

CLAIMS

1. A bispecific activatable antibody (BAA) comprising the following structure:

- a. an IgG antibody (AB1) that specifically binds to an EGFR wherein the AB1 comprises two heavy chains (AB1 HCs) and two light chains (AB1 LCs), wherein the AB1 comprises a VH CDR1 amino acid sequence comprising NYGVH (SEQ ID NO:60), a VH CDR2 amino acid sequence comprising VIWSGGNTDYNTFPFTS (SEQ ID NO:61), a VH CDR3 amino acid sequence comprising ALTYDYEFAY (SEQ ID NO:62), a VL CDR1 amino acid sequence comprising RASQSIGTNIH (SEQ ID NO:57), a VL CDR2 amino acid sequence comprising YASESIS (SEQ ID NO:58), and a VL CDR3 amino acid sequence comprising QQNNNWPTT (SEQ ID NO:59);

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; and

wherein the amino terminus of each of the two AB1 LCs is linked to the carboxyl terminus of a MM1-CM1 construct, wherein each MM1-CM1 construct comprises:

- i. a first masking moiety (MM1), the MM1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-84; and
ii. a first cleavable moiety (CM1), the CM1 being a substrate for a first protease;

wherein the MM1 is linked in an N- to C-terminal direction to the CM1, to form each MM1-CM1 construct; and

- b. two single chain variable antibody fragments (scFvs) (each an AB2), wherein each of the AB2s 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; and

wherein the carboxyl terminus of each AB2 is linked to the amino terminus of a AB1 heavy chain; and

wherein each AB2 is linked to:

- i. a second masking moiety (MM2), the MM2 comprising the amino acid sequence SEQ ID NO: 12; and
- ii. a second cleavable moiety (CM2), the CM2 being a substrate for a second protease;

wherein the MM2 is linked to the CM2 to form a MM2-CM2 construct, wherein the carboxyl terminus of each MM2-CM2 construct is linked to the amino terminus of a corresponding AB2.

- 2. The BAA of claim 1, wherein the MM1 comprises an amino acid sequence of SEQ ID NO: 78.
- 3. The BAA of claim 1, wherein the CM1 or CM2 comprises one of the following characteristics:
 - (a) amino acid sequence of SEQ ID NO: 14,
 - (b) amino acid sequence of SEQ ID NO: 17, or
 - (c) amino acid sequence of SEQ ID NO: 16.
- 4. The BAA of claim 1, wherein the CM1 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 14 and SEQ ID NO: 16.
- 5. The BAA of claim 1, wherein the CM2 comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 14 and SEQ ID NO: 17.
- 6. The BAA of claim 1, wherein the AB1 comprises at least one of the following characteristics:
 - (a) amino acid substitutions in at least two of amino acid positions L234, L235, N297 and P331.
 - (b) amino acid substitutions in at least three of amino acid positions L234, L235, N297 and P331.

- (c) amino acid substitutions at amino acid positions L234, L235, N297 and P331.
 - (d) amino acid substitutions at amino acid positions L234F, L235E, and P331S.
 - (e) amino acid substitutions at amino acid positions L234F, L235E, N297Q and P331S.
7. The BAA of claim 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.
 8. The BAA of claim 1, wherein the BAA is selected from the group consisting of:
 - a) comprising a heavy chain amino acid sequence of SEQ ID NO: 130 and a light chain amino acid sequence of SEQ ID NO: 132 or a heavy chain amino acid sequence of SEQ ID NO: 197 and a light chain amino acid sequence of SEQ ID NO: 199,
 - b) comprising a heavy chain amino acid sequence of SEQ ID NO: 134 and a light chain amino acid sequence of SEQ ID NO: 136 or a heavy chain amino acid sequence of SEQ ID NO: 201 and a light chain amino acid sequence of SEQ ID NO: 203,
 - c) comprising a heavy chain amino acid sequence of SEQ ID NO: 121 and a light chain amino acid sequence of SEQ ID NO: 122 or a heavy chain amino acid sequence of SEQ ID NO: 192 and a light chain amino acid sequence of SEQ ID NO: 193, and
 - d) comprising a heavy chain amino acid sequence of SEQ ID NO: 124 and a light chain amino acid sequence of SEQ ID NO: 108 or a heavy chain amino acid sequence of SEQ ID NO: 195 and a light chain amino acid sequence of SEQ ID NO: 182.
 9. The BAA of claim 1, comprising the sequence of a heavy chain amino acid sequence of SEQ ID NO: 130 and a light chain amino acid sequence of SEQ ID NO: 132.
 10. The BAA of claim 1, comprising the sequence of a heavy chain amino acid sequence of SEQ ID NO: 134 and a light chain amino acid sequence of SEQ ID NO: 136.

11. The BAA of claim 1, comprising the sequence of a heavy chain amino acid sequence of SEQ ID NO: 121 and a light chain amino acid sequence of SEQ ID NO: 122.
12. The BAA of claim 1, comprising the sequence of a heavy chain amino acid sequence of SEQ ID NO: 124 and a light chain amino acid sequence of SEQ ID NO: 108.
13. The BAA of claim 1, wherein the antibody is a rodent antibody, a chimeric antibody, a humanized antibody, or a fully human monoclonal antibody.
14. A pharmaceutical composition comprising the BAA of claim 1 and a carrier.
15. The pharmaceutical composition of claim 14, comprising an additional agent.
16. The pharmaceutical composition of claim 15, wherein the additional agent is a therapeutic agent.
17. A method of producing the BAA of claim 1 by culturing a cell to express the BAA.
18. 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.