. HT29-luc2 were harvested, washed, and resuspended in a final volume of 60 µL of FACS Stain Buffer containing 2.5 µg/ml 7-AAD (BD Biosciences, Catalog 559925). Cells stained with secondary antibody alone were used as a negative control. Data was acquired on a MACSQuant[®] Analyzer 10 (Miltenyi) and the median fluorescence intensity (MFI) of viable cells was calculated using FlowJo[®] V10 (Treestar). Background subtracted MFI data was graphed in GraphPad Prism 6 using curve fit analysis.

[0354] Jurkats growing in suspension were harvested, washed, plated in 96 well plates at 150000 cells/well and resuspended in 50 µl of primary antibody. Staining and data acquisition were carried out as described for HT29-luc2 cells above.

[0355] FIG. 1A demonstrates that incorporation of the h20GG CD3ε masking peptide into the EGFR masked BAAs CI106 and CI107 significantly reduced binding to Jurkat cells relative to CI011. In some embodiments, the reduction in binding to Jurkat cells was more than 5,000-fold. In some embodiments, an scFv of the disclosure also led to reduced binding. A reduction in binding to EGFR+ HT29-luc2 cells was also evident for CI106 and CI107 relative to CI011 (FIG. 1B). In some embodiments, the reduction in binding to EGFR+ HT29-luc2 cells was more than 1,000-fold. In FIG. 1A and FIG. 1B, the dually masked, BAAs exhibit reduced binding relative to the activated bispecific antibodies.

EXAMPLE 3. EGFR-dependent cytotoxicity of dually masked BAAs

[0356] To determine if the CD3ε and EGFR masks and the protease substrates in CI106 and CI107 could further attenuate cell killing relative to CI011 and CI040, a cytotoxicity assay was performed. Human PBMCs were purchased in frozen aliquots (HemaCare) and co-cultured with EGFR expressing HT29-luc2 cells at a ratio of 10:1 in RPMI-1640+glutamax supplemented with 5% heat inactivated human serum (Sigma, Catalog H3667). Titrations of the following bispecific, activated antibodies and dually masked BAAs were tested: CI011, CI040, activated CI104, CI106, and CI107. In addition, non-EGFR binding, masked bispecific, AAs CI127 and CI128 were used to demonstrate the EGFR dependence of cytotoxicity. After 48 hours, cytotoxicity was evaluated using

the ONE-Glo™ Luciferase Assay System (Promega, Catalog E6130). Luminescence was measured on the Infinite M200 Pro (Tecan). Percent cytotoxicity was calculated and plotted in GraphPad PRISM with curve fit analysis. EGFR receptor number on a panel of cell lines was quantified by flow cytometry using QIFIKIT (Dako).

[0357] FIG. 2A demonstrates that killing of EGFR+ HT29-luc2 cells was further attenuated by CI106 and CI107 relative to CI011 and CI040. FIG. 2B shows that no cytotoxicity was observed when cells were treated with CI127 and CI128 demonstrating the dependence of EGFR targeting for cell killing. Additionally, FIG. 2B depicts a more than 300,000 fold EC50 shift of the dually masked bispecific antibodies CI106 and CI107 relative to the protease activated bispecific antibody act-104. FIG. 2C depicts the EGFR receptor number on a panel of cell lines that includes HT29. The approximate EGFR receptor number on HT29 cells was 75,000, indicating that high antigen density was not required for potent cytotoxicity of the tested antibodies.

EXAMPLE 4. Primary T cell activation by dually masked BAAs

[0358] To determine if the CD3ε and EGFR masks in CI106 and CI107 could attenuate primary T cell activation relative to CI011 and CI040, a flow cytometry assay was performed. Human PBMCs and U266 cells were co-cultured according to the conditions described in Example 3. After a 48 hour incubation, cells were pelleted, media was removed, and cells were resuspended in 50 µl of a cocktail containing anti-CD45 VioBlue® (Miltenyi, Catalog 130-002-880), anti-CD8 APC-Vio770 (Miltenyi, Catalog 130-096-561) and anti-CD69 PE (BD Pharmingen, Catalog 555531) in FACS Stain Buffer + 2% FBS. Cells were stained for 1 h at 4°C with shaking, harvested, washed, and resuspended in a final volume of 60 µL FACS Buffer. Data was acquired on a MACSQuant® Analyzer 10 (Miltenyi) and activation was quantified in FlowJo® V10 (Treestar) as the percentage of CD8+ T cells with expression of CD69 above the PE isotype control. Data was plotted in GraphPad PRISM 6 with curve fit analysis.

[0359] FIG. 3A demonstrates that activation of primary CD8+ T cells was attenuated by CI106 and CI107, relative to CI011 and CI040. FIG. 3B demonstrates that dually masked antibodies display a shifted dose response curve for T cell activation relative to protease activated bispecific antibody act-104 indicating that masking attenuates T cell activation.

EXAMPLE 5. Dually masked, bispecific, AAs of the embodiments induced regression of established HT29-luc2 tumors in mice

[0360] In this example, dually masked BAAs CI106 and CI107 targeting EGFR and CD3 ϵ were analyzed for the ability to induce regression or reduce growth of established HT-29-Luc2 xenograft tumors in human T-cell engrafted NSG mice.

[0361] The human colon cancer cell line HT29-luc2 was obtained from Perkin Elmer, Inc., Waltham, MA (formerly Caliper Life Sciences, Inc.) and cultured according to established procedures. Purified, frozen human PBMCs were obtained from Hemacare, Inc., Van Nuys, CA. NSGTM (NOD.Cg-Prkdcscid Il2rg^{tm1Wjl}/SzJ) mice were obtained from The Jackson Laboratories, Bar Harbor, ME.

[0362] On day 0, each mouse was inoculated subcutaneously at the right flank with 2×10^6 HT29-luc2 cells in 100 μ L RPMI + Glutamax, serum-free medium. Previously frozen PBMCs from a single donor were administered (i.p.) on day 3 at a CD3+ T cell to tumor cell ratio of 1:1. When tumor volumes reached 200 mm³ (approximately day 12), mice were randomized, assigned to treatment groups and dosed i.v. according to Table 12. Tumor volume and body weights were measured twice weekly.

Table 12. Groups and doses for HT2-9luc2 xenograft study.

Group	Count	Treatment	Dose (mg/kg)
1	7	PBS	N/A
2	7	CI106	0.5
3	7	CI106	1.5
4	7	CI107	0.5
5	7	CI107	1.5

[0363] FIG. 4, which plots tumor volume versus days post initial treatment dose, demonstrates a dose-dependent effect of CI106 and CI107 dually masked, bispecific, AAs on the growth of HT29-

luc2 xenograft tumors. The most efficacious dose tested was 1.5 mg/kg, resulting in tumor regression. Statistical analysis (RMANOVA with Dunnett's vs. PBS control) was carried out in GraphPad PRISM. * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.0001$.

EXAMPLE 6. Dually masked, bispecific, AAs and bispecific antibodies of the embodiments reduce growth of established HCT116 tumors in mice

[0364] In this example, bispecific antibody, activated CI104, and dually masked BAAs CI106 and CI107 targeting EGFR and CD3 ϵ were analyzed for the ability to induce regression or reduce growth of established HCT116 xenograft tumors in human T-cell engrafted NSG mice. The human colon cancer cell line HCT116 was obtained from ATCC and was cultured in RPMI + Glutamax + 10% FBS according to established procedures. The tumor model was carried out as described in Example 5. Mice were dosed according to Table 13.

Table 13. Groups and doses for HCT116 xenograft study.

Group	Count	Treatment	Dose (mg/kg)
1	8	PBS	N/A
2	8	CI106	0.3
3	8	CI106	1.0
4	8	CI107	0.3
5	8	CI107	1.0
6	8	act-104	0.3

[0365] FIG. 5 which plots tumor volume versus days post initial treatment dose, demonstrated a dose-dependent effect of CI106 and CI107 dually masked bispecific, AAs on the growth of HCT116 xenograft tumors. The most efficacious dose tested was 1.0 mg/kg, resulting in tumor stasis. Act-104 dosed at 0.3 mg/kg also resulted in tumor stasis, demonstrating a 3 fold difference in efficacy between dually masked and protease activated bispecific antibodies. Statistical analysis

(RMANOVA with Dunnett's vs. PBS control) was carried out in GraphPad PRISM. * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.0001$.

EXAMPLE 7. Cross reactivity of dually masked bispecific, AAs to cynomolgus monkey T cells

[0366] To confirm that Cynomolgus monkey is a relevant toxicity species, protease activated CI104, CI106 and CI107 were used in a flow cytometry based cell binding assay and a HT29-luc2 cytotoxicity assay using Cynomolgus pan T cells (BioreclamationIVT) and the potency was compared to human PBMCs. Protocol was as described in Examples 2 and 3.

[0367] FIG. 6A and FIG. 6B demonstrate that the EC50s of the tested dually masked and protease activated bispecific antibodies in a cytotoxicity assay are similar when either human (6A) or cyno (6B) effector cells are used. FIG. 6C and FIG. 6D demonstrate that binding of the protease-activated and dually masked antibodies to human (6C) and cyno (6D) T cells is similar.

[0368] Therefore, cynomolgus monkey was determined to be a relevant species for tolerability studies.

EXAMPLE 8. Mutations in the Fc region affect tolerability of dually masked, bispecific, AAs in cynomolgus monkeys

[0369] In this example, CI079 and CI090 were dosed at 600, $\mu\text{g/kg}$ in naïve cynomolgus monkeys ($n=1$) to assess tolerability. The starting dose of 600 $\mu\text{g/kg}$ was chosen based on the MTD of CI011 as previously established. The monkeys were of Chinese origin and ranged in weight from 2.5 to 4 kg. Each study animal was monitored for a minimum of 7 days. Tolerability was evaluated based on clinical signs, body weight, and food consumption. This study was conducted in compliance with standard operating procedures at SNBL USA, Ltd. (Everett, WA).

[0370] Table 14 describes clinical observations following dosing of CI079 and CI090 dually masked, bispecific, AAs (BAAs) that differ only in their Fc regions (Table 15). CI079 contains Fc mutations L234F, L235E, and P331S. CI090 contains those Fc mutations and N297Q mutation. No clinical observations were noted following dosing of CI090 at 600 $\mu\text{g/kg}$, whereas, emesis was noted in the first 24 hours following dosing of CI079, demonstrating that mutations in the Fc region contribute to tolerability of these molecules.

Table 14

BAA	Dose (μ pk)	Observations
CI079	600	emesis during 1 st 24 hours
CI090	600	no clinical observations

Table 15

BAA	EGFR Mask & Substrate	CD3 Mask &Substrate	Fc
CI079	3954 0001	h20GG 0001	Fcmt3
CI090	3954 0001	h20GG 0001	Fcmt4

EXAMPLE 9. Tolerability of dually masked BAAs in cynomolgus monkeys

[0371] In this example, CI106 and CI107 were dosed at 600, 2000, 4000 μ g/kg (CI107 only), or 6000 μ g/kg (CI107 only) to establish the maximum tolerated dose (MTD) following a single IV bolus administration to naïve cynomolgus monkeys (n=1). The starting dose of 600 μ g/kg was chosen based on the MTD of CI011 as previously established. The monkeys were of Chinese origin and ranged in weight from 2.5 to 4 kg. Each study animal was monitored for a minimum of 7 days. Tolerability was evaluated based on clinical signs, body weight, food consumption and laboratory analyses that included serum chemistry, hematology, cytokine analysis, and flow cytometry to evaluate T cell activation. Blood was collected for standard serum chemistry and hematology analysis once during acclimation and at pre-dose, 48h, 72h (hematology only), and 7 days post dose. Blood was collected for cytokine analysis pre-dose and at 1h, 4h, 8h, and 24h post dose. Flow cytometry was performed on peripheral blood pre-dose, 72h, and 7 days post dose. This study was conducted in compliance with standard operating procedures at SNBL USA, Ltd. (Everett, WA).

[0372] CI107 dosed at 6000 μ g/kg was fatal within 24 hours post dose. In the other groups, abnormal clinical signs including emesis and reduced food intake were observed in cynos treated with CI106 and CI107 at doses of 2000 μ g/kg and above. These findings, when present, were transient and generally confined to the 48 h post-dose period. Serum chemistry findings at these doses included mild elevations of alanine transaminase (ALT) and aspartate aminotransferase (AST) at 48 h that did not exceed normal ranges. In CI107 treated animals at 2000 and 4000 μ g/kg, total bilirubin increased outside of normal range at 48 hours and was fully reversed by day 8. In both

CI106 and CI107 treated animals, transient increases in serum cytokines IL-2, IL-6, and IFN γ were observed after dosing and were resolved by 24h post dose. An increase in the percentage of T cells expressing CD69, Ki67, and PD-1 was observed at 72h post-dose and, generally, the percentage of positive cells was greater for CI107 treated animals.

[0373] FIGS. 7A-7C depict pre-dose, 48 h, and 7 days post-dose serum concentration of ALT (7A), AST (7B), and total bilirubin (7C). With the exception of total bilirubin at 2000 and 4000 $\mu\text{g/kg}$, all values are within established normal ranges for cynomolgus monkeys. Only pre-dose data was available for CI107 at 6000 $\mu\text{g/kg}$, and was not included.

[0374] FIGS. 8A-8C plot the increase in serum cytokine levels for IL-2 (8A), IL-6 (8B), and IFN- γ (8C).

[0375] FIGS. 9A-9C depict T cell activation as measured by CD69 (9A), Ki67 (9B), and PD-1 (9B) expression on CD4 $^{+}$ T cells.

EXAMPLE 10. Dually masked, bispecific, AAs are safer in cynomolgus monkeys than activated bispecific antibodies.

[0376] In this example, protease activated CI104 and dually masked CI106 and CI107 were dosed in cynomolgus monkeys (n=1) at 60, 180 (activated CI104 only), 600, 2000, 4000 $\mu\text{g/kg}$ (CI107 only), or 6000 $\mu\text{g/kg}$ (CI107 only) to compare the tolerability of masked and unmasked antibodies following a single IV bolus. Tolerability evaluation and blood collections were as described in Example 9. Dually masked, BAAs CI106 and CI107 were tolerated at 30-60 fold higher dose level than the protease activated, bispecific antibody.

[0377] FIGS. 10A-E plot dose dependent increases in AST at 48h post dose (10A), ALT at 48h post dose (10B), IL-6 at 8h post dose (10C), IFN γ at 8h post dose (10D), and Ki67 at 72h post dose (10E). The dose response curve for all parameters was shifted for the dually masked antibodies indicating improved tolerability and decreased pharmacodynamics effects relative to the protease activated bispecific antibody. In some embodiments, the IL-6 dose response curve was shifted by more than 60-fold.

EXAMPLE 11. Tolerability of EGFR binding, dually masked, bispecific, AAs is dependent on EGFR.

[0378] In this example, dually masked bispecific antibody, CI107, targeting EGFR and CD3 ϵ and CI128, targeting RSV and CD3 ϵ were dosed at 2000 μ g/kg in cynomolgus monkeys (n=1).

Tolerability evaluation and blood collections were as described in Example 9 above. There was no effect of CI128 on measures of acute organ toxicity (total bilirubin) and T cell activation (IL-6, PD-1) demonstrating that the toxicity observed in cyno was dependent on EGFR binding. These data also demonstrate that CD3 ϵ binding alone was not sufficient to induce toxicity.

[0379] FIGS. 11A-11C compares the effects of EGFR binding CI107 and non EGFR binding CI128 on increases in total bilirubin (11A), IL-6 (11B), and PD-1 expressing CD4⁺ T cells (11C).

EXAMPLE 12. Humanization of anti-CD3 variants v12, v16, and v19 which have different affinities and potencies.

[0380] This example describes anti-CD3 antibody variants v12, v16, and v19. These three variants were derived from the parent antibody hSP34

[0381] Humanization of the anti-human CD3 single-chain variable fragment (scFv) was performed by selectively mutating the framework. Briefly, CDRs were grafted into a series of light chain (LC) and heavy chain (HC) human IgG scaffolds and a number of amino acids in the variable region framework was selectively mutated. Immunoglobulins were expressed in all possible combinations of LC and HC, and then evaluated for expression level, percent monomer, and CD3 affinity using ELISA and on-cell binding to Jurkat cells. The variable regions of desirable combinations were expressed as scFv in the bispecific antibody (TCB) format and then evaluated for expression levels, percent monomer, CD3 affinity and function in cell cytotoxicity assays.

[0382] The affinity of v12, v16, and v19 variant was measured using surface plasmon resonance (SPR). Surfaces were HC200m, carboxylated hydrogel based on a linear, synthetic polycarboxylate. Surface channels were activated with a standard EDC/NHS amine coupling protocols. Channels 1 and 2 were blank, Channels 3 and 4 were various anti-human CD3 antibodies. Surfaces were generated by diluting v12, v16, v19 and MM194 antibodies to 5 μ g/ml in 1.0 mL 10 mM Sodium Acetate pH 4.5.

[0383] Kinetic analysis was performed in PBST (10 mM Sodium Phosphates, pH 7.4, 150 mM Sodium Chloride, 0.05% Tween-20) at 20°C. Regeneration was a series of three injections; a single 5 µl injection of 20 mM Sodium Hydroxide followed by two 5µL injection of 10 mM Sodium Hydroxide freshly-made.

[0384] The configuration was run with an inverse 3-fold serial dilution alternating with buffer blanks. Human CD3egFc was from Sino Biological Inc., (Beijing, China, Catalog# CT041-H0305H) reconstituted with sterile water from a lyophilized formulation based on PBS and stabilizers. Serial dilutions with the analyte in solution from concentrations starting at 300 nM or 100 nM human CD3. Processing was done with Scrubber software.

[0385] These variants were also engineered using described methods into dually masked, bispecific, AAs targeting EGFR and CD3 and used in an *in vitro* cytotoxicity assay as described in example 3.

[0386] FIG. 12A depicts the affinity measurements of v12, v16, and v19 relative to hSP34. V12 was the highest affinity at 12nM while v16 was the lowest affinity at 70nM.

[0387] FIG. 12B depicts the cytotoxicity of activated or dually masked, bispecific antibodies on HT29-luc2 cells. There were slight differences in the potency of cell killing of the activated molecules with v16 being the most potent. There are also slight differences in protection against cell killing for the dually masked molecules.

EXAMPLE 13. Dually masked BAAs enable extended PK in cynomolgus monkeys.

[0388] In this example, protease activated, bispecific antibody act-104 and dually masked, bispecific antibody CI107 were dosed at 60 µg/kg, 180 µg/kg (act-104) or 2000 µg/kg (CI107) in cynomolgus monkey. Plasma samples were collected at 5 min (act-104 only), 30 min, 4h (act-104 only), 24h, 48h (act-104 only), 96h, and 168h. Plasma concentration was measured by ELISA using an anti-idiotypic antibody to capture, a horseradish peroxidase (HRP) labeled anti-human IgG (Fc) for detection, and visualized using 3,3',5,5'-tetramethylbenzidine (TMB). Plasma concentration values were interpolated from a standard curve and plotted using GraphPad PRISM. Area under the curve (AUC) analysis was also performed.

[0389] FIG. 13 depicts the extended PK of the dually masked molecule, CI107, relative to the protease activated molecule, act-104. Exposure (AUC) of CI107 was 448 day*nM and act-104 (60 µg/kg) was 0.04 day*nM representing a greater than 10,000 fold difference in plasma exposure.

EXAMPLE 14. Sensitivity to protease cleavage of dually masked, bispecific, AAs correlates to tumor efficacy and tumor T cell infiltration.

[0390] This example describes anti-tumor efficacy and tumor T cell infiltration in a HT29-luc2 xenograft model. The model was carried out as described in example 5. In the tumor T cell infiltration study, mice received a single dose of test article and tumors were harvested 7 days post dose. Formalin fixed paraffin embedded (FFPE) blocks were created to use for histology. Test articles used are CI011, CI020 (a dually masked bispecific antibody devoid of a cleavable substrate), CI040, and CI048. Protease sensitivity and substrate cleavability of the test articles is as follows: CI040 > CI011 > CI020. Mice were dosed according to Table 16.

Table 16. Groups and doses for HT29-luc2 xenograft study.

Group	Count	Treatment	Dose (mg/kg)	Study
1	8	PBS	N/A	Efficacy
2	8	CI011	0.3	Efficacy
3	8	CI020	0.3	Efficacy
4	8	CI040	0.3	Efficacy
5	8	CI048	0.3	Efficacy
6	5	PBS	N/A	Infiltration
7	5	CI011	1.0	Infiltration
8	5	CI020	1.0	Infiltration
9	5	CI040	1.0	Infiltration
10	5	CI048	1.0	Infiltration

[0391] FIG. 14A depicts efficacy in a HT29-luc2 tumor intervention model in PBMC engrafted NSG mice. Anti-tumor potency in this example correlates to protease sensitivity and substrate cleavability of the test articles, with the most efficacious test article being the fully protease activated CI048.

[0392] FIG. 14B depicts staining of tumor sections for CD3 (dark staining) as a measure of the degree of T cell infiltration into tumors. Tumor T cell infiltration correlates with protease sensitivity and substrate cleavability of the test articles.

EXAMPLE 15. Second generation dually masked, bispecific, AAs are safer in cynomolgus monkeys than first generation molecules.

[0393] In this example, cynomolgus tolerability data was compared for CI011, CI040, CI048 (first generation molecules), act-104, CI106, and CI107 (second generation molecules). Data presented in this example was compiled from two cyno tolerability studies. Protease activated CI104 and CI048 were dosed in cynomolgus monkeys at 20 (CI048 only), 60 or 180 µg/kg (act-104 only). Dually masked CI011, CI040, CI106 and CI107 were dosed at 600, 2000, 4000 (CI107 only), or 6000 (CI107 only) µg/kg to compare the tolerability of dually masked and activated bispecific antibodies following a single IV bolus. Tolerability evaluation was as described in Example 8.

[0394] Table 17 summarizes the clinical observations following a single dose of test article. Second generation, protease activated bispecific antibody act-104 was tolerated at 2-fold higher dose than first generation protease activated bispecific antibody CI048. CI106 and CI107 were tolerated at 30-60-fold higher dose than first generation antibodies CI011 and CI040.

Table 17

BAA	Dose (µg/kg)	Clinical Observations
CI048	20 (n=2)	1. Emesis and hunching in 1 st 24 hrs 2. None
	60	Emesis at 4 hr; hunching lasting 4 days and inappetence lasting 2 days; 10% weight loss

CI011	600 (n=3) 2000	1. None 2. Emesis at 4 hrs, inappetence on day 4 3. Hunching and inappetence through day 5. 5% weight loss Emesis days 1-2, inappetence days 2-4
CI040	600 (n=2) 2000	1. Emesis at 4 hr 2. Emesis within 12 hrs, inappetence on day 4 Emesis days 1-2, moribund and euthanized day 2
act-104	60	Emesis and hunching in 1 st 24 hrs
CI104	180	Severe emesis; hunching, paleness, inappetence lasting 3 days
CI106	600 2000	none none
CI107	600 2000 4000 6000	None Emesis, once in 1 st 24 hrs Emesis, multiple incidences in 1 st 24 hrs Severe emesis; bloody diarrhea

EXAMPLE 16. Evaluation of Masking Efficiencies of Activatable anti-EGFR Antibodies

[0395] Masking the ability of an antibody to bind to its antigen is an example of inhibition of binding and is enumerated herein as masking efficiency (ME). Masking efficiency can be calculated as the K_D for binding of the AA divided by the K_D for binding of the antibody measured under the same conditions. The extent of inhibition is dependent on the affinity of the antibody for its antigen, the affinity of the inhibitor (i.e., the masking moiety) for the antibody and the concentration of all reactants. Local concentrations of the tethered masking moiety peptide (inhibitor) is very high in the AA context, on the order of 10 mM, therefore moderate affinity peptides would effectively mask AA antigen binding.

[0396] The general outline for this assay is as follows: Nunc, Maxisorp™ plates are coated overnight at 4°C with 100 µl/well of a 1 µg/mL solution of human EGFR (R and D Systems) in PBS, pH 7.4. Plates are washed 3X PBST (PBS, pH 7.4, 0.05% Tween-20), and wells are blocked with 200 µl/well, 10 mg/mL BSA in PBST for 2 hours at RT. Plates are washed 3X PBST (PBS, pH 7.4, 0.05% Tween-20). Dilution curves can be prepared, in 10 mg/mL BSA in PBST, as

illustrated below in Table 18. In this example the highest concentrations are 10 nM for the parental antibody and 400 nM for the AAs, however, the top concentrations can be increased or decreased to give full saturation binding curves for AAs with stronger or weaker masking.

Table 18. Plate layout for masking efficiency assay.

	[Antibody] = nM Columns 1-3	[AA 1] = nM Columns 4-6	[AA 2] = nM Columns 7-9	[AA 3] = nM Columns 10-12
A	10	400	400	400
B	2	100	100	100
C	0.625	25	25	25
D	0.156	6.25	6.25	6.25
E	0.039	1.56	1.56	1.56
F	0.0097	0.39	0.39	0.39
G	0.0024	0.098	0.098	0.098
H	0.0006	0.024	0.024	0.024

[0397] The binding solutions are added to the plates, which are then incubated for 1 hour at room temperature, and then washed 3X PBST (PBS, pH 7.4, 0.05% Tween-20). 100 μ l/well 1:4000 dilution goat-anti-human IgG (Fab specific, Sigma cat # A0293) in 10 mg/mL BSA in PBST is added, and the plate is incubated for 1 hour at room temperature. The plate is developed with TMB and 1N HCL. Shown in FIG. 15 and FIG. 16 are plots of binding isotherms for activatable anti-EGFR C225v5 antibodies of the disclosure, for activatable anti-EGFR antibody 3954-2001-C225v5 described above, and for anti-EGFR antibody C225v5. Plots are generated in GraphPad PRISM and the data are fit to a model of single site saturation and a K_D is determined. The K_D and ME values are provided in Table 19.

Table 19. K_D and ME values calculated from the binding isotherms shown in FIG. 15 and FIG. 16.

	K_D (nM)	ME
3954-2001-C225v5	13	130
CF08-2001-C225v5	0.2	2
CF13-2001-C225v5	0.3	3
CF19-2001-C225v5	27	270

CF22-2001-C225v5	78	780
CF41-2001-C225v5	52	520
CF46-2001-C225v5	1	10

EXAMPLE 17. Pharmacokinetics of dually masked BAAs in cynomolgus monkeys.

[0398] In this example, dually masked bispecific antibody CI107 was dosed at 600 µg/kg, 2000 µg/kg, or 4000 µg/kg in cynomolgus monkey. Plasma samples were collected at 30 min, 4h (600 µg/kg only), 24h, 48h (600 and 4000 µg/kg only), 96h, and 168h. Plasma concentration was measured by ELISA as in example 13.

[0399] FIG. 20 depicts the PK of the dually masked BAA CI107 following a single i.v. dose of either 600, 2000, or 4000 µg/kg.

EXAMPLE 18. EGFR-dependent cytotoxicity of dually masked, bispecific, activatable antibodies

[0400] To determine whether the anti-CD3ε, CD3 mask, and protease substrates in CI090 and CI091 could further attenuate cell killing relative to CI011, a cytotoxicity assay was performed using the method described in Example 3. Titrations of the following bispecific, activated antibodies and dually masked, bispecific, activatable antibodies were tested: CI011, CI090, CI091, activated CI090, and CI048. In addition, non-EGFR binding, bispecific, activatable antibody CI064 was used to demonstrate the EGFR dependence of cytotoxicity.

[0401] FIG. 21 demonstrates that killing of EGFR+ HT29-luc2 cells was further attenuated by CI090 and CI091 relative to CI011, however the potency of the activated CI090 is equivalent to CI048. The EC50 shift of CI090 and CI091 relative to activated bispecific antibody is increased representing increased masking efficiency of these molecules relative to CI011. No cytotoxicity was observed when cells were treated with CI064, demonstrating the dependence of EGFR targeting for cell killing.

EXAMPLE 19. Primary T cell activation by dually masked, bispecific, activatable antibodies

[0402] To determine if the anti-CD3 ϵ , CD3 mask, and protease substrates in CI090 and CI091 could attenuate primary T cell activation relative to CI011, a flow cytometry assay was performed as described in Example 4.

[0403] FIG. 22 demonstrates that activation of primary CD8⁺ T cells was further attenuated by CI090 and CI091, relative to CI011.

EXAMPLE 20. Dually masked, bispecific, activatable antibodies of the embodiments induced regression of established HT29-luc2 tumors in mice

[0404] In this example, bispecific activatable antibodies CI011, CI090, and CI091 were analyzed for the ability to induce regression or reduce growth of established HT-29-Luc2 xenograft tumors in human PBMC engrafted NSG mice. The method is as described in Example 5.

Table 20. Groups and doses for HT-29-luc2 xenograft study.

Group	Count	Treatment	Dose (mg/kg)
1	7	PBS	N/A
2	7	CI011	1.0
3	7	CI090	1.0
4	7	CI091	1.0

[0405] FIG. 23, which plots tumor volume versus days post initial treatment dose, demonstrates that a 1 mg/kg, weekly dose induced tumor regression for all bispecific activatable antibodies tested.

EXAMPLE 21. Dually masked, bispecific, activatable antibodies elicit less cytokine release than activated bispecific antibodies in cynomolgus monkey.

[0406] In this example, protease activated CI104, and dually masked CI011, CI090, and CI091 were dosed in cynomolgus monkeys (n=1) at 0.06, 0.18 (activated CI104), or 600 mg/kg (CI011,

CI090, CI091). Blood was collected for cytokine analysis pre-dose and at 1h, 4h, 8h, and 24h post dose. Samples were analyzed using Life Technologies Monkey Magnetic 29-Plex Panel Kit (Product No. LCP0005M). Data was acquired on a BioRad BioPlex 200 instrument. This analysis was conducted in compliance with standard operating procedures at SNBL USA, Ltd. (Everett, WA).

[0407] FIG. 24 plots IL-6 levels at 8h post dose. The dually masked bispecific activatable antibody CI011 induces significantly less cytokine release than activated CI104 even when delivered at a higher dose, demonstrating the effect of masking on T cell activation. IL-6 is even further reduced in CI090 and CI091 treated animals reflecting the increased masking efficiency of these molecules relative to CI011.

Other Embodiments

[0408] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following.