

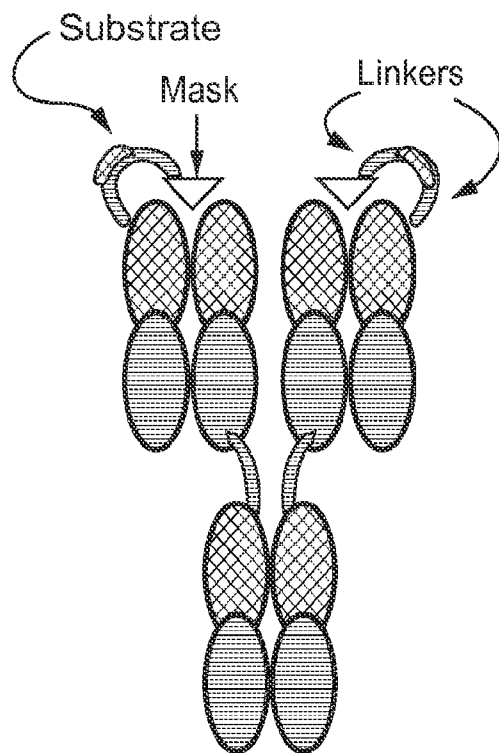


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- (71) Applicant: **CYTOMX THERAPEUTICS, INC.**
[US/US]; 343 Oyster Point Blvd., Suite 100, South San Francisco, California 94080-1913 (US).
- (72) Inventors: **LOWMAN, Henry Bernard**; PO Box 2556, El Granada, California 94018-2556 (US). **DESNOYERS, Luc Roland**; 250 Claremont Boulevard, San Francisco, California 94127 (US). **LIU, Shouchun**; 1795 Hunt Drive, Burlingame, California 94010 (US).
- (74) Agents: **ELRIFI, Ivor** et al.; Cooley LLP, 1299 Pennsylvania Avenue, Suite 700, Washington, District of Columbia 20004 (US).
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(54) Title: ACTIVATABLE ANTIBODIES THAT BIND EPIDERMAL GROWTH FACTOR RECEPTOR AND METHODS OF USE THEREOF

FIGURE 1



(57) Abstract: The invention relates generally to variant activatable antibodies that include a masking moiety (MM), a cleavable moiety (CM), and an antibody (AB) that specifically binds to epidermal growth factor receptor (EGFR), and to methods of making and using these variant anti-EGFR activatable antibodies in a variety of therapeutic, diagnostic and prophylactic indications.



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ACTIVATABLE ANTIBODIES THAT BIND EPIDERMAL GROWTH FACTOR RECEPTOR AND METHODS OF USE THEREOF

Related Applications

[0001] This application claims the benefit of U.S. Provisional Application No. 61/897,385, filed October 30, 2013; U.S. Provisional Application No. 61/897,391, filed October 30, 2013; U.S. Provisional Application No. 61/897,418, filed October 30, 2013; U.S. Provisional Application No. 61/897,442, filed October 30, 2013; and U.S. Provisional Application No. 61/897,465, filed October 30, 2013 the contents of each of which are incorporated herein by reference in their entireties.

Field of the Invention

[0001] The invention relates generally to activatable antibodies that specifically bind to epidermal growth factor receptor (EGFR), to conjugated activatable antibodies that specifically bind to EGFR, and to methods of making and using these anti-EGFR activatable antibodies and/or conjugated anti-EGFR activatable antibodies in a variety of therapeutic, diagnostic and prophylactic indications.

Background of the Invention

[0002] Antibody-based therapies have proven effective treatments for some 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.

[0003] 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.

[0004] 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

[0005] The activatable antibodies provided herein, in an uncleaved state, include a masked light chain that includes at least an antibody or antigen-binding fragment thereof (AB) that specifically binds Epidermal Growth Factor Receptor (EGFR) coupled to a masking moiety (MM) via a cleavable moiety (CM) that includes a substrate for a protease, such that coupling of the MM to the AB reduces the ability of the AB to bind EGFR. In an activated state, e.g., a cleaved state, the activatable antibodies bind EGFR. In an uncleaved state, e.g., non-activated state, the activatable antibodies provided herein include an antibody or an antigen binding fragment thereof (AB) that specifically binds to EGFR that includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence - Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence is selected from the group consisting of GQSGQ (SEQ ID NO: 14), QSGQ (SEQ ID NO: 16), SGQ (SEQ ID NO: 18), GQ (SEQ ID NO: 20), and Q (SEQ ID NO: 22). In some embodiments, the two linking peptides need not be identical to each other. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a masked light chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0006] In some embodiments, the activatable antibodies provided herein include an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID

NO: 28), and the Spacer Sequence includes the amino acid sequence GQSGQ (SEQ ID NO: 14). In some embodiments, the two linking peptides need not be identical to each other. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 4. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 2 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 4. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 34 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 4. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 36 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 4.

[0007] In some embodiments, the activatable antibodies provided herein include an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence QSGQ (SEQ ID NO: 16). In some embodiments, the two linking peptides need not be identical to each other. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 6. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 2 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 6. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 34 and a masked light chain that includes or

is derived from the amino acid sequence of SEQ ID NO: 6. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 36 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 6.

[0008] In some embodiments, the activatable antibodies provided herein include an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence SGQ (SEQ ID NO: 18). In some embodiments, the two linking peptides need not be identical to each other. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 8. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 2 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 8. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 34 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 8. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 36 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 8.

[0009] In some embodiments, the activatable antibodies provided herein include an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-

Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence GQ (SEQ ID NO: 20). In some embodiments, the two linking peptides need not be identical to each other. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 10. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 2 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 10. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 34 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 10. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 36 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 10.

[0010] In some embodiments, the activatable antibodies provided herein include an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence Q (SEQ ID NO: 22). In some embodiments, the two linking peptides need not be identical to each other. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 12. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 2 and a masked light chain that includes or is derived from the amino acid

sequence of SEQ ID NO: 12. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 34 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 12. In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a heavy chain that includes or is derived from the amino acid sequence of SEQ ID NO: 36 and a masked light chain that includes or is derived from the amino acid sequence of SEQ ID NO: 12.

[0011] The invention also provides conjugated activatable antibodies in which an anti-EGFR activatable antibody provided herein is conjugated to one or more additional agents. In an activated state, e.g., a cleaved state, the conjugated activatable antibodies bind EGFR. In some embodiments, the conjugated activatable antibody in an uncleaved state includes a masked light chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0012] In some embodiments, the conjugated activatable antibody in an uncleaved state includes a masked light chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 4.

[0013] In some embodiments, the conjugated activatable antibody in an uncleaved state includes a masked light chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 6.

[0014] In some embodiments, the conjugated activatable antibody in an uncleaved state includes a masked light chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 8.

[0015] In some embodiments, the conjugated activatable antibody in an uncleaved state includes a masked light chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 10.

[0016] In some embodiments, the conjugated activatable antibody in an uncleaved state includes a masked light chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 12.

[0017] The activatable anti-EGFR antibodies and the conjugated activatable anti-EGFR antibodies provided herein are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in other tissue(s), e.g., normal tissue, healthy tissue, and/or

diseased tissue at a site not intended for therapy, and, when activated, exhibit binding to EGFR that is at least comparable to the corresponding, unmodified antibody.

[0018] In some embodiments of the activatable antibody and/or conjugated activatable antibody, at least one of LP1 or LP2 includes an amino acid sequence selected from the group consisting of $(GS)_n$, $(GGS)_n$, $(GSGGS)_n$ (SEQ ID NO: 66) and $(GGGS)_n$ (SEQ ID NO: 67), where n is an integer of at least one. In some embodiments of the activatable antibody and/or conjugated activatable antibody, at least one of LP1 or LP2 includes an amino acid sequence selected from the group consisting of GGSG (SEQ ID NO: 68), GGSGG (SEQ ID NO: 69), GSGSG (SEQ ID NO: 70), GSGGG (SEQ ID NO: 71), GGGSG (SEQ ID NO: 72), and GSSSG (SEQ ID NO: 73).

[0019] In some embodiments of the activatable antibody and/or conjugated activatable antibody, LP1 includes the amino acid sequence GSSGSGGSGGSG (SEQ ID NO: 26).

[0020] In some embodiments of the activatable antibody and/or conjugated activatable antibody, LP2 includes the amino acid sequence GSSGT (SEQ ID NO: 30) or GSSG (SEQ ID NO: 65).

[0021] In some embodiments of the activatable antibody and/or conjugated activatable antibody, the AB 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, or a single domain light chain antibody. In some embodiments, such an antibody or immunologically active fragment thereof that binds EGFR is a mouse, chimeric, humanized or fully human monoclonal antibody.

[0022] In some embodiments of the activatable antibody and/or conjugated activatable antibody, the AB has an equilibrium dissociation constant of about 100 nM or less for binding to EGFR.

[0023] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 2, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence -Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY

(SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence is selected from the group consisting of GQSGQ (SEQ ID NO: 14), QSGQ (SEQ ID NO: 16), SGQ (SEQ ID NO: 18), GQ (SEQ ID NO: 20), and Q (SEQ ID NO: 22).

[0024] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 2, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence GQSGQ (SEQ ID NO: 14).

[0025] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 2, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence QSGQ (SEQ ID NO: 16).

[0026] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 2, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer

Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence SGQ (SEQ ID NO: 18).

[0027] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 2, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence GQ (SEQ ID NO: 20).

[0028] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 2, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence Q (SEQ ID NO: 22).

[0029] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 34, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID

NO: 28), and the Spacer Sequence is selected from the group consisting of GQSGQ (SEQ ID NO: 14), QSGQ (SEQ ID NO: 16), SGQ (SEQ ID NO: 18), GQ (SEQ ID NO: 20), and Q (SEQ ID NO: 22).

[0030] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 34, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence GQSGQ (SEQ ID NO: 14).

[0031] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 34, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence QSGQ (SEQ ID NO: 16).

[0032] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 34, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY

(SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence SGQ (SEQ ID NO: 18).

[0033] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 34, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence GQ (SEQ ID NO: 20).

[0034] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 34, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence Q (SEQ ID NO: 22).

[0035] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence is selected from the group consisting of GQSGQ (SEQ

ID NO: 14), QSGQ (SEQ ID NO: 16), SGQ (SEQ ID NO: 18), GQ (SEQ ID NO: 20), and Q (SEQ ID NO: 22).

[0036] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 10, and a light chain amino acid sequence that includes SEQ ID NO: 36, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence QSGQ (SEQ ID NO: 14).

[0037] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 10, and a light chain amino acid sequence that includes SEQ ID NO: 36, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence QSGQ (SEQ ID NO: 16).

[0038] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 10, and a light chain amino acid sequence that includes SEQ ID NO: 36, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID

NO: 28), and the Spacer Sequence includes the amino acid sequence SGQ (SEQ ID NO: 18).

[0039] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 10, and a light chain amino acid sequence that includes SEQ ID NO: 36, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence GQ (SEQ ID NO: 20).

[0040] In some embodiments, the activatable antibody and/or conjugated activatable antibody includes an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence that includes SEQ ID NO: 10, and a light chain amino acid sequence that includes SEQ ID NO: 36, wherein the activatable antibody and/or conjugated activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence includes the amino acid sequence Q (SEQ ID NO: 22).

[0041] In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12. In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%

identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0042] In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a heavy chain that includes the amino acid sequence of SEQ ID NO: 2, and a masked light chain that includes the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12. In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid of SEQ ID NO: 2, and a masked light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0043] In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a heavy chain that includes the amino acid sequence of SEQ ID NO: 34, and a masked light chain that includes the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12. In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid of SEQ ID NO: 6, and a masked light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0044] In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a heavy chain that includes the amino acid sequence of SEQ ID NO: 36, and a masked light chain that includes the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12. In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid of SEQ ID NO: 36, and a masked light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0045] In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a combination of a heavy chain amino acid sequence and a masked light chain amino acid sequence selected from the group consisting of (i) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 4, (ii) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 6, (iii) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 8, (iv) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 10, (v) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 12, (vi) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 4, (vii) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 6, (viii) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 8, (ix) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 10, (x) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 12, (xi) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 4, (xii) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 6, (xiii) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 8, (xiv) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 10, and (xv) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 12.

[0046] In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a combination of a heavy chain amino acid sequence and a masked light chain amino acid sequence selected from the group consisting of (i) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 4, (ii) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 6, (iii) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 8, (iv) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 10, (v) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 12, (vi) a heavy chain sequence of SEQ ID NO: 34 and a masked

light chain amino acid sequence of SEQ ID NO: 4, (vii) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 6, (viii) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 8, (ix) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 10, (x) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 12, (xi) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 4, (xii) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 6, (xiii) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 8, (xiv) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 10, and (xv) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 12.

[0047] In some embodiments of the activatable antibody and/or conjugated activatable antibody, the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24) and is a polypeptide of no more than 40 amino acids long.

[0048] In some embodiments of the activatable antibody and/or conjugated activatable antibody, the MM has an equilibrium dissociation constant for binding to the AB that is greater than the equilibrium dissociation constant of the AB to EGFR.

[0049] In some embodiments of the activatable antibody and/or conjugated activatable antibody, the MM has an equilibrium dissociation constant for binding to the AB that is no more than the equilibrium dissociation constant of the AB to EGFR.

[0050] In some embodiments of the activatable antibody and/or conjugated activatable antibody, the MM does not interfere or compete with the AB of the activatable antibody in a cleaved state for binding to EGFR.

[0051] In some embodiments of the activatable antibody and/or conjugated activatable antibody, the coupling of the MM to the AB reduces the ability of the AB to bind EGFR such that the dissociation constant (K_d) of the AB when coupled to the MM towards EGFR is at least 20 times greater than the K_d of the AB when not coupled to the MM towards EGFR. In some embodiments of the activatable antibody and/or conjugated activatable antibody, the coupling of the MM to the AB reduces the ability of the AB to bind EGFR such that the dissociation constant (K_d) of the AB when coupled to the MM towards EGFR is at least 40 times greater than the K_d of the AB when not coupled to the

MM towards EGFR. In some embodiments of the activatable antibody and/or conjugated activatable antibody, the coupling of the MM to the AB reduces the ability of the AB to bind EGFR such that the dissociation constant (K_d) of the AB when coupled to the MM towards EGFR is at least 50 times greater than the K_d of the AB when not coupled to the MM towards EGFR. In some embodiments of the activatable antibody and/or conjugated activatable antibody, the coupling of the MM to the AB reduces the ability of the AB to bind EGFR such that the K_d of the AB when coupled to the MM towards EGFR is at least 100 times greater than the K_d of the AB when not coupled to the MM towards EGFR. In some embodiments of the activatable antibody and/or conjugated activatable antibody, the coupling of the MM to the AB reduces the ability of the AB to bind EGFR such that the K_d of the AB when coupled to the MM towards EGFR is at least 1000 times greater than the K_d of the AB when not coupled to the MM towards EGFR. In some embodiments of the activatable antibody and/or conjugated activatable antibody, the coupling of the MM to the AB reduces the ability of the AB to bind EGFR such that the K_d of the AB when coupled to the MM towards EGFR is at least 10,000 times greater than the K_d of the AB when not coupled to the MM towards EGFR.

[0052] In some embodiments of the activatable antibody and/or conjugated activatable antibody, in the presence of EGFR, the MM reduces the ability of the AB to bind EGFR by at least 90% when the CM is uncleaved, as compared to when the CM is cleaved when assayed *in vitro* using a target displacement assay such as, for example, the assay described in PCT Publication Nos. WO 2009/025846 and WO 2010/081173.

[0053] In some embodiments of the activatable antibody and/or conjugated activatable antibody, the CM is a substrate for a protease. In some embodiments of the activatable antibody and/or conjugated activatable antibody, the protease is co-localized with EGFR in a tissue, and the protease cleaves the CM when the activatable antibody and/or conjugated activatable antibody is exposed to the protease. In some embodiments, the protease is not active or is significantly less active in tissues that do not significantly express EGFR. In some embodiments, the protease is not active or is significantly less active in healthy, *e.g.*, non-diseased tissues. In some embodiments, the protease is not active or is significantly less active in diseased tissues not intended for therapy.

[0054] In some embodiments, the CM is positioned in the activatable antibody and/or conjugated activatable antibody such that in the uncleaved state, binding to EGFR by the activatable antibody is reduced to occur with an equilibrium dissociation constant that is

at least 20-fold greater than the equilibrium dissociation constant of an unmodified AB binding to EGFR, and whereas the AB of the activatable antibody and/or the conjugated activatable antibody in the cleaved state binds EGFR.

[0055] In some embodiments, the CM is positioned in the activatable antibody and/or the conjugated activatable antibody such that in the uncleaved state, binding to EGFR by the activatable antibody is reduced to occur with an equilibrium dissociation constant that is at least 40-fold greater than the equilibrium dissociation constant of an unmodified AB binding to EGFR, and whereas the AB of the activatable antibody and/or the conjugated activatable antibody in the cleaved state binds EGFR.

[0056] In some embodiments, the CM is positioned in the activatable antibody and/or the conjugated activatable antibody such that in the uncleaved state, binding to EGFR by the activatable antibody is reduced to occur with an equilibrium dissociation constant that is at least 50-fold greater than the equilibrium dissociation constant of an unmodified AB binding to EGFR, and whereas the AB of the activatable antibody and/or the conjugated activatable antibody in the cleaved state binds EGFR.

[0057] In some embodiments, the CM is positioned in the activatable antibody and/or the conjugated activatable antibody such that in the uncleaved state, binding to EGFR by the activatable antibody is reduced to occur with an equilibrium dissociation constant that is at least 100-fold greater than the equilibrium dissociation constant of an unmodified AB binding to EGFR, and whereas the AB of the activatable antibody and/or the conjugated activatable antibody in the cleaved state binds EGFR.

[0058] In some embodiments, the CM is positioned in the activatable antibody and/or the conjugated activatable antibody such that in the uncleaved state, binding to EGFR by the activatable antibody is reduced to occur with an equilibrium dissociation constant that is at least 200-fold greater than the equilibrium dissociation constant of an unmodified AB binding to EGFR, and whereas the AB of the activatable antibody and/or the conjugated activatable antibody in the cleaved state binds EGFR.

[0059] In some embodiments, the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28) and is a polypeptide of up to 15 amino acids in length.

[0060] In some embodiments, the CM is a substrate for an enzyme selected from the group consisting of uPA, legumain and MT-SP1. In some embodiments, the enzyme comprises uPA. In some embodiments, the enzyme comprises legumain. In some embodiments, the enzyme comprises MT-SP1.

[0061] In some embodiments, the activatable antibody also includes an agent conjugated to the AB. In some embodiments, the agent is a therapeutic agent. In some embodiments, the agent is an antineoplastic agent. In some embodiments, the agent is a toxin or fragment thereof. As used herein, a fragment of a toxin is a fragment that retains toxic activity. In some embodiments, the agent is conjugated to the AB via a cleavable linker. In some embodiments, the agent is conjugated to the AB via a linker that includes at least one MMP-cleavable substrate sequence. In some embodiments, the agent is conjugated to the AB via a noncleavable 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 agent is an agent selected from the group listed in Table 1. In some embodiments, the agent is a dolastatin. In some embodiments, the agent is an auristatin or derivative thereof. In some embodiments, the agent is auristatin E or a derivative thereof. In some embodiments, the agent is monomethyl auristatin E (MMAE). In some embodiments, the agent is monomethyl auristatin D (MMAD). In some embodiments, the agent is a maytansinoid or maytansinoid derivative. In some embodiments, the agent is DM1 or DM4. In some embodiments, the agent is a duocarmycin or derivative thereof. In some embodiments, the agent is a calicheamicin or derivative thereof. In some embodiments, the agent is a pyrrolobenzodiazepine.

[0062] In some embodiments, the agent is conjugated to the AB via a linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the linker is selected from the group consisting of the linkers shown in Tables 2 and 3.

[0063] In some embodiments, the activatable antibody also includes a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent. In some embodiments, the detectable moiety is a conjugatable detection reagent. In some embodiments, the detectable moiety is, for example, a fluorescent label or radioisotope. In some embodiments, the detectable moiety is an imaging agent, a contrasting agent, an enzyme, a fluorescent label, a chromophore, a dye, one or more metal ions, and/or a ligand-based label.

[0064] In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is longer than that of the corresponding antibody; e.g., the pK of the activatable antibody and/or conjugated activatable antibody is longer than that of the corresponding antibody. In some embodiments, the serum half-life of the activatable

antibody and/or conjugated activatable antibody is similar to that of the corresponding antibody. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 15 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 12 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 11 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 10 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 9 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 8 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 7 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 6 days when administered to an organism. In some examples of any of these activatable antibody and/or conjugated activatable antibody embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 5 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 4 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 3 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 2 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 24 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 20 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 18 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 16 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 14 hours when administered to an

organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 12 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 10 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 8 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 6 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 4 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody and/or conjugated activatable antibody is at least 3 hours when administered to an organism.

[0065] In some embodiments, the activatable anti-EGFR antibody is monospecific. In some embodiments, the activatable anti-EGFR antibody is multispecific, *e.g.*, by way of non-limiting example, bispecific or trifunctional. In some embodiments, the activatable anti-EGFR antibody is formulated as part of a activatable antibody and/or conjugated activatable antibody, such as a T-cell engaging activatable antibody and/or conjugated activatable antibody. In some embodiments, the activatable anti-EGFR antibody is formulated as part of a pro-Bispecific T Cell Engager (BITE) molecule. In some embodiments, the activatable anti-EGFR antibody is formulated as part of a pro-Chimeric Antigen Receptor (CAR) modified T cell or other engineered receptor.

[0066] The invention also provides an isolated nucleic acid molecule encoding an activatable anti-EGFR antibody described herein, as well as vectors that include these isolated nucleic acid sequences. The invention provides methods of producing an activatable antibody by culturing a cell under conditions that lead to expression of the activatable antibody, wherein the cell includes such a vector.

[0067] The invention also provides a method of manufacturing an activatable antibody that in an activated state binds Epidermal Growth Factor Receptor (EGFR) by (a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody under conditions that lead to expression of the activatable antibody, wherein the activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid

sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12; and (b) recovering the activatable antibody.

[0068] The invention also provides a method of manufacturing a conjugated activatable antibody that in an activated state binds Epidermal Growth Factor Receptor (EGFR) by (a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody under conditions that lead to expression of the activatable antibody, wherein the activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12; (b) recovering the activatable antibody; and (c) conjugating the activatable antibody to one or more additional agents.

[0069] The invention provides methods of treating, preventing and/or delaying the onset or progression of, or alleviating a symptom of an indication, *e.g.*, disease or disorder, associated with aberrant expression and/or activity of EGFR, *e.g.*, an EGFR-related disorder or EGFR-related disease, in a subject using a therapeutic molecule, *e.g.*, activatable antibodies that bind EGFR and/or conjugated activatable antibodies that bind EGFR, particularly activatable antibodies and/or conjugated activatable antibodies that bind and neutralize or otherwise inhibit at least one biological activity of EGFR and/or EGFR-mediated signaling.

[0070] In some embodiments, the indication, *e.g.*, disease or disorder, associated with aberrant expression and/or activity of EGFR is a cancer. In some embodiments, the cancer is a breast cancer, *e.g.*, by way of non-limiting example, the breast cancer is a triple-negative breast cancer. In some embodiments, the cancer is a triple-negative breast cancer. In some embodiments, the cancer is colorectal cancer. In some embodiments, the cancer is gastric cancer. In some embodiments, the cancer is glioblastoma. In some embodiments, the cancer is a head and neck cancer, *e.g.*, by way of non-limiting example, esophageal cancer. In some embodiments, the cancer is an esophageal cancer. In some embodiments, the cancer is a lung cancer, *e.g.*, by way of non-limiting example, non-small cell lung cancer. In some embodiments, the cancer is a non-small cell lung cancer. In some embodiments, the cancer is ovarian/endometrial cancer. In some embodiments, the cancer is ovarian cancer. In some embodiments, the cancer is endometrial cancer. In some embodiments, the cancer is pancreatic cancer. In some embodiments, the cancer is prostate

cancer. In some embodiments, the cancer is a renal cancer. In some embodiments, the cancer is a sarcoma, *e.g.*, by way of non-limiting example, osteosarcoma. In some embodiments, the cancer is an osteosarcoma. In some embodiments, the cancer is a skin cancer, *e.g.*, by way of non-limiting example, squamous cell cancer, basal cell carcinoma, and/or melanoma. In some embodiments, the cancer is a squamous cell cancer. In some embodiments, the cancer is a basal cell carcinoma. In some embodiments, the cancer is a melanoma.

[0071] In some embodiments, the indication, *e.g.*, disease or disorder, associated with aberrant expression and/or activity of EGFR is an inflammatory disorder and/or an autoimmune disease. In some embodiments, the inflammatory and/or autoimmune disease is psoriasis.

[0072] The therapeutic molecule, *e.g.*, activatable anti-EGFR antibody and/or conjugated anti-EGFR activatable antibody, can be administered at any stage of the disease. For example, a therapeutic molecule can be administered to a patient suffering cancer of any stage, from early to metastatic. For example, a therapeutic molecule can be administered to a patient suffering from an inflammatory disorder and/or autoimmune disease of any stage, from early onset to an advanced stage. It is to be understood that the terms subject and patient are used interchangeably herein.

[0073] The therapeutic molecules, *e.g.* activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies, are also useful in other therapeutic indications and treatment regimens. For example, the therapeutic molecules of the embodiments provided herein can be used in a treatment regimen that includes neoadjuvant therapy.

[0074] In some embodiments, the therapeutic molecules, *e.g.* activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies, are administered during and/or after treatment in combination with one or more additional agents such as, by way of non-limiting example, a chemotherapeutic agent. In some embodiments, the therapeutic molecule and the additional agent(s) are administered simultaneously. For example, the therapeutic molecule and the additional agent(s) can be formulated in a single composition or administered as two or more separate compositions. In some embodiments, the therapeutic molecule and the additional agent(s) are administered sequentially.

[0075] The invention also provides methods of preventing, delaying the progression of, treating, alleviating a symptom of, or otherwise ameliorating an EGFR-related disorder,

for example, cancer, in a subject by administering a therapeutically effective amount of an activatable anti-EGFR antibody described herein to a subject in need thereof.

[0076] The invention also provides methods of inhibiting angiogenesis in a subject by administering a therapeutically effective amount of an activatable anti-EGFR antibody described herein to a subject in need thereof.

[0077] In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human mammal, such as a rodent, a non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal.

[0078] The activatable anti-EGFR antibody and therapeutic formulations thereof are administered to a subject suffering from or susceptible to a disease or disorder associated with aberrant EGFR expression and/or activity. A subject suffering from or susceptible to a disease or disorder associated with aberrant EGFR expression and/or activity is identified using any of a variety of methods known in the art. For example, subjects suffering from cancer or other neoplastic condition are identified using any of a variety of clinical and/or laboratory tests such as, physical examination and blood, urine and stool analysis to evaluate health status.

[0079] The invention also provides methods of using activatable antibodies that bind EGFR (*i.e.*, activatable anti-EGFR antibodies, also referred to herein as anti-EGFR activatable antibodies) in a variety of diagnostic and/or prophylactic indications, as well as kits for use in these methods. For example, the invention provides methods of detecting presence or absence of a cleaving agent and EGFR in a subject or a sample by (i) contacting a subject or sample with an anti-EGFR activatable antibody, and (ii) measuring a level of activated anti-EGFR activatable antibody in the subject or sample, wherein a detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent and EGFR are present in the subject or sample and wherein no detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent, EGFR or both the cleaving agent and EGFR are absent in the subject or sample. As used herein, "absent" can refer to the state of being not present at all in a subject or sample, as well as the state of being not present at a significant level in a subject or sample. Such an anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group

consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0080] The invention also provides kits for use in methods of detecting presence or absence of a cleaving agent and a target of interest (EGFR) in a subject or a sample, where the kits include at least an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody described herein for use in contacting a subject or sample and means for detecting the level of activated anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody in the subject or sample, wherein a detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent and EGFR are present in the subject or sample and wherein no detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent, EGFR or both the cleaving agent and EGFR are absent in the subject or sample. Such an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0081] The invention also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or sample with an anti-EGFR activatable antibody in the presence of EGFR, and (ii) measuring a level of activated anti-EGFR activatable antibody in the subject or sample, wherein a detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent is present in the subject or sample and wherein no detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent is absent in the subject or sample. Such an anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0082] The invention also provides kits for use in methods of detecting presence or absence of a cleaving agent in a subject or a sample, where the kits include at least an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody described herein for use in contacting a subject or sample and means for detecting the level of

activated anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody in the subject or sample, wherein a detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent is present in the subject or sample and wherein no detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent is absent in the subject or sample. Such an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0083] The invention provides methods of detecting presence or absence of a cleaving agent and EGFR in a subject or a sample by (i) contacting a subject or sample with an anti-EGFR activatable antibody, wherein the anti-EGFR activatable antibody includes a detectable label that is positioned on a portion of the anti-EGFR activatable antibody that is released following cleavage of the CM and (ii) measuring a level of activated anti-EGFR activatable antibody in the subject or sample, wherein a detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent, EGFR or both the cleaving agent and EGFR are absent in the subject or sample, and wherein no detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent and EGFR are present in the subject or sample. Such an anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0084] The invention also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or sample with an anti-EGFR activatable antibody, wherein the anti-EGFR activatable antibody includes a detectable label that is positioned on a portion of the anti-EGFR activatable antibody that is released following cleavage of the CM; and (ii) measuring a level of detectable label in the subject or sample, wherein a detectable level of the detectable label in the subject or sample indicates that the cleaving agent is absent in the subject or sample and wherein no detectable level of the detectable label in the subject or sample indicates that the cleaving agent is present in the subject or sample. Such an anti-EGFR activatable antibody includes a heavy

chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0085] The invention also provides kits for use in methods of detecting presence or absence of a cleaving agent and a target of interest (EGFR) in a subject or a sample, where the kits include at least an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody described herein for use in contacting a subject or sample and means for detecting the level of activated anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody in the subject or sample, wherein a detectable level of the detectable label in the subject or sample indicates that the cleaving agent is absent in the subject or sample and wherein no detectable level of the detectable label in the subject or sample indicates that the cleaving agent is present in the subject or sample. Such an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0086] In some embodiments of these methods and/or kits, the anti-EGFR activatable antibody includes a detectable label. In some embodiments, the detectable label is positioned on the AB. In some embodiments, measuring the level of activatable anti-EGFR antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label. In some embodiments of these methods and/or kits, the detectable label includes an imaging agent, a radioisotope, a contrasting agent, an enzyme, a fluorescent label, a chromophore, a dye, one or more metal ions, or a ligand-based label. In some embodiments of these methods and/or kits, the imaging agent comprises a radioisotope. In some embodiments of these methods, the radioisotope is indium or technetium. In some embodiments of these methods, the radioisotope is or is derived from iodine. In some embodiments of these methods, the radioisotope is ^{125}I or ^{133}I . In some embodiments of these methods and/or kits, the contrasting agent comprises iodine, gadolinium or iron oxide. In some embodiments of these methods and/or kits, the enzyme comprises horseradish

peroxidase, alkaline phosphatase, or β -galactosidase. In some embodiments of these methods and/or kits, the fluorescent label comprises yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), green fluorescent protein (GFP), modified red fluorescent protein (mRFP), red fluorescent protein tdimer2 (RFP tdimer2), HCRED, or a europium derivative. In some embodiments of these methods and/or kits, the luminescent label comprises an N-methylacrydium derivative. In some embodiments of these methods and/or kits, the label comprises an Alexa Fluor[®] label, such as Alex Fluor[®] 680 or Alexa Fluor[®] 750. In some embodiments of these methods and/or kits, the ligand-based label comprises biotin, avidin, streptavidin or one or more haptens.

[0087] In some embodiments of these methods and/or kits, the subject is a mammal. In some embodiments of these methods and/or kits, the subject is a human. In some embodiments, the subject is a non-human mammal, such as a rodent, a non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal.

[0088] In some embodiments of these methods, the method is an *in vivo* method. In some embodiments of these methods, the method is an *in situ* method. In some embodiments of these methods, the method is an *ex vivo* method. In some embodiments of these methods, the method is an *in vitro* method.

[0089] In some embodiments of the methods and/or kits, the method and/or kit is used to identify or otherwise refine, e.g., stratify, a patient population suitable for treatment with an anti-EGFR activatable antibody of the disclosure. For example, patients that test positive for both the target (e.g., EGFR) and a protease that cleaves the substrate in the cleavable moiety (CM) of the anti-EGFR activatable antibody being tested in these methods are identified as suitable candidates for treatment with such an anti-EGFR activatable antibody comprising such a CM. Likewise, patients that test negative for either or both of the target (e.g., EGFR) and the protease that cleaves the substrate in the CM in the activatable antibody being tested using these methods might be identified as suitable candidates for another form of therapy (i.e., not suitable for treatment with the anti-EGFR activatable antibody being tested). In some embodiments, such patients can be tested with other anti-EGFR activatable antibodies until a suitable anti-EGFR activatable antibody for treatment is identified (e.g., an anti-EGFR activatable antibody comprising a CM that is cleaved by the patient at the site of disease). Such an anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence

selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0090] The invention also provides kits for use in methods of identifying or otherwise refining a patient population, where the kits include at least (i) an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody described herein for use in contacting a subject or sample, (ii) means for detecting the level of activated anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody in the subject or sample, wherein a detectable level of activated anti-EGFR activatable antibody in the sample indicates that the sample is positive for the presence of EGFR and a cleaving agent that cleaves the substrate in the cleavable moiety (CM) of the anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody and (iii) means for identifying and selecting one or more subjects that test positive for the presence of EGFR and the cleaving agent thereby identifying or refining a patient population. Such an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12. In some embodiments, the kit also includes instructions for administering a therapeutically effective amount of an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody described herein to the one or more subjects in the patient population that test positive for the presence of EGFR and the cleaving agent. In some embodiments, the kit also includes instructions for administering a therapeutically effective amount of another anti-EGFR therapeutic agent described herein to the one or more subjects in the patient population that did not test positive for the presence of both EGFR and the cleaving agent. In some embodiments, the anti-EGFR activatable antibody comprises a detectable label. In some embodiments, the detectable label is positioned on the AB. In some embodiments, measuring the level of activatable anti-EGFR antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label. In some embodiments, the detectable label comprises an imaging agent, a radioisotope, a contrasting agent, an enzyme, a fluorescent label, a chromophore, a dye, one or more metal ions, or a ligand-based label. In some embodiments, the EGFR-related

disorder is cancer. In some embodiments, the cancer is breast cancer, colorectal cancer, gastric cancer, glioblastoma, head and neck cancer, lung cancer, ovarian cancer, endometrial cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, or skin cancer. In some embodiments, the EGFR-related disorder is psoriasis.

[0091] The invention also provides methods of using the anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies (*i.e.*, activatable anti-EGFR antibody conjugates, also referred to herein as conjugated activatable anti-EGFR antibodies and/or conjugated anti-EGFR activatable antibodies) in a variety of diagnostic and/or prophylactic indications. For example, the invention provides methods of detecting presence or absence of a cleaving agent and a target of interest (EGFR) in a subject or a sample by (i) contacting a subject or sample with an anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody and (ii) measuring a level of anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody in the subject or sample, wherein a detectable level of activated anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody in the subject or sample indicates that the cleaving agent and EGFR are present in the subject or sample and wherein no detectable level of activated anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody in the subject or sample indicates that the cleaving agent, EGFR or both the cleaving agent and EGFR are absent in the subject or sample. Such an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0092] The invention also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or sample with an anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody in the presence of EGFR, and (ii) measuring a level of activated anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody in the subject or sample, wherein a detectable level of activated anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody in the subject or sample indicates that the cleaving agent is present in the subject or sample and wherein no detectable level of anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody in the subject or sample indicates that the cleaving agent is absent in the subject or sample. Such an anti-EGFR activatable antibody and/or conjugated anti-EGFR

activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0093] The invention also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or sample with an anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody; and (ii) measuring a level of detectable label in the subject or sample, wherein a detectable level of the detectable label in the subject or sample indicates that the cleaving agent is absent in the subject or sample and wherein no detectable level of the detectable label in the subject or sample indicates that the cleaving agent is present in the subject or sample. Such an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0094] In some embodiments of these methods, the anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody includes a detectable label selected from the group consisting of an imaging agent, a radioisotope, a contrasting agent, an enzyme, a fluorescent label, a chromophore, a dye, one or more metal ions, and a ligand-based label. In some embodiments of these methods, the imaging agent comprises a radioisotope. In some embodiments of these methods, the radioisotope is indium or technetium. In some embodiments of these methods, the contrasting agent comprises iodine, gadolinium or iron oxide. In some embodiments of these methods, the enzyme comprises horseradish peroxidase, alkaline phosphatase, or β -galactosidase. In some embodiments of these methods, the fluorescent label comprises yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), green fluorescent protein (GFP), modified red fluorescent protein (mRFP), red fluorescent protein tdimer2 (RFP tdimer2), HCRED, or a europium derivative. In some embodiments of these methods, the luminescent label comprises an N-methylacrydium derivative. In some embodiments of these methods, the label comprises an Alexa Fluor[®] label, such as Alex Fluor[®] 680 or Alexa Fluor[®] 750. In some embodiments of these methods, the ligand-based label comprises biotin, avidin, streptavidin or one or more haptens. In some embodiments, the detectable label is positioned on the AB. In some

embodiments, measuring the level of activatable anti-EGFR antibody in the subject or sample is accomplished using a secondary reagent that specifically binds to the activated antibody, wherein the reagent comprises a detectable label. In some embodiments, the secondary reagent is an antibody comprising a detectable label.

[0095] In some embodiments of these methods, the subject is a mammal. In some embodiments of these methods, the subject is a human. In some embodiments, the subject is a non-human mammal, such as a rodent, a non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal.

[0096] In some embodiments of these methods, the method is an *in vivo* method. In some embodiments of these methods, the method is an *in situ* method. In some embodiments of these methods, the method is an *ex vivo* method. In some embodiments of these methods, the method is an *in vitro* method.

[0097] In some embodiments of the methods, the method is used to identify or otherwise refine a patient population suitable for treatment with an anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody of the disclosure. For example, patients that test positive for both the target (e.g., EGFR) and a protease that cleaves the substrate in the cleavable moiety (CM) of the anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody being tested in these methods are identified as suitable candidates for treatment with such anti-EGFR antibody and/or such a conjugated activatable anti-EGFR antibody comprising such a CM. Likewise, patients that test negative for either or both of the target (e.g., EGFR) and the protease that cleaves the substrate in the CM in the activatable antibody being tested using these methods might be identified as suitable candidates for another form of therapy (i.e., not suitable for treatment with the anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody being tested). In some embodiments, such patients can be tested with other anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody until a suitable anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody for treatment is identified (e.g., an anti-EGFR antibody and/or conjugated activatable anti-EGFR antibody comprising a CM that is cleaved by the patient at the site of disease). Such an anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[0098] Pharmaceutical compositions according to the invention can include an antibody and/or conjugated antibody of the invention and a carrier. These pharmaceutical compositions can be included in kits, such as, for example, diagnostic kits.

Brief Description of the Drawings

[0099] Figure 1 is a schematic representation of an activatable antibody.

Detailed Description of the Invention

[00100] The present invention provides activatable monoclonal antibodies (mAbs) that specifically bind human epidermal growth factor receptor (EGFR), also known as EGF receptor, human EGF receptor-1 (HER1), erbB, erbB1, and species antigen 7 (SA-7). The activatable antibodies in an uncleaved state include a masked light chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12. The activatable antibodies include a heavy chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36.

[00101] In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a combination of a heavy chain amino acid sequence and a masked light chain amino acid sequence selected from the group consisting of (i) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 4, (ii) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 6, (iii) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 8, (iv) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 10, (v) a heavy chain sequence of SEQ ID NO: 2 and a masked light chain amino acid sequence of SEQ ID NO: 12, (vi) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 4, (vii) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 6, (viii) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 8, (ix) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 10, (x) a heavy chain sequence of SEQ ID NO: 34 and a masked light chain amino acid sequence of SEQ ID NO: 12, (xi) a heavy chain sequence of

SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 4, (xii) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 6, (xiii) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 8, (xiv) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 10, and (xv) a heavy chain sequence of SEQ ID NO: 36 and a masked light chain amino acid sequence of SEQ ID NO: 12.

[00102] The activatable anti-EGFR antibodies, also referred to herein as anti-EGFR activatable antibodies or EGFR activatable antibodies, are used in methods of treating, preventing, delaying the progression of, ameliorating and/or alleviating a symptom of a disease or disorder associated with aberrant EGFR expression and/or activity. For example, the activatable anti-EGFR antibodies are used in methods of treating, preventing, delaying the progression of, ameliorating and/or alleviating a symptom of a cancer or other neoplastic condition.

[00103] In an activated state, e.g., a cleaved state, the activatable antibodies bind EGFR. In an uncleaved state, e.g., non-activated state, the activatable antibodies provided herein include an antibody or an antigen binding fragment thereof (AB) that specifically binds to EGFR that includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence -Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence is selected from the group consisting of GQSGQ (SEQ ID NO: 14), QSGQ (SEQ ID NO: 16), SGQ (SEQ ID NO: 18), GQ (SEQ ID NO: 20), and Q (SEQ ID NO: 22).

[00104] The MM is coupled to the AB via a CM sequence that includes a substrate for a protease, for example, a protease that is co-localized with EGFR at a treatment site in a subject. Numerous studies have demonstrated the correlation of aberrant protease levels, e.g., uPA, legumain, MT-SPI, matrix metalloproteases (MMPs), in solid tumors. (*See e.g.*, Murthy RV, et al. "Legumain expression in relation to clinicopathologic and biological variables in colorectal cancer." Clin Cancer Res. 11 (2005): 2293-2299; Nielsen BS, et al.

“Urokinase plasminogen activator is localized in stromal cells in ductal breast cancer.” Lab Invest 81 (2001): 1485–1501; Mook OR, et al. “In situ localization of gelatinolytic activity in the extracellular matrix of metastases of colon cancer in rat liver using quenched fluorogenic DQ-gelatin.” J Histochem Cytochem. 51 (2003): 821-829).

[00105] The activatable anti-EGFR antibodies provided herein include a substrate for a protease, which is useful in leveraging the protease activity in tumor cells for targeted antibody activation at the site of treatment and/or diagnosis. The substrate selection process is used to identify substrates that have a number of desirable characteristics. For example, the selected substrates are systemically stable (*i.e.*, stable in the systemic circulation of a subject), are generally not susceptible to cleavage by circulating proteases such as plasmin, thrombin, tissue plasminogen activator (tPA) or a kallikrein (KLK) such as KLK-5 and/or KLK-7, are non-toxic, are generally not susceptible to cleavage at potential sites of toxicity such as the skin by proteases such as ADAM 9, ADAM 10, ADAM 17 and/or kallikreins, such as KLK-5 and KLK-7, and are active at an intended site of treatment and/or diagnosis. In some embodiments, the identified substrates are selected for proteases that are overexpressed at an intended site of therapy and/or diagnosis but are not typically expressed at or in normal, healthy or otherwise non-diseased or damaged tissue or in diseased or damaged tissue at a site not intended for therapy and/or diagnosis, and then the selected substrates are subsequently counter-screened against proteases expressed in normal, *e.g.*, non-diseased, tissue or in diseased or damaged tissue at a site not intended for therapy and/or diagnosis.

[00106] The activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies described herein overcome a limitation of antibody therapeutics, particularly antibody therapeutics that are known to be toxic to at least some degree *in vivo*. Target-mediated toxicity constitutes a major limitation for the development of therapeutic antibodies. This is best exemplified by the skin rash that afflicts 88% of patients treated with cetuximab, an antibody that specifically binds epidermal growth factor receptor (EGFR) and has been approved for the treatment of colorectal and head and neck cancer. The activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies provided herein are designed to address the toxicity associated with the inhibition of the target in normal tissues by traditional therapeutic antibodies. These activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies remain masked until proteolytically activated at the site of disease. Starting with cetuximab as a parental

therapeutic antibody, the activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies provided herein were engineered by coupling the antibody to an inhibitory mask through a linker that incorporates a protease substrate. In studies performed *in vitro*, the binding to EGFR and the cell-based activity of the activatable anti-EGFR antibody is diminished compared to cetuximab. In studies performed *in vivo*, the activatable anti-EGFR antibody remains masked in normal tissues, but is activated and accumulates in the tumor environment. The tumor activation of the activatable anti-EGFR antibody translates into an *in vivo* efficacy that is equivalent to the efficacy of cetuximab.

[00107] The activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies provided herein satisfy a significant clinical need. EGFR is a clinically validated target that has been shown to promote proliferation, angiogenesis and invasion/metastasis, as well as to inhibit apoptosis of tumor cells. Antibodies and small molecule tyrosine kinase inhibitors targeting EGFR have been approved for cancer treatment (Nature Rev Cancer 5 (2005) 341; Curr Opin Cell Biol 21, (2009) 177). However, current anti-EGFR therapies, also referred to as EGFR inhibitors (EGFRi), have been shown to exhibit a number of adverse events post-treatment, including for example, papulopustular rash, particularly in the face and upper trunk of human subjects; dry and itchy skin; inflammation around the nails, loss of hair on the scalp; and increased growth of eyelashes and facial hair. Cutaneous toxicities that results from treatment with EGFRi have been shown to affect 45-100% of patients. (See e.g., Segal and Van Cutsem. Ann Oncol. 16(9) (2005):1425-33).

[00108] Exemplary activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies provided herein include, for example, the activatable antibodies referred to herein as the Des-1-3954-1204-C225v5 activatable antibody, the Des-2-3954-1204-C225v5 activatable antibody, the Des-3-3954-1204-C225v5 activatable antibody, the Des-4-3954-1204-C225v5 activatable antibody, and the Des-5-3954-1204-C225v5 activatable antibody, each of which binds epidermal growth factor receptor (EGFR) and includes the heavy chain sequence shown below as "C225v5 Activatable Antibody Heavy Chain." For example, the Des-1-3954-1204-C225v5 activatable antibody includes the Des-1-3954-1204-C225v5 light chain sequence shown below, and the C225v5 heavy chain sequence shown below; the Des-2-3954-1204-C225v5 activatable antibody includes the Des-2-3954-1204-C225v5 light chain sequence shown below, and the C225v5 heavy chain sequence shown below; the Des-3-3954-1204-C225v5 activatable antibody includes the

Des-3-3954-1204-C225v5 light chain sequence shown below, and the C225v5 heavy chain sequence shown below; the Des-4-3954-1204-C225v5 activatable antibody includes the Des-4-3954-1204-C225v5 light chain sequence shown below, and the C225v5 heavy chain sequence shown below; and the Des-5-3954-1204-C225v5 activatable antibody includes the Des-5-3954-1204-C225v5 light chain sequence shown below, and the C225v5 heavy chain sequence shown below.

[00109] The following annotations are used throughout the activatable antibody sequences shown below:

Bold:	Spacer sequence
<u>Underline:</u>	Mask sequence
<i>Italics and Underline:</i>	Linker 1 sequence
<u>Bold and Underline:</u>	1204 Substrate sequence
<u><i>Bold, Italics and Underline:</i></u>	Linker 2 sequence
Normal text:	anti-EGFR antibody derived sequence

C225v5 Activatable Antibody Heavy Chain Nucleotide Sequence:

[C225v5 (SEQ ID NO: 1)]

[caggtgcagctgaaacagagcggcccgggcctgggtgcagccgagccagagcctgagcatt
acctgcacogtgagcggccttttagcctgaccaactatggcgtgcattgggtgcgccagagcc
cgggcaaaggcctggaatggctgggcgtgatttgagcggcggaacaccgattataacac
cccgtttaccagcgcctgagcattaacaaagataacagcaaaagccaggtgttttttaa
atgaacagcctgcaaagccaggataaccgcgattttattattgcgcgcgcgcgctgaactatt
atgattatgaattttgcgtattggggccagggcaccctgggtgaccgtgagcgcggctagcac
caagggcccatcggtcttccccctggcacccctcctccaagagcaccctctgggggcacagcg
gccttgggctgcctgggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcag
gcgccctgaccagcggcgtgcacaccttcccggtgtcctacagtcctcaggactctactc
cctcagcagcgtgggtgaccgtgcctccagcagcttgggcacccagacctacatctgcaac
gtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaattcttgtagaca
aaactcacacatgcccaccgtgcccagcaccctgaactcctggggggaccgtcagtccttct
cttccccccaaaacccaaggacaccctcatgatctcccggaaccctgaggtcacatgcgtg
gtggtggacgtgagccacgaagaccctgaggtcaagttcaactggtacgtggacggcgtgg
aggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggt

cagcgctcctcaccgctcctgcaccaggactggctgaatggcaaggagtacaagtgcaaggctc
tccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagcccc
gagaaccacaggtgtacaccctgcccccatcccgggatgaactgaccaagaaccaggtcag
cctgacctgcttgggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaat
gggcagccgggagaacaactacaagaccacgcctcccgtgctggactccgacgggtccttct
tctctacagcaagctcaccggtggacaagagcaggtggcagcaggggaacgtcttctcatg
ctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctccctgtctccg
ggtaaatga] (SEQ ID NO: 1)

C225v5 Activatable Antibody Heavy Chain Amino Acid Sequence:

[C225v5 (SEQ ID NO: 2)]
[QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTDYN
TPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLTVTVSAAS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK*] (SEQ ID NO: 2)

Des-1-3954-1204-C225v5 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 13)][**Mask** (SEQ ID NO: 23)][**Linker 1** (SEQ
ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][**Linker 2** (SEQ ID
NO: 29)][C225 (SEQ ID NO: 31)]
[ggccagtcctggccag][tgcattctcacctcgtggttgtccggacggcccatacgtcatgt
acggctcgagcgggtggcagcgggtggtctctggtggatccggt][ctgagcggccgttccgat
aatcat][ggcagtagcgggtacc][cagatcttgcctgacccagagcccggtgattctgagc
gtgagcccgggcgaaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccaca
ttcattggtatcagcagcgcaccaacggcagcccgcgctgctgattaaatatgcgagcga
aagcattagcggcattccgagccgcttttagcggcagcggcagcggcaaccgattttaccctg
agcattaacagcgtggaaagcgaagatattgctgattattattgccagcagaacaacaact
ggccgaccacctttggcgcgggcaccaaactggaactgaaacgtacgggtggctgcaccatc
tgtcttcattcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgc

ctgctgaataaacttctatcccagagaggccaaagtacagtggaaggtggataacgcctcc
aatcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcct
cagcagcaccttgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaa
gtcaccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag]
(SEQ ID NO: 3)

Des-1-3954-1204-C225v5 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 14)][Mask (SEQ ID NO: 24)][Linker 1 (SEQ
ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][Linker 2 (SEQ ID
NO: 30)][C225 (SEQ ID NO: 32)]
[**GQSGQ**][CISPRGCPDGPYVMY][GSSGGSGGSGSG][**LSGRSDNH**][GSSGT][QILL
TQSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRFSGS
GSGTDFTLSINSVESEDIADYYCQQNNWNWPTTFGAGTKLELKRTVAAPSVFIFFPSDEQLK
SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNREGC*] (SEQ ID NO: 4)

Des-2-3954-1204-C225v5 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 15)][Mask (SEQ ID NO: 23)][Linker 1 (SEQ
ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][Linker 2 (SEQ ID
NO: 29)][C225 (SEQ ID NO: 31)]
[**cagtctggccag**][tgcattctcacctcgtggttggtccggacggcccatatcgtcatgtacg
gctcgagcgggtggcagcgggtggctctggtggatccggt][**ctgagcggccgttccgataat**
cat][ggcagtagcgggtacc][cagatcttgctgacccagagcccgggtgattctgagcgtg
agcccgggccaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccacacattc
attgggtatcagcagcgcaccaacggcagcccgcgcctgctgattaaatatgcgagcgaaag
cattagcggcattccgagccgcttttagcggcagcggcagcggcaccgattttaccctgagc
attaacagcgtggaaagcgaagatattgoggattattattgccagcagaacaacaactggc
cgaccacctttggcgcgggcaccaaactggaactgaaacgtacggtggctgcaccatctgt
cttcattcttcccgccatctgatgagcagttgaaatctggaactgcctctgttggtgtgcctg
ctgaataaacttctatcccagagaggccaaagtacagtggaaggtggataacgcctccaat
cgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcag
cagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtc

acccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag]
(SEQ ID NO: 5)

Des-2-3954-1204-C225v5 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 16)][**Mask** (SEQ ID NO: 24)][**Linker 1** (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][**Linker 2** (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]
[**QSGQ**][**CISPRGCPDGPYVMY**][**GSSGGSGGSGGSG**][**LSGRSDNH**][**GSSGT**][QILLT
QSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLSINSVESEDIADYYCQQNNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEK
HKVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 6)

Des-3-3954-1204-C225v5 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 17)][**Mask** (SEQ ID NO: 23)][**Linker 1** (SEQ ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][**Linker 2** (SEQ ID NO: 29)][C225 (SEQ ID NO: 31)]
[**tctggccag**][**tgcattctcacctcggtggttgctccggacggcccatagctcatgtacggct**
cgagcgggtggcagcgggtggctctggtggatccggt][**ctgagcggccggttccgataatcat**
][**ggcagtagcgggtacc**][cagatcttgctgacccagagcccggtgattctgagcgtgagc
ccgggcgaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccaacattcatt
ggtatcagcagcgcaccaacggcagcccgccgctgctgattaaatatgagcgcgaaagcat
tagcggcattccgagccgcttttagcggcagcggcagcggcaccgattttaccctgagcatt
aacagcgtggaaagcgaagatattgcggattattattgccagcagaacaacaactggccga
ccacctttggcgcgggcaccaaactggaactgaaacgtacgggtggctgcaccatctgtctt
catcttcccgccatctgatgagcagttgaaatctggaactgcctctgttggtgtgcctgctg
aataacttctatcccagagaggccaaagtacagtggaaggtggataacgccctccaatcgg
gtaactcccaggagagtggtcacagagcaggacagcaaggacagcacctacagcctcagcag
caccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacc
catcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag] (SEQ ID NO: 7)

Des-3-3954-1204-C225v5 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 18)][**Mask** (SEQ ID NO: 24)][**Linker 1** (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][**Linker 2** (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]

[**SGQ**][CISPRGCPDGPYVMY][GSSGGSGGSGSG][LSGRSDNH][GSSGT][QILLTQ
SPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSGS
GTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 8)

Des-4-3954-1204-C225v5 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 19)][**Mask** (SEQ ID NO: 23)][**Linker 1** (SEQ ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][**Linker 2** (SEQ ID NO: 29)][C225 (SEQ ID NO: 31)]

[**ggccag**][tgcattctcacctcgtggttgccggacggcccatacgtcatgtacggctcga
gcggtggcagcgggtggctctggtggatccggt][ctgagcggccgttccgataatcat][g
gcagtagcgggtacc][cagatcttgctgaccagagcccgggtgattctgagcgtgagccg
ggcgaaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccacattcattggt
atcagcagcgcaccaacggcagcccgcgcctgctgattaaatatgcgagcgaaagcattag
cggcattccgagccgcttttagcggcagcggcagcggcaccgattttaccctgagcattaac
agcgtggaaagcgaagatattgctgattattattgccagcagaacaacaactggccgacca
cctttggcgcgggcaccaaactggaactgaaacgtacgggtggctgcaccatctgtcttcat
cttcccgcctctgatgagcagttgaaatctggaactgcctctgttggtgtgctgctgaat
aacttctatcccagagaggccaaagtacagtggaaaggtggataacgcctccaatcgggta
actcccaggagagtggtcacagagcaggacagcaaggacagcacctacagcctcagcagcac
cctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccat
cagggcctgagctcgcccgtcacaagagcttcaacaggggagagtggttag] (SEQ ID NO: 9)

Des-4-3954-1204-C225v5 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 20)][**Mask** (SEQ ID NO: 24)][**Linker 1** (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][**Linker 2** (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]

[**GQ**] [CISPRGCPDGPYVMY] [GSSGGSGGSGGSG] [LSGRSDNH] [GSSGT] [QILLTQS
PVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSG
TDFTLINSINSEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDESTYLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 10)

Des-5-3954-1204-C225v5 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 21)] [Mask (SEQ ID NO: 23)] [Linker 1 (SEQ
ID NO: 25)] [**1204 Substrate** (SEQ ID NO: 27)] [Linker 2 (SEQ ID
NO: 29)] [C225 (SEQ ID NO: 31)]
[**cag**] [tgcattctcactcgtggttggtccggacggcccatcagtcattgtacggctcgagcg
gtggcagcggtggctctggtggatccgggt] [ctgagcggccggttccgataatcat] [ggca
gtagcgggtacc] [cagattcttgctgacccagagcccggtgattctgagcgtgagcccgggc
gaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccaacattcattggtatc
agcagcgcaccaacggcagcccgcgccctgctgattaaatatgcgagcgaaagcattagcgg
cattccgagccgcttttagcggcagcggcagcggcacccgattttaccctgagcattaacagc
gtggaaagcgaagatattgcggtattattattgccagcagaacaacaactggccgaccacct
ttggcgcgggcaccaaaactggaactgaaacgtacgggtggctgcaccatctgtcttcatctt
cccgccatctgatgagcagttgaaatctggaactgcctctgttggtgtgcctgctgaataac
ttctatccagagaggccaaagtacagtggaagggtggataacggccctccaatcgggtaact
cccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagcacct
gacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatcag
ggcctgagctcgcccgctcaciaaagagcttcaacaggggagagtggttag] (SEQ ID
NO: 11)

Des-5-3954-1204-C225v5 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 22)] [Mask (SEQ ID NO: 24)] [Linker 1 (SEQ
ID NO: 26)] [**1204 Substrate** (SEQ ID NO: 28)] [Linker 2 (SEQ ID
NO: 30)] [C225 (SEQ ID NO: 32)]
[**Q**] [CISPRGCPDGPYVMY] [GSSGGSGGSGGSG] [LSGRSDNH] [GSSGT] [QILLTQSP
VILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSGT
DFTLSINSINSEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA

SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 12)

[00110] Exemplary activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies provided herein include, for example, the activatable antibodies referred to herein as the Des-1-3954-1204-C225v4 activatable antibody, the Des-2-3954-1204-C225v4 activatable antibody, the Des-3-3954-1204-C225v4 activatable antibody, the Des-4-3954-1204-C225v4 activatable antibody, and the Des-5-3954-1204-C225v4 activatable antibody, each of which binds epidermal growth factor receptor (EGFR) and includes the heavy chain sequence shown below as “C225v4 Activatable Antibody Heavy Chain.” For example, the Des-1-3954-1204-C225v4 activatable antibody includes the Des-1-3954-1204-C225v4 light chain sequence shown below, and the C225v4 heavy chain sequence shown below; the Des-2-3954-1204-C225v4 activatable antibody includes the Des-2-3954-1204-C225v4 light chain sequence shown below, and the C225v4 heavy chain sequence shown below; the Des-3-3954-1204-C225v4 activatable antibody includes the Des-3-3954-1204-C225v4 light chain sequence shown below, and the C225v4 heavy chain sequence shown below; the Des-4-3954-1204-C225v4 activatable antibody includes the Des-4-3954-1204-C225v4 light chain sequence shown below, and the C225v4 heavy chain sequence shown below; and the Des-5-3954-1204-C225v4 activatable antibody includes the Des-5-3954-1204-C225v4 light chain sequence shown below, and the C225v4 heavy chain sequence shown below.

C225v4 Activatable Antibody Heavy Chain Nucleotide Sequence:

[C225v4 (SEQ ID NO: 33)]

[caggtgcagctgaaacagagcggcccggtggtgcagccgagccagagcctgagcatt
acctgcaccgtgagcggcttttagcctgaccaactatggcgtgcattgggtgcgccagagcc
cgggcaaaggcctggaatggctgggcgtgatttggagcggcggcaacaccgattataaac
cccgtttaccagccgctgagcattaacaaagataacagcaaaagccaggtgttttttaa
atgaacagcctgcaaagcaacgataaccgcgatttattattgcgcgcgcgcgctgacctatt
atgattatgaatttgctattggggccagggcaccctggtgaccgtgagcgcggctagcac
caagggcccatcggtcttccccctggcaccctcctccaagagcacctctgggggcacagcg
gccctgggctgctggtcaaggactacttccccgaaccggtgacggtgtcgtggaactcag
gcgcctgaccagcggcgtgcacaccttcccggtgtcctacagtcctcaggactctactc

cctcagcagcgtggtgaccgtgccctecagcagcttgggcacccagacctacatctgcaac
 gtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaaattcttgtagaca
 aaactcacacatgccaccgtgccagcacctgaactcctggggggaccgtcagttcttct
 ctccccccaaaacccaaggacacctcatgatctcccggaaccttgaggtcacatgcggtg
 gtggtggacgtgagccacgaagacctgaggtcaagttcaactggtacgtggacggcggtgg
 aggtgcataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgtgtggt
 cagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgaaggtc
 tccaacaaagccctcccagcccccatcgagaaaaccatctccaaagccaaagggcagcccc
 gagaaccacaggtgtacacctgcccccatcccgggatgaactgaccaagaaccaggtcag
 cctgaacctgcttgggtcaaaggcttctatcccagcgacatcgccgtggagtgaggagcaat
 gggcagcgggagaacaactacaagaccacgcctcccggtgctggactccgacgggtccttct
 tctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgtcttctcatg
 ctccgtgatgcatgaggtctctgcacaaccactacaacgcagaagagcctctccctgtotccg
 ggtaaatga] (SEQ ID NO: 33)

C225v4 Activatable Antibody Heavy Chain Amino Acid Sequence:

[C225v4 (SEQ ID NO: 34)]

[QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTDYN
 TPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTVSAAS
 TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
 SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
 LFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRV
 VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
 SLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
 CSVMHEALHNHYTQKSLSLSPGK*] (SEQ ID NO: 34)

Des-1-3954-1204-C225v4 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 13)][Mask (SEQ ID NO: 23)][Linker 1 (SEQ
 ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][Linker 2 (SEQ ID
 NO: 29)][C225 (SEQ ID NO: 31)]

[ggccaggtctggccag][tgcattctcacotcgtggttgctccggacggcccatacgtcatgt
ac][ggctcgagcgggtggcagcgggtggctctggtggatccggt][ctgagcggccggtccg
ataatcat][ggcagtagcgggtacc][cagatcttgctgaccagagcccggtgattctga

gcgtagagcccgggcggaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccac
 cattcattggtatcagcagcgcaccaacggcagcccgcgccctgctgattaaatatgcgagc
 gaaagcattagcggcattccgagccgcttttagcggcagcggcagcggcaccgattttaccc
 tgagcattaacagcgtggaaagcgaagatattgcccattattattgccagcagaacaacaa
 ctggcgaccacctttggcgcgggcaccaaaactggaactgaaacgtacgggtggctgcacca
 tctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttggtgt
 gcctgctgaataacttctatcccagagaggccaaagtacagtggaaggtggataacgcctt
 ccaatcgggtaactcccaggagagtggtcacagagcaggacagcaaggacagcacctacagc
 ctcagcagcacctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcg
 aagtcaccatcaggggctgagctcgcccgctcacaaagagcttcaacagggggagagtggtta
 g] (SEQ ID NO: 3)

Des-1-3954-1204-C225v4 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 14)][**Mask** (SEQ ID NO: 24)][**Linker 1** (SEQ
 ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][**Linker 2** (SEQ ID
 NO: 30)][C225 (SEQ ID NO: 32)]
 [GQSGQ][CISPRGCPDGPYVMY][GSSGGSGGSGSG][LSGRSDNH][GSSGT][QILL
 TQSPVILSVSPGERVVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGS
 GSGTDFTLSINSVESEDIADYYCQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLK
 SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYE
 KHKVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 4)

Des-2-3954-1204-C225v4 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 15)][**Mask** (SEQ ID NO: 23)][**Linker 1** (SEQ
 ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][**Linker 2** (SEQ ID
 NO: 29)][C225 (SEQ ID NO: 31)]
 [cagtctggccag][tgcattctacctcgtggttggtcgcgaacggcccatatcgtcatgtac]
 [ggctcgagcgggtggcagcgggtggctctggtggatccggt][ctgagcggccgttccgata
 atcat][ggcagtagcgggtacc][cagatcttgcctgacccagagcccggtgattctgagcg
 tgagcccgggcggaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccacat
 tcattggtatcagcagcgcaccaacggcagcccgcgccctgctgattaaatatgcgagcga
 agcattagcggcattccgagccgcttttagcggcagcggcagcggcaccgattttaccctga
 gcattaacagcgtggaaagcgaagatattgcccattattattgccagcagaacaacaactg

gccgaccacctttggcgcgggcaccaaaactggaactgaaacgtacggtgggtgcaccatct
gtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcc
tgctgaataactttctatcccagagaggccaaagtacagtgggaaggtggataacgccctcca
atcgggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctc
agcagcaccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaag
tcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag]
(SEQ ID NO: 5)

Des-2-3954-1204-C225v4 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 16)][**Mask** (SEQ ID NO: 24)][**Linker 1** (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][**Linker 2** (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]
[**QSGQ**][**CISPRGCPDGPYVMY**][**GSSGGSGSGSGSG**][**LSGRSDNH**][**GSSGT**][QILLT
QSPVILSVSPGERVSFSCRASQSIGTNIHWYQRTNGSPRLLIKYASESISGIPSRFSGSG
SGTDFTLSINSVESEDIADYYCQQNNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKS
GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEK
HKVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 6)

Des-3-3954-1204-C225v4 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 17)][**Mask** (SEQ ID NO: 23)][**Linker 1** (SEQ ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][**Linker 2** (SEQ ID NO: 29)][C225 (SEQ ID NO: 31)]
[**tctggccag**][**tgcattcacctcgtggttgctccggacggcccatagctcatgtac**][**gg**
ctcgagcgggtggcagcgggtggctctggtggatccggt][**ctgagcggccggttccgataatc**
at][**ggcagtagcgggtacc**][cagatcttgctgacccagagcccggtgattctgagcgtga
gcccgggcgaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccaacattca
ttggtatcagcagcgcaccaacggcagcccgcgccctgctgattaaatatgagagcgaaagc
attagcggcattccgagccgcttttagcggcagcggcagcggcaccgattttaccctgagca
ttaacagcgtggaaagcgaagatattgcggtattattattgccagcagaacaacaactggcc
gaccacctttggcgcgggcaccaaaactggaactgaaacgtacggtgggtgcaccatctgtc
ttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcctgc
tgaataactttctatcccagagaggccaaagtacagtgggaaggtggataacgccctccaatc
gggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagc

agcacccctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtca
cccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag]
(SEQ ID NO: 7)

Des-3-3954-1204-C225v4 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 18)][Mask (SEQ ID NO: 24)][Linker 1 (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][Linker 2 (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]
[**SGQ**][CISPRGCPDGPYVMY][GSSGSGGSGGSG][**LSGRSDNH**][GSSGT][QILLTQ
SPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSGS
GTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 8)

Des-4-3954-1204-C225v4 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 19)][Mask (SEQ ID NO: 23)][Linker 1 (SEQ ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][Linker 2 (SEQ ID NO: 29)][C225 (SEQ ID NO: 31)]
[**ggccag**][tgcattctcacctcgtggttgccggacggcccatacgtcatgtac][ggctc
gagcggtggcgagcggtggctctggtggatccggt][ctgagcgccggttccgataatcat]
[ggcagtagcggtacc][cagatcttgctgacccagagcccgggtgattctgagcgtgagcc
cgggcgaacgtgtgagcttttagctgcgcgcgagccagagcattggcaccaacattcattg
gtatcagcagcgcaccaacggcagcccgcgccctgctgattaaatatgcgagcgaaagcatt
agcggcattccgagccgcttttagcggcagcggcagcggcaccgattttaccctgagcatta
acagcgtggaaagcgaagatattgctgattattattgccagcagaacaacaactggccgac
cacctttggcgcgggcaccaaaactggaactgaaacgtacggtggctgcaccatctgtcttc
atcttcccgccatctgatgagcagttgaaatctggaactgcctctgttggtgctgctga
ataacttctatcccagagaggccaaagtacagtgggaaggtggataacgcctccaatcggg
taactcccaggagagtggtcacagagcaggacagcaaggacagcacctacagcctcagcagc
accctgaacgtgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcaccc
atcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag] (SEQ ID NO: 9)

Des-4-3954-1204-C225v4 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 20)][**Mask** (SEQ ID NO: 24)][**Linker 1** (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][**Linker 2** (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]

[**GQ**][CISPRGCPDGPVVMY][GSSGGSGGSGGSG][LSGRSDNH][GSSGT][QILLTQS
 PVILSVSPGERVVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSG
 TDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGT
 ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
 VYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 10)

Des-5-3954-1204-C225v4 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 21)][**Mask** (SEQ ID NO: 23)][**Linker 1** (SEQ ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][**Linker 2** (SEQ ID NO: 29)][C225 (SEQ ID NO: 31)]

[**cag**][tgcattctcacctcgtggttggtccggacggcccatatcgatcatgtac][ggctcgag
cggtaggcagcgggtggctctggtggatccgggt][ctgagcggccggttccgataatcat][gg
cagtagcgggtacc][cagatcttgctgacccagagcccgggtgattctgagcgtgagcccgg
 gcgaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccacattcattggta
 tcagcagcgcaccaacggcagcccgcgcctgctgattaaatatgcgagcgaaagcattagc
 ggcatccgagcgcgttttagcggcagcggcagcggcacccgattttaccctgagcattaaca
 gcgtggaaagcgaagatattgcggattattattgccagcagaacaacaactggccgaccac
 ctttggcgcgggcaccaaactggaactgaaacgtacgggtggctgcaccatctgtcttcac
 tccccgccatctgatgagcagttgaaatctggaactgcctctgttggtgctgctgaata
 acttctatcccagagaggccaaagtacagtggaaaggtggataacgccctccaatcgggtaa
 ctcccaggagagtggtcacagagcaggacagcaaggacagcacctacagcctcagcagcacc
 ctgacgctgagcaaacgagactacgagaaacacaaagtctacgcctgcgaagtcacccatc
 agggcctgagctcgcgccgtcacaaagagcttcaacaggggagagtgtag] (SEQ ID NO: 11)

Des-5-3954-1204-C225v4 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 22)][**Mask** (SEQ ID NO: 24)][**Linker 1** (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][**Linker 2** (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]

[Q] [CISPRGCPDGPYVMY] [GSSGGSGGSGGSG] [LSGRSDNH] [GSSGT] [QILLTQSP
VILSVSPGERVSFSCRASQSIGTNIHWYQQRNNGSPRLLIKYASESISGIPSRFSGSGSGT
DFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYFPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 12)

[00111] Exemplary activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies provided herein include, for example, the activatable antibody referred to herein as the Des-1-3954-1204-C225v6 activatable antibody, the Des-2-3954-1204-C225v6 activatable antibody, the Des-3-3954-1204-C225v6 activatable antibody, the Des-4-3954-1204-C225v6 activatable antibody, and the Des-5-3954-1204-C225v6 activatable antibody, each of which binds epidermal growth factor receptor (EGFR) and includes the heavy chain sequence shown below as “C225v4 Activatable Antibody Heavy Chain.” For example, the Des-1-3954-1204-C225v6 activatable antibody includes the Des-1-3954-1204-C225v6 light chain sequence shown below, and the C225v6 heavy chain sequence shown below; the Des-2-3954-1204-C225v6 activatable antibody includes the Des-2-3954-1204-C225v6 light chain sequence shown below, and the C225v6 heavy chain sequence shown below; the Des-3-3954-1204-C225v6 activatable antibody includes the Des-3-3954-1204-C225v6 light chain sequence shown below, and the C225v6 heavy chain sequence shown below; the Des-4-3954-1204-C225v6 activatable antibody includes the Des-4-3954-1204-C225v6 light chain sequence shown below, and the C225v6 heavy chain sequence shown below; and the Des-5-3954-1204-C225v6 activatable antibody includes the Des-5-3954-1204-C225v6 light chain sequence shown below, and the C225v6 heavy chain sequence shown below.

C225v6 Activatable Antibody Heavy Chain Nucleotide Sequence:

[C225v6 (SEQ ID NO: 35)]

[cagggtgcagctgaaacagagcgcccgccgctggtgcagccgagccagagcctgagcatt
acctgcaccgtgagcggttttagcctgaccaactatggcgtgcattgggtgcgccagagcc
cgggcaaaggcctggaatggctgggcgtgatttggagcggcggaacacccgattataaac
cccgtttaccagccgctgagcattaacaaagataacagcaaaagccaggtgttttttaaa
atgaacagcctgcaaagccaggataccgcgatttattattgcgcgcgcgcgcgtgacotatt
atgattatgaatttgctattggggccagggcaccctggtgaccgtgagcgcggcgtagcac

caagggcccatcggtcttccccctggcaacctctccaagagcacctctgggggcacagcg
gccctgggctgectgggtcaaggactacttccccgaaccgggtgaagggtgctgtggaactcag
gcgccctgaccagcggtgcacaccttcccggtgtcctacagtcctcaggactctactc
cctcagcagcggtgggtgacggtgccctccagcagcttgggcacccagacctacatctgcaac
gtgaatcacaagcccagcaaaccaagggtggacaagaaagttgagcccaaattctgtgaca
aaactcacacatgcccacggtgccagcacctgaactcctgggggggaccgtcagtccttct
cttccccccaaaaccaaggacacctcatgatctcccggaaccttgagggtcacatgcggtg
gtggtggacgtgagccacgaagacctgagggtcaagttcaactggtagctggacggcggtg
aggtgcataatgccaagacaaagccgcgggaggagcagtagccagcacgtaccgtgtggt
cagcgtcctcaccgctcctgcaccaggactgggtgaatggcaaggagtacaagtgaaggtc
tccaacaaagccctcccagcccccatcgagaaaacctctccaagccaaagggcagcccc
gagaaccacaggtgtacacctgcccccatcccgggatgaactgaccaagaaccagggtcag
cctgaactgectgggtcaaaggcttctatccagcgacatcgccgtggagtgaggagcaat
gggcagccggagaacaactacaagaccacgcctcccggtgctggactccgacggctccttct
tctctacagcaagctcaccggtggacaagagcaggtggcagcaggggaacgtcttctcatg
ctccgtgatgcatgaggctctgcacaaccactacacgcagaagagcctctcctgtctccg
ggtaaatga] (SEQ ID NO: 35)

C225v6 Activatable Antibody Heavy Chain Amino Acid Sequence:

[C225v6 (SEQ ID NO: 36)]
[QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTDYN
TPFTSRLSINKDNSKSQVFFKMNSLQSQDTAIYYCARALTYDYEFAYWGQGLVTVSAAS
TKGPSVFPPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRV
VSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQV
SLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS
CSVMHEALHNHYTQKSLSLSPGK*] (SEQ ID NO: 36)

Des-1-3954-1204-C225v6 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 13)][Mask (SEQ ID NO: 23)][Linker 1 (SEQ
ID NO: 25)][1204 Substrate (SEQ ID NO: 27)][Linker 2 (SEQ ID
NO: 29)][C225 (SEQ ID NO: 31)]

[ggccagctctggccag] [tgcattctcacotcgtgggttgteccggacggcccatacgtcatgt
ac] [ggctcgagcgggtggcagcgggtggctctgggtggatccgggt] [ctgagcggccgttccg
ataatcat] [ggcagtagcgggtacc] [cagatcttgctgaccagagcccgggtgattctga
 gcgtgagcccggggcgaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccac
 cattcattgggtatcagcagcgcaccaacggcagcccgcgcctgctgattaaatatgcgagc
 gaaagcattagcggcattcccgagcgcgttttagcggcagcggcagcggcaccgatttttacc
 tgagcattaacagcgtggaaagcgaagatattgcggattattattgccagcagaacaacaa
 ctggccgaccaccttttgccgcgggcaccaaactggaactgaaacgtacgggtggctgcacca
 tctgtcttcatcttcccgccatctgatgagcagttgaaatctggaactgcctctgttggtgt
 gcctgtgaataaacttctatcccagagaggccaaagtacagtggaagggtggataacgcct
 ccaatcgggtaactcccaggagagtggtcacagagcaggacagcaaggacagcacctacagc
 ctgagcagcacctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcg
 aagtcacccatcagggcctgagctcgcccgctcacaagagcttcaacaggggagagtggtta
 g] (SEQ ID NO: 3)

Des-1-3954-1204-C225v6 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 14)] [**Mask** (SEQ ID NO: 24)] [**Linker 1** (SEQ
 ID NO: 26)] [**1204 Substrate** (SEQ ID NO: 28)] [**Linker 2** (SEQ ID
 NO: 30)] [C225 (SEQ ID NO: 32)]
 [GQSGQ] [CISPRGCPDGPYVMY] [GSSGGSGGSGSG] [LSGRSDNH] [GSSGT] [QILL
 TQSPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGS
 GSGTDFTLSINSVESEDIADYYCQNNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLK
 SGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYE
 KHKVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 4)

Des-2-3954-1204-C225v6 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 15)] [**Mask** (SEQ ID NO: 23)] [**Linker 1** (SEQ
 ID NO: 25)] [**1204 Substrate** (SEQ ID NO: 27)] [**Linker 2** (SEQ ID
 NO: 29)] [C225 (SEQ ID NO: 31)]
 [cagctctggccag] [tgcattctcacctcgtgggttgteccggacggcccatacgtcatgtac]
 [ggctcgagcgggtggcagcgggtggctctgggtggatccgggt] [ctgagcggccgttccgata
atcat] [ggcagtagcgggtacc] [cagatcttgctgaccagagcccgggtgattctgagcg
 tgagcccgggcgaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccacat

tcattggtatcagcagcgcaccaacggcagcccgcgccctgctgattaaatatgcgagcgaa
 agcattagcggcattccgagccgctttagcggcagcggcagcggcacccgattttaccctga
 gcattaacagcgtggaaagcgaagatattgcggattattattgccagcagaacaacaactg
 gccgaaccacctttggcgcgggcaccaaaactggaactgaaacgtacgggtggctgcaccatct
 gtcttcattcttcccgccatctgatgagcagttgaaatctggaactgcctctgttgtgtgcc
 tgctgaataactttctatcccagagaggccaaagtacagtggaagggtggataacgccctcca
 atcgggtaactcccaggagagtggtcacagagcaggacagcaaggacagcacctacagcctc
 agcagcaccctgaacgtgagcaaagcagactacgagaaacacaaagtctacgcctgcgaag
 tcacccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgtag]
 (SEQ ID NO: 5)

Des-2-3954-1204-C225v6 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 16)][Mask (SEQ ID NO: 24)][Linker 1 (SEQ
 ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][Linker 2 (SEQ ID
 NO: 30)][C225 (SEQ ID NO: 32)]
 [QSGQ][CISPRGCPDGPYVMY][GSSGGSGGSGSG][LSGRSDNH][GSSGT][QILLT
 QSPVILSVSPGERVVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSG
 SGTDFTLTINSVESEDIADYYCQQNNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKS
 GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLTLSKADYEK
 HKVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 6)

Des-3-3954-1204-C225v6 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 17)][Mask (SEQ ID NO: 23)][Linker 1 (SEQ
 ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][Linker 2 (SEQ ID
 NO: 29)][C225 (SEQ ID NO: 31)]
 [tctggccag][tgcattctcacctcgttggttgccggacggcccatacgtcatgtac][gg
ctcgagcgggtggcagcgggtggctctggtggatccggt][ctgagcggccggtccgataatc
at][ggcagtagcgggtacc][cagatcttgctgaccagagcccggtgattctgagcgtga
 gccggggcgaaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccacattca
 ttggtatcagcagcgcaccaacggcagcccgcgccctgctgattaaatatgcgagcgaaagc
 attagcggcattccgagccgcttttagcggcagcggcagcggcacccgattttaccctgagca
 ttaacagcgtggaaagcgaagatattgcggattattattgccagcagaacaacaactggcc
 gaccaacctttggcgcgggcaccaaaactggaactgaaacgtacgggtggctgcaccatctgtc

ttcatotttcccgcocatctgatgagcagttgaaatctggaactgcctctgttggtgtgcctgc
tgaataactttctatcccagagaggccaaagtacagtggagggtggataacgccctccaatc
gggtaactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagc
agcaccttgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtca
cccatcagggcctgagctcgcccgtcacaaagagcttcaacaggggagagtgttag]
(SEQ ID NO: 7)

Des-3-3954-1204-C225v6 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 18)][Mask (SEQ ID NO: 24)][Linker 1 (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][Linker 2 (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]
[**SGQ**][CISPRGCPDGPYVMY][GSSGGSGGSGGSG][**LSGRSDNH**][GSSGT][QILLTQ
SPVILSVSPGERVSFSCRASQSIGTNIHWYQORTNGSPRLLIKYASESISGIPSRFSGSGS
GTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKH
KVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 8)

Des-4-3954-1204-C225v6 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 19)][Mask (SEQ ID NO: 23)][Linker 1 (SEQ ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][Linker 2 (SEQ ID NO: 29)][C225 (SEQ ID NO: 31)]
[**ggccag**][tgcattctcacctcgtggttgctcggagcggcccatatcgatgtac][ggctc
gagcgggtggcagcgggtggctctggtggatccggt][ctgagcggccgttccgataatcat]
[ggcagtagcgggtacc][cagatcttgctgaccagagcccgggtgattctgagcgtgagcc
cgggcgaacgtgtgagcttttagctgcgcgcgagccagagcattggcaccaacatttcattg
gtatcagcagcgcaccaacggcagcccgcgcctgctgattaaatatgcgagcgaagcatt
agcggcattccgagccgcttttagcggcagcggcagcggcacccgattttaccctgagcatta
acagcgtggaaagcgaagatattgcggtattattattgccagcagaacaacaactggccgac
cacctttggcgcgggcaccaaactggaaactgaaacgtacgggtggctgcaccatctgtcttc
atcttcccgccatctgatgagcagttgaaatctggaactgcctctgttggtgtgcctgctga
ataactttctatcccagagaggccaaagtacagtggagggtggataacgccctccaatcggg
taactcccaggagagtgtcacagagcaggacagcaaggacagcacctacagcctcagcagc
accctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcaccc

atcaggggactgagctcgcccgctcacaagagcttcaacaggggagagtgttag] (SEQ ID NO: 9)

Des-4-3954-1204-C225v6 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 20)][**Mask** (SEQ ID NO: 24)][**Linker 1** (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][**Linker 2** (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]
 [GQ][CISPRGCPDGPVVMY][GSSGGSGGSGGSG][LSGRSDNH][GSSGT][QILLTQS
 PVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSGSGSG
 TDFTLINSINSEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGT
 ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEEKHK
 VYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 10)

Des-5-3954-1204-C225v6 Activatable Antibody Light Chain Nucleotide Sequence:

[**Spacer** (SEQ ID NO: 21)][**Mask** (SEQ ID NO: 23)][**Linker 1** (SEQ ID NO: 25)][**1204 Substrate** (SEQ ID NO: 27)][**Linker 2** (SEQ ID NO: 29)][C225 (SEQ ID NO: 31)]
 [cag][tgcattctcaactcgtggttggtccggacggcccatcagtcattgtac][ggctcgag
 cggtgagcagcggtggctctggtggatccggt][ctgagcggccgttccgataatcat][gg
 cagtagcgggtacc][cagatcttgcgtgacccagagcccggtgattctgagcgtgagcccg
 gcgaacgtgtgagcttttagctgccgcgcgagccagagcattggcaccacattcattggtat
 tcagcagcgcaccaacggcagcccgccgcctgctgattaaatatgcgagcgaagcattagc
 ggcattccgagcgcgttttagcggcagcggcagcggcaccgattttacccctgagcattaaca
 gcgtggaaagcgaagatattgcggattattattgccagcagaacaacaactggccgaccac
 ctttggcgcgggcaccaaactggaactgaaacgtacgggtggctgcaccatctgtcttcac
 tccccgccatctgatgagcagttgaaatctggaactgcctctgttggtgctgctgaata
 acttctatcccagagaggccaaagtacagtggaaaggtggataacgccctccaatcgggtaa
 ctcccaggagagtggtcacagagcaggacagcaaggacagcacctacagcctcagcagcacc
 ctgacgctgagcaaagcagactacgagaaacacaaagtctacgcctgcgaagtcacccatc
 agggcctgagctcgcccgctcacaagagagcttcaacaggggagagtgttag] (SEQ ID NO: 11)

Des-5-3954-1204-C225v6 Activatable Antibody Light Chain Amino Acid Sequence:

[**Spacer** (SEQ ID NO: 22)][**Mask** (SEQ ID NO: 24)][**Linker 1** (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][**Linker 2** (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]

[**Q**][**CISPRGCPDGPYVMY**][**GSSGGSGGSGSG**][**LSGRSDNH**][**GSSGT**][QILLTQSP
VILSVSPGERVSFSCRASQSIGTNIHWYQQRRTNGSPRLLIKYASESISGIPSRFSGSGSGT
DFTLSINSVESEDIADYYCQONNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNFPYFREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSSTLTLSKADYEKHKV
YACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 12)

[00112] In some embodiments, the anti-EGFR activatable antibody and/or conjugated anti-EGFR activatable antibody includes a heavy chain selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32 coupled to a spacer that has an amino acid sequence selected from the group consisting of GQSGQ (SEQ ID NO: 14), QSGQ (SEQ ID NO: 16), SGQ (SEQ ID NO: 18), GQ (SEQ ID NO: 20), and Q (SEQ ID NO: 22) and is linked to a masking moiety (MM) that includes a sequence selected from the group consisting of CISPRG (SEQ ID NO: 76); CISPRGCG (SEQ ID NO: 77); CISPRGCPDGPYVMY (SEQ ID NO: 24); CISPRGCPDGPYVM (SEQ ID NO: 78); CISPRGCEPGTYVPT (SEQ ID NO: 79); CISPRGCPGQIWHPP (SEQ ID NO: 80); GSHCLIPINMGAPSC (SEQ ID NO: 81); CISPRGCGSSASQSGQGSHCLIPINMGAPSC (SEQ ID NO: 82); CNHHYFYTCGCISPRGCPG (SEQ ID NO: 83); ADHVFWGSYGCISPRGCPG (SEQ ID NO: 84); CHHVYWGHCISPRGCPG (SEQ ID NO: 85); CPHFTTTSCGCISPRGCPG (SEQ ID NO: 86); CNHHYHYTCGCISPRGCPG (SEQ ID NO: 87); CPHVSFGSCGCISPRGCPG (SEQ ID NO: 88); CPYYTSLSYCGCISPRGCPG (SEQ ID NO: 89); CNHVYFGTCGCISPRGCPG (SEQ ID NO: 90); CNHFTLTTCGCISPRGCPG (SEQ ID NO: 91); CHHFTLTTCGCISPRGCPG (SEQ ID NO: 92); YNPCATPMCCISPRGCPG (SEQ ID NO: 93); CNHHYFYTCGCISPRGCG (SEQ ID NO: 94); CNHHYHYTCGCISPRGCG (SEQ ID NO: 95); CNHVYFGTCGCISPRGCG (SEQ ID NO: 96); CHHVYWGHCISPRGCG (SEQ ID NO: 97); CPHFTTTSCGCISPRGCG (SEQ ID NO: 98); CNHFTLTTCGCISPRGCG (SEQ ID NO: 99); CHHFTLTTCGCISPRGCG (SEQ ID NO: 100); CPYYTSLSYCGCISPRGCG (SEQ ID NO: 101); CPHVSFGSCGCISPRGCG (SEQ ID NO: 102);

ADHVFWSYGCISPRGCG (SEQ ID NO: 103); YNPCATPMCCISPRGCG (SEQ ID NO: 104); CHHVYWGHCISPRGCG (SEQ ID NO: 105); C(N/P)H(H/V/F)(Y/T)(F/W/T/L)(Y/G/T/S)(T/S/Y/H)CGCISPRGCG (SEQ ID NO: 106); CISPRGCGQPIPSVK (SEQ ID NO: 107); CISPRGCTQPYHVS (SEQ ID NO: 108); and/or CISPRGCNAVSGGLGS (SEQ ID NO: 109).

[00113] In some embodiments, the CM is a substrate for an MMP and includes the sequence ISSGLLSS (SEQ ID NO: 110); QNQALRMA (SEQ ID NO: 111); AQNLLGMV (SEQ ID NO: 112); STFPFGMF (SEQ ID NO: 113); PVGYTSSL (SEQ ID NO: 114); DWLYWPGI (SEQ ID NO: 115); MIAPVAYR (SEQ ID NO: 116); RPSPMWAY (SEQ ID NO: 117); WATPRPMR (SEQ ID NO: 118); FRLLDWQW (SEQ ID NO: 119); LKAAPRWA (SEQ ID NO: 120); GPSHLVLT (SEQ ID NO: 121); LPGGLSPW (SEQ ID NO: 122); MGLFSEAG (SEQ ID NO: 123); SPLPLRVP (SEQ ID NO: 124); RMHLRSLG (SEQ ID NO: 125); LAAPLGLL (SEQ ID NO: 126); AVGLLAPP (SEQ ID NO: 127); LLAPSHRA (SEQ ID NO: 128); PAGLWLD (SEQ ID NO: 129); and/or ISSGLSS (SEQ ID NO: 130).

[00114] In some embodiments, the CM includes the sequence TGRGPSWV (SEQ ID NO: 131); SARGPSRW (SEQ ID NO: 132); TARGPSFK (SEQ ID NO: 133); LSGRSDNH (SEQ ID NO: 134); GGWHTGRN (SEQ ID NO: 135); HTGRSGAL (SEQ ID NO: 136); PLTGRSGG (SEQ ID NO: 137); AARGPAIH (SEQ ID NO: 138); RGPANPM (SEQ ID NO: 139); SSRGPAYL (SEQ ID NO: 140); RGPATPIM (SEQ ID NO: 141); RGPA (SEQ ID NO: 142); GGQPSGMWGW (SEQ ID NO: 143); FPRPLGITGL (SEQ ID NO: 144); VHMPGLGLP (SEQ ID NO: 145); SPLTGRSG (SEQ ID NO: 146); SAGFSLPA (SEQ ID NO: 147); LAPLGLQRR (SEQ ID NO: 148); SGGPLGVR (SEQ ID NO: 149); and/or PLGL (SEQ ID NO: 150).

[00115] In some embodiments, the activatable anti-EGFR antibodies described herein also include an agent conjugated to the activatable antibody. In some embodiments, the conjugated agent is a therapeutic agent, such as an antineoplastic agent. In such embodiments, the agent is conjugated to a carbohydrate moiety of the activatable antibody, and in some embodiments, where the carbohydrate moiety is located outside the antigen-binding region of the antibody or antigen-binding fragment in the activatable antibody. In some embodiments, the agent is conjugated to a sulfhydryl group of the antibody or antigen-binding fragment in the activatable antibody. In some embodiments, the agent is conjugated to an amino group of the antibody or antigen-binding fragment of the activatable antibody.

In some embodiments the agent is conjugated to a carboxylic acid group of the antibody or antigen-binding fragment of the activatable antibody.

[00116] In some embodiments, the agent is a cytotoxic agent such as a toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate). Suitable cytotoxic agents include, for example, any of the cytotoxic agents listed in Table 1. In some embodiments, the cytotoxic agent is a dolastatin or a derivative thereof (*e.g.* auristatin E, AFP, MMAF, MMAE, DMAF, DMAE). For example, the cytotoxic agent is monomethyl auristatin E (MMAE).

[00117] In some embodiments, the conjugated activatable antibody can be modified for site-specific conjugation through modified amino acid sequences inserted or otherwise included in the activatable antibody sequence. These modified amino acid sequences are designed to allow for controlled placement and/or dosage of the conjugated agent within a conjugated activatable anti-EGFR antibody. For example, the activatable antibody can be engineered to include cysteine substitutions at positions on light and heavy chains that provide reactive thiol groups and do not negatively impact protein folding and assembly, nor alter antigen binding. In some embodiments, the activatable antibody can be engineered to include or otherwise introduce one or more non-natural amino acid residues within the activatable antibody to provide suitable sites for conjugation. In some embodiments, the activatable antibody can be engineered to include or otherwise introduce enzymatically activatable peptide sequences within the activatable antibody sequence.

[00118] Methods of preparing a conjugate of an activatable anti-EGFR antibody having one or more interchain disulfide bonds in the AB and one or more intrachain disulfide bonds in the MM, and a drug reactive with free thiols are provided. These methods generally include partially reducing interchain disulfide bonds in the activatable antibody with a reducing agent, such as, for example, TCEP; and conjugating the drug reactive with free thiols to the partially reduced activatable antibody. As used herein, the term partial reduction refers to situations where an activatable anti-EGFR antibody is contacted with a reducing agent and less than all disulfide bonds, *e.g.*, less than all possible sites of conjugation are reduced. In some embodiments, less than 99%, 98%, 97%, 96%, 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10% or less than 5% of all possible sites of conjugation are reduced.

[00119] In yet other embodiments, a method of reducing and conjugating an agent, e.g., a drug, to an activatable anti-EGFR antibody resulting in selectivity in the placement of the agent is provided. The method generally includes partially reducing the activatable anti-EGFR antibody with a reducing agent such that any conjugation sites in the masking moiety or other non-AB portion of the activatable antibody are not reduced, and conjugating the agent to interchain thiols in the AB. The conjugation site(s) are selected so as to allow desired placement of an agent to allow conjugation to occur at a desired site. The reducing agent is, for example, TCEP. The reduction reaction conditions such as, for example, the ratio of reducing agent to activatable antibody, the length of incubation, the temperature during the incubation, the pH of the reducing reaction solution, etc., are determined by identifying the conditions that produce a conjugated activatable antibody in which the MM retains the ability to effectively and efficiently mask the AB of the activatable antibody in an uncleaved state. The ratio of reduction agent to activatable anti-EGFR antibody will vary depending on the activatable antibody. In some embodiments, the ratio of reducing agent to activatable anti-EGFR antibody will be in a range from about 20:1 to 1:1, from about 10:1 to 1:1, from about 9:1 to 1:1, from about 8:1 to 1:1, from about 7:1 to 1:1, from about 6:1 to 1:1, from about 5:1 to 1:1, from about 4:1 to 1:1, from about 3:1 to 1:1, from about 2:1 to 1:1, from about 20:1 to 1:1.5, from about 10:1 to 1:1.5, from about 9:1 to 1:1.5, from about 8:1 to 1:1.5, from about 7:1 to 1:1.5, from about 6:1 to 1:1.5, from about 5:1 to 1:1.5, from about 4:1 to 1:1.5, from about 3:1 to 1:1.5, from about 2:1 to 1:1.5, from about 1.5:1 to 1:1.5, or from about 1:1 to 1:1.5. In some embodiments, the ratio is in a range of from about 5:1 to 1:1. In some embodiments, the ratio is in a range of from about 5:1 to 1.5:1. In some embodiments, the ratio is in a range of from about 4:1 to 1:1. In some embodiments, the ratio is in a range from about 4:1 to 1.5:1.

[00120] In some embodiments, a method of reducing interchain disulfide bonds in the AB of an activatable anti-EGFR antibody and conjugating an agent, e.g., a thiol-containing agent such as a drug, to the resulting interchain thiols to selectively locate agent(s) on the AB is provided. The method generally includes partially reducing the AB with a reducing agent to form at least two interchain thiols without forming all possible interchain thiols in the activatable antibody; and conjugating the agent to the interchain thiols of the partially reduced AB. For example, the AB of the activatable antibody is partially reduced for about 1 hour at about 37°C at a desired ratio of reducing agent:activatable antibody. In some embodiments, the ratio of reducing agent to activatable antibody will be in a range from

about 20:1 to 1:1, from about 10:1 to 1:1, from about 9:1 to 1:1, from about 8:1 to 1:1, from about 7:1 to 1:1, from about 6:1 to 1:1, from about 5:1 to 1:1, from about 4:1 to 1:1, from about 3:1 to 1:1, from about 2:1 to 1:1, from about 20:1 to 1:1.5, from about 10:1 to 1:1.5, from about 9:1 to 1:1.5, from about 8:1 to 1:1.5, from about 7:1 to 1:1.5, from about 6:1 to 1:1.5, from about 5:1 to 1:1.5, from about 4:1 to 1:1.5, from about 3:1 to 1:1.5, from about 2:1 to 1:1.5, from about 1.5:1 to 1:1.5, or from about 1:1 to 1:1.5. In some embodiments, the ratio is in a range of from about 5:1 to 1:1. In some embodiments, the ratio is in a range of from about 5:1 to 1.5:1. In some embodiments, the ratio is in a range of from about 4:1 to 1:1. In some embodiments, the ratio is in a range from about 4:1 to 1.5:1.

[00121] The thiol-containing reagent can be, for example, cysteine or N-acetyl cysteine. The reducing agent can be, for example, TCEP. In some embodiments, the reduced activatable antibody can be purified prior to conjugation, using for example, column chromatography, dialysis, or diafiltration. Alternatively, the reduced antibody is not purified after partial reduction and prior to conjugation.

[00122] Also provided are partially reduced activatable anti-EGFR antibodies in which at least one interchain disulfide bond in the activatable antibody has been reduced with a reducing agent without disturbing any intrachain disulfide bonds in the activatable antibody, wherein the activatable antibody includes an antibody or an antigen binding fragment thereof (AB) that specifically binds to EGFR, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the EGFR target, and 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 is coupled to the AB via the CM. In some embodiments, one or more intrachain disulfide bond(s) of the activatable antibody is not disturbed by the reducing agent. In some embodiments, one or more intrachain disulfide bond(s) of the MM within the activatable antibody is not disturbed by the reducing agent. In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM. In some embodiments, reducing agent is TCEP.

[00123] In an uncleaved state, e.g., non-activated state, the activatable antibodies provided herein include an AB that specifically binds to EGFR that includes a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a light chain amino acid sequence that includes SEQ ID NO: 32, wherein the activatable antibody in the uncleaved state has the structural arrangement from

N-terminus to C-terminus as follows: Spacer Sequence-Masking Moiety (MM)-Linking Peptide 1 (LP1)-Cleavable Moiety (CM)-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence, wherein the MM includes the amino acid sequence CISPRGCPDGPYVMY (SEQ ID NO: 24), the CM includes the amino acid sequence LSGRSDNH (SEQ ID NO: 28), and the Spacer Sequence is selected from the group consisting of GQSGQ (SEQ ID NO: 14), QSGQ (SEQ ID NO: 16), SGQ (SEQ ID NO: 18), GQ (SEQ ID NO: 20), and Q (SEQ ID NO: 22). In some embodiments, the two linking peptides need not be identical to each other.

[00124] In some embodiments, the activatable antibodies provided herein, in an uncleaved state, include a masked light chain that includes or is derived from an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12. In some embodiments, the activatable antibody and/or conjugated activatable antibody in an uncleaved state includes a heavy chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34, and SEQ ID NO: 36, and a masked light chain amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12.

[00125] In some embodiments, the agent is a detectable moiety such as, for example, a label or other marker. For example, the agent is or includes a radiolabeled amino acid, one or more biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods), one or more radioisotopes or radionuclides, one or more fluorescent labels, one or more enzymatic labels, and/or one or more chemiluminescent agents. In some embodiments, detectable moieties are attached by spacer molecules.

[00126] Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcun, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of

radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi , ^{131}I , ^{111}In , ^{90}Y , and ^{186}Re .

[00127] Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., Science 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. (See WO94/11026).

[00128] Those of ordinary skill in the art will recognize that a large variety of possible moieties can be coupled to the resultant antibodies of the invention. (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).

[00129] Table 1 lists some of the exemplary pharmaceutical agents that may be employed in the herein described invention but in no way is meant to be an exhaustive list.

Table 1: Exemplary Pharmaceutical Agents for Conjugation

CYTOTOXIC AGENTS

Auristatins	Ampicillin
Auristatin E	Sulfanilamide
Monomethyl auristatin D (MMAD)	Polymyxin
Monomethyl auristatin E (MMAE)	Chloramphenicol
Desmethyl auristatin E (DMAE)	Turbostatin
Auristatin F	Phenstatins
Monomethyl auristatin F (MMAF)	Hydroxyphenstatin
Desmethyl auristatin F (DMAF)	Spongistatin 5
Auristatin derivatives, <i>e.g.</i> , amides thereof	Spongistatin 7
Auristatin tyramine	Halistatin 1
Auristatin quinoline	Halistatin 2
Dolastatins	Halistatin 3
Dolastatin derivatives	Modified Bryostatins
Dolastatin 16 DmJ	Halocomstatins
Dolastatin 16 Dpv	Pyrrolobenzimidazoles (PBI)

Maytansinoids, e.g. DM-1; DM-4

Maytansinoid derivatives

Duocarmycin

Duocarmycin derivatives

Alpha-amanitin

Anthracyclines

Doxorubicin

Daunorubicin

Bryostatins

Camptothecin

Camptothecin derivatives

7-substituted Camptothecin

10, 11-

Difluoromethylenedioxycamptothecin

Combretastatins

Debromoaplysiatoxin

Kahalalide-F

Discodermolide

Ecteinascidins

ANTIVIRALS

Acyclovir

Vira A

Symmetrel

ANTIFUNGALS

Nystatin

ADDITIONAL ANTI-NEOPLASTICS

Adriamycin

Cerubidine

Bleomycin

Alkeran

Velban

Oncovin

Fluorouracil

Methotrexate

Thiotepa

Bisantrene

Novantrone

Thioguanine

Procarabazine

Cytarabine

ANTI-BACTERIALSAminoglycosides

Streptomycin

Cibrostatin6

Doxaliform

Anthracyclins analogues

Cemadotin analogue (CemCH2-SH)

Pseudomonas toxin A (PE38) variant

Pseudomonas toxin A (ZZ-PE38) variant

ZJ-101

OSW-1

4-Nitrobenzyloxycarbonyl Derivatives of

O6-Benzylguanine

Topoisomerase inhibitors

Hemiasterlin

Cephalotaxine

Homoharringtonine

Pyrrolobenzodiazepine dimers (PBDs)

Functionalized pyrrolobenzodiazepenes

Calicheamicins

Podophyllotoxins

Taxanes

Vinca alkaloids

CONJUGATABLE DETECTION**REAGENTS**

Fluorescein and derivatives thereof

Fluorescein isothiocyanate (FITC)

RADIOPHARMACEUTICALS¹²⁵I¹³¹I⁸⁹Zr¹¹¹In¹²³I¹³¹I^{99m}Tc²⁰¹Tl¹³³Xe¹¹C⁶²Cu¹⁸F⁶⁸Ga¹³N¹⁵O³⁸K⁸²Rb^{99m}Tc (Technetium)

Neomycin
 Kanamycin
 Amikacin
 Gentamicin
 Tobramycin
 Streptomycin B
 Spectinomycin

HEAVY METALS

Barium
 Gold
 Platinum

ANTI-MYCOPLASMAS

Tylosine
 Spectinomycin

[00130] Coupling may be accomplished by any chemical reaction that will bind the two molecules so long as the antibody and the other moiety retain their respective activities. This linkage can include many chemical mechanisms, for instance covalent binding, affinity binding, intercalation, coordinate binding and complexation. In some embodiments, the binding is, however, covalent binding. Covalent binding can be achieved either by direct condensation of existing side chains or by the incorporation of external bridging molecules. Many bivalent or polyvalent linking agents are useful in coupling protein molecules, such as the antibodies of the present invention, to other molecules. For example, representative coupling agents can include organic compounds such as thioesters, carbodiimides, succinimide esters, diisocyanates, glutaraldehyde, diazobenzenes and hexamethylene diamines. This listing is not intended to be exhaustive of the various classes of coupling agents known in the art but, rather, is exemplary of the more common coupling agents. (See Killen and Lindstrom, *Jour. Immun.* 133:1335-2549 (1984); Jansen et al., *Immunological Reviews* 62:185-216 (1982); and Vitetta et al., *Science* 238:1098 (1987).

[00131] Suitable linkers are described in the literature. (See, for example, Ramakrishnan, S. et al., *Cancer Res.* 44:201-208 (1984) describing use of MBS (M-maleimidobenzoyl-N-hydroxysuccinimide ester). See also, U.S. Patent No. 5,030,719, describing use of halogenated acetyl hydrazide derivative coupled to an antibody by way of an oligopeptide linker. For example, suitable linkers include: (i) SMPT (4-succinimidylloxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G); (ii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido]hexanoate (Pierce Chem. Co., Cat #21651G); and (iii) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propionamide] hexanoate (Pierce Chem. Co. Cat. #2165-G.

[00132] The linkers described above contain components that have different attributes, thus leading to conjugates with differing physio-chemical properties. For example, the linker SMPT contains a sterically hindered disulfide bond, and can form

conjugates with increased stability. Disulfide linkages, are in general, less stable than other linkages because the disulfide linkage is cleaved *in vitro*, resulting in less conjugate available.

[00133] The reagent EDC (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride) is useful to create a carboxamide starting with a carboxylic acid and a primary or secondary amine. Thus, EDC may be used to link lysine residues in an antibody with a carboxylic acid in a linker or toxin, or to link aspartate or glutamate residues in an antibody with an amine in a linker or toxin. Such conjugation reactions utilizing EDC may be enhanced by addition of NHS (N-hydroxysuccinimide) or sulfo-NHS (N-hydroxy-3-oxy-sulfonylsuccinimide). Addition of NHS or sulfo-NHS to such conjugation reactions may enhance the rate, completeness, selectivity, and/or reproducibility of the conjugation reactions.

[00134] In some embodiments, the linkers are cleavable. In some embodiments, the linkers are non-cleavable. In some embodiments, two or more linkers are present. The two or more linkers are all the same, *e.g.*, cleavable or non-cleavable, or the two or more linkers are different, *e.g.*, at least one cleavable and at least one non-cleavable.

[00135] The present invention utilizes several methods for attaching agents to ABs: (a) attachment to the carbohydrate moieties of the AB, or (b) attachment to sulfhydryl groups of the AB, or (c) attachment to amino groups of the AB, or (d) attachment to carboxylate groups of the AB. According to the invention, ABs may be covalently attached to an agent through an intermediate linker having at least two reactive groups, one to react with AB and one to react with the agent. The linker, which may include any compatible organic compound, can be chosen such that the reaction with AB (or agent) does not adversely affect AB reactivity and selectivity. Furthermore, the attachment of linker to agent might not destroy the activity of the agent. Suitable linkers for reaction with oxidized antibodies or oxidized antibody fragments include those containing an amine selected from the group consisting of primary amine, secondary amine, hydrazine, hydrazide, hydroxylamine, phenylhydrazine, semicarbazide and thiosemicarbazide groups. Such reactive functional groups may exist as part of the structure of the linker, or may be introduced by suitable chemical modification of linkers not containing such groups.

[00136] According to the present invention, suitable linkers for attachment to reduced ABs include those having certain reactive groups capable of reaction with a sulfhydryl group of a reduced antibody or fragment. Such reactive groups include, but are not limited

to: reactive haloalkyl groups (including, for example, haloacetyl groups), p-mercuribenzoate groups and groups capable of Michael-type addition reactions (including, for example, maleimides and groups of the type described by Mitra and Lawton, 1979, J. Amer. Chem. Soc. 101: 3097-3110).

[00137] According to the present invention, suitable linkers for attachment to neither oxidized nor reduced Abs include those having certain functional groups capable of reaction with the primary amino groups present in unmodified lysine residues in the Ab. Such reactive groups include, but are not limited to, NHS carboxylic or carbonic esters, sulfo-NHS carboxylic or carbonic esters, 4-nitrophenyl carboxylic or carbonic esters, pentafluorophenyl carboxylic or carbonic esters, acyl imidazoles, isocyanates, and isothiocyanates.

[00138] According to the present invention, suitable linkers for attachment to neither oxidized nor reduced AB's include those having certain functional groups capable of reaction with the carboxylic acid groups present in aspartate or glutamate residues in the AB, which have been activated with suitable reagents. Suitable activating reagents include EDC, with or without added NHS or sulfo-NHS, and other dehydrating agents utilized for carboxamide formation. In these instances, the functional groups present in the suitable linkers would include primary and secondary amines, hydrazines, hydroxylamines, and hydrazides.

[00139] The agent may be attached to the linker before or after the linker is attached to the AB. In certain applications it may be desirable to first produce an AB-linker intermediate in which the linker is free of an associated agent. Depending upon the particular application, a specific agent may then be covalently attached to the linker. In other embodiments the AB is first attached to the MM, CM and associated linkers and then attached to the linker for conjugation purposes.

[00140] *Branched Linkers:* In specific embodiments, branched linkers that have multiple sites for attachment of agents are utilized. For multiple site linkers, a single covalent attachment to an AB would result in an AB-linker intermediate capable of binding an agent at a number of sites. The sites may be aldehyde or sulfhydryl groups or any chemical site to which agents can be attached.

[00141] Alternatively, higher specific activity (or higher ratio of agents to AB) can be achieved by attachment of a single site linker at a plurality of sites on the AB. This plurality of sites may be introduced into the AB by either of two methods. First, one may generate

multiple aldehyde groups and/or sulfhydryl groups in the same AB. Second, one may attach to an aldehyde or sulfhydryl of the AB a "branched linker" having multiple functional sites for subsequent attachment to linkers. The functional sites of the branched linker or multiple site linker may be aldehyde or sulfhydryl groups, or may be any chemical site to which linkers may be attached. Still higher specific activities may be obtained by combining these two approaches, that is, attaching multiple site linkers at several sites on the AB.

[00142] *Cleavable Linkers:* Peptide linkers that are susceptible to cleavage by enzymes of the complement system, such as but not limited to urokinase, tissue plasminogen activator, trypsin, plasmin, or another enzyme having proteolytic activity may be used in one embodiment of the present invention. According to one method of the present invention, an agent is attached via a linker susceptible to cleavage by complement. The antibody is selected from a class that can activate complement. The antibody-agent conjugate, thus, activates the complement cascade and releases the agent at the target site. According to another method of the present invention, an agent is attached via a linker susceptible to cleavage by enzymes having a proteolytic activity such as a urokinase, a tissue plasminogen activator, plasmin, or trypsin. These cleavable linkers are useful in conjugated activatable antibodies that include an extracellular toxin, *e.g.*, by way of non-limiting example, any of the extracellular toxins shown in Table 1.

[00143] Non-limiting examples of cleavable linker sequences are provided in Table 2.

Table 2: Exemplary Linker Sequences for Conjugation

Types of Cleavable Sequences	Amino Acid Sequence
<u>Plasmin cleavable sequences</u>	
Pro-urokinase	PRFKIIGG (SEQ ID NO: 37)
	PRFRIIGG (SEQ ID NO: 38)
TGFβ	SSRHRRALD (SEQ ID NO: 39)
Plasminogen	RKSSIIIRMRDVVL (SEQ ID NO: 40)
Staphylokinase	SSSFDKGKYKKGDDA (SEQ ID NO: 41)
	SSSFDKGKYKRGDDA (SEQ ID NO: 42)
<u>Factor Xa cleavable sequences</u>	
	IEGR (SEQ ID NO: 43)
	IDGR (SEQ ID NO: 44)
	GGSIDGR (SEQ ID NO: 45)
<u>MMP cleavable sequences</u>	
Gelatinase A	PLGLWA (SEQ ID NO: 46)

Collagenase cleavable sequences

Calf skin collagen ($\alpha 1$ (I) chain)	GPQGIAGQ (SEQ ID NO: 47)
Calf skin collagen ($\alpha 2$ (I) chain)	GPQGLLGA (SEQ ID NO: 48)
Bovine cartilage collagen ($\alpha 1$ (II) chain)	GIAGQ (SEQ ID NO: 49)
Human liver collagen ($\alpha 1$ (III) chain)	GPLGIAGI (SEQ ID NO: 50)
Human α_2 M	GPEGLRVG (SEQ ID NO: 51)
Human PZP	YGAGLGVV (SEQ ID NO: 52)
	AGLGVVVER (SEQ ID NO: 53)
	AGLGISST (SEQ ID NO: 54)
Rat α_1 M	EPQALAMS (SEQ ID NO: 55)
	QALAMSAI (SEQ ID NO: 56)
Rat α_2 M	AAYHLVSQ (SEQ ID NO: 57)
	MDAFLESS (SEQ ID NO: 58)
Rat α_1 I ₃ (2J)	ESLPVVAV (SEQ ID NO: 59)
Rat α_1 I ₃ (27J)	SAPAVESE (SEQ ID NO: 60)
Human fibroblast collagenase	DVAQFVLT (SEQ ID NO: 61)
<u>(autolytic cleavages)</u>	VAQFVLTE (SEQ ID NO: 62)
	AQFVLTEG (SEQ ID NO: 63)
	PVQPIGPQ (SEQ ID NO: 64)

[00144] In addition, agents may be attached via disulfide bonds (for example, the disulfide bonds on a cysteine molecule) to the AB. Since many tumors naturally release high levels of glutathione (a reducing agent) this can reduce the disulfide bonds with subsequent release of the agent at the site of delivery. In certain specific embodiments the reducing agent that would modify a CM would also modify the linker of the conjugated activatable antibody.

[00145] *Spacers and Cleavable Elements:* In still another embodiment, it may be necessary to construct the linker in such a way as to optimize the spacing between the agent and the AB of the activatable antibody. This may be accomplished by use of a linker of the general structure:



wherein

W is either --NH--CH₂-- or --CH₂--;

Q is an amino acid, peptide; and

n is an integer from 0 to 20.

[00146] In still other embodiments, the linker may comprise a spacer element and a cleavable element. The spacer element serves to position the cleavable element away from the core of the AB such that the cleavable element is more accessible to the enzyme

responsible for cleavage. Certain of the branched linkers described above may serve as spacer elements.

[00147] Throughout this discussion, it should be understood that the attachment of linker to agent (or of spacer element to cleavable element, or cleavable element to agent) need not be particular mode of attachment or reaction. Any reaction providing a product of suitable stability and biological compatibility is acceptable.

[00148] *Serum Complement and Selection of Linkers:* According to one method of the present invention, when release of an agent is desired, an AB that is an antibody of a class that can activate complement is used. The resulting conjugate retains both the ability to bind antigen and activate the complement cascade. Thus, according to this embodiment of the present invention, an agent is joined to one end of the cleavable linker or cleavable element and the other end of the linker group is attached to a specific site on the AB. For example, if the agent has an hydroxy group or an amino group, it may be attached to the carboxy terminus of a peptide, amino acid or other suitably chosen linker via an ester or amide bond, respectively. For example, such agents may be attached to the linker peptide via a carbodiimide reaction. If the agent contains functional groups that would interfere with attachment to the linker, these interfering functional groups can be blocked before attachment and deblocked once the product conjugate or intermediate is made. The opposite or amino terminus of the linker is then used either directly or after further modification for binding to an AB that is capable of activating complement.

[00149] Linkers (or spacer elements of linkers) may be of any desired length, one end of which can be covalently attached to specific sites on the AB of the activatable antibody. The other end of the linker or spacer element may be attached to an amino acid or peptide linker.

[00150] Thus when these conjugates bind to antigen in the presence of complement the amide or ester bond that attaches the agent to the linker will be cleaved, resulting in release of the agent in its active form. These conjugates, when administered to a subject, will accomplish delivery and release of the agent at the target site, and are particularly effective for the in vivo delivery of pharmaceutical agents, antibiotics, antimetabolites, antiproliferative agents and the like as presented in but not limited to those in Table 1.

[00151] *Linkers for Release without Complement Activation:* In yet another application of targeted delivery, release of the agent without complement activation is desired since activation of the complement cascade will ultimately lyse the target cell.

Hence, this approach is useful when delivery and release of the agent should be accomplished without killing the target cell. Such is the goal when delivery of cell mediators such as hormones, enzymes, corticosteroids, neurotransmitters, genes or enzymes to target cells is desired. These conjugates may be prepared by attaching the agent to an AB that is not capable of activating complement via a linker that is mildly susceptible to cleavage by serum proteases. When this conjugate is administered to an individual, antigen-antibody complexes will form quickly whereas cleavage of the agent will occur slowly, thus resulting in release of the compound at the target site.

[00152] *Biochemical Cross Linkers:* In other embodiments, the activatable antibody may be conjugated to one or more therapeutic agents using certain biochemical cross-linkers. Cross-linking reagents form molecular bridges that tie together functional groups of two different molecules. To link two different proteins in a step-wise manner, hetero-bifunctional cross-linkers can be used that eliminate unwanted homopolymer formation.

[00153] Peptidyl linkers cleavable by lysosomal proteases are also useful, for example, Val-Cit, Val-Ala or other dipeptides. In addition, acid-labile linkers cleavable in the low-pH environment of the lysosome may be used, for example: bis-sialyl ether. Other suitable linkers include cathepsin-labile substrates, particularly those that show optimal function at an acidic pH.

[00154] Exemplary hetero-bifunctional cross-linkers are referenced in Table 3.

Table 3: Exemplary Hetero-Bifunctional Cross Linkers

<u>HETERO-BIFUNCTIONAL CROSS-LINKERS</u>			
Linker	Reactive Toward	Advantages and Applications	Spacer Arm Length after cross-linking (Angstroms)
SMPT	Primary amines Sulfhydryls	Greater stability	11.2 Å
SPDP	Primary amines Sulfhydryls	Thiolation Cleavable cross-linking	6.8 Å
LC-SPDP	Primary amines Sulfhydryls	Extended spacer arm	15.6 Å
Sulfo-LC-SPDP	Primary amines Sulfhydryls	Extender spacer arm Water-soluble	15.6 Å
SMCC	Primary amines Sulfhydryls	Stable maleimide reactive group Enzyme-antibody conjugation Hapten-carrier protein	11.6 Å

Sulfo-SMCC	Primary amines	conjugation Stable maleimide reactive group	11.6 Å
	Sulfhydryls	Water-soluble	
MBS	Primary amines	Enzyme-antibody conjugation	9.9 Å
	Sulfhydryls	Hapten-carrier protein conjugation	
Sulfo-MBS	Primary amines	Water-soluble	9.9 Å
	Sulfhydryls		
SIAB	Primary amines	Enzyme-antibody conjugation	10.6 Å
	Sulfhydryls		
Sulfo-SIAB	Primary amines	Water-soluble	10.6 Å
	Sulfhydryls		
SMPB	Primary amines	Extended spacer arm	14.5 Å
	Sulfhydryls	Enzyme-antibody conjugation	
Sulfo-SMPB	Primary amines	Extended spacer arm	14.5 Å
	Sulfhydryls	Water-soluble	
EDE/Sulfo-NHS	Primary amines	Hapten-Carrier conjugation	0
	Carboxyl groups		
ABH	Carbohydrates	Reacts with sugar groups	11.9 Å
	Nonselective		

[00155] *Non-Cleavable Linkers or Direct Attachment:* In still other embodiments of the invention, the conjugate may be designed so that the agent is delivered to the target but not released. This may be accomplished by attaching an agent to an AB either directly or via a non-cleavable linker.

[00156] These non-cleavable linkers may include amino acids, peptides, D-amino acids or other organic compounds that may be modified to include functional groups that can subsequently be utilized in attachment to ABs by the methods described herein. A general formula for such an organic linker could be



wherein

W is either --NH--CH₂-- or --CH₂--;

Q is an amino acid, peptide; and

n is an integer from 0 to 20.

[00157] *Non-Cleavable Conjugates:* Alternatively, a compound may be attached to ABs that do not activate complement. When using ABs that are incapable of complement activation, this attachment may be accomplished using linkers that are susceptible to cleavage by activated complement or using linkers that are not susceptible to cleavage by activated complement.

Definitions:

[00158] Unless otherwise defined, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures utilized in connection with, and techniques of, cell and tissue culture, molecular biology, and protein and oligo- or polynucleotide chemistry and hybridization described herein are those well-known and commonly used in the art. Standard techniques are used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (*e.g.*, electroporation, lipofection). Enzymatic reactions and purification techniques are performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.*, Sambrook *et al.* *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The nomenclatures utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well-known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[00159] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[00160] As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. By "specifically bind" or "immunoreacts with" or "immunospecifically bind" is meant that the antibody reacts with one or more antigenic determinants of the desired antigen and does not react with other polypeptides or binds at much lower affinity ($K_d > 10^{-6}$ M). Antibodies include, but are not limited to, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, domain antibodies, single chain antibodies, Fab, and F(ab')₂ fragments, scFvs, and an Fab expression library.

[00161] The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

[00162] The term "monoclonal antibody" (mAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs contain an antigen binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

[00163] The term "antigen-binding site" or "binding portion" refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. Three highly divergent stretches within the V regions of the heavy and light chains, referred to as "hypervariable regions," are interposed between more conserved flanking stretches known as "framework regions," or "FRs". Thus, the term "FR" refers to amino acid sequences that are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as "complementarity-determining regions," or "CDRs." The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987 and

1991)), or Chothia & Lesk J. Mol. Biol. 196:901-917 (1987), Chothia *et al.* Nature 342:878-883 (1989).

[00164] As used herein, the term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin, an scFv, or a T-cell receptor. The term “epitope” includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor. Epitopic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. For example, antibodies may be raised against N-terminal or C-terminal peptides of a polypeptide. An antibody is said to specifically bind an antigen when the dissociation constant is $\leq 1 \mu\text{M}$; in some embodiments $\leq 100 \text{ nM}$, and in some embodiments $\leq 10 \text{ nM}$.

[00165] As used herein, the terms “specific binding,” “immunological binding,” and “immunological binding properties” refer to the non-covalent interactions of the type that occur between an immunoglobulin molecule and an antigen for which the immunoglobulin is specific. The strength, or affinity of immunological binding interactions can be expressed in terms of the dissociation constant (K_d) of the interaction, wherein a smaller K_d represents a greater affinity. Immunological binding properties of selected polypeptides can be quantified using methods well known in the art. One such method entails measuring the rates of antigen-binding site/antigen complex formation and dissociation, wherein those rates depend on the concentrations of the complex partners, the affinity of the interaction, and geometric parameters that equally influence the rate in both directions. Thus, both the “on rate constant” (K_{on}) and the “off rate constant” (K_{off}) can be determined by calculation of the concentrations and the actual rates of association and dissociation. (See Nature 361:186-87 (1993)). The ratio of K_{off}/K_{on} enables the cancellation of all parameters not related to affinity, and is equal to the dissociation constant K_d . (See, generally, Davies et al. (1990) Annual Rev Biochem 59:439-473). An antibody of the present invention is said to specifically bind to EGFR, when the equilibrium binding constant (K_d) is $\leq 1 \mu\text{M}$, in some embodiments $\leq 100 \text{ nM}$, in some embodiments $\leq 10 \text{ nM}$, and in some embodiments $\leq 100 \text{ pM}$ to about 1 pM , as measured by assays such as radioligand binding assays or similar assays known to those skilled in the art.

[00166] The term “isolated polynucleotide” as used herein shall mean a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which

by virtue of its origin the “isolated polynucleotide” (1) is not associated with all or a portion of a polynucleotide in which the “isolated polynucleotide” is found in nature, (2) is operably linked to a polynucleotide that it is not linked to in nature, or (3) does not occur in nature as part of a larger sequence. Polynucleotides in accordance with the invention include the nucleic acid molecules encoding the heavy chain immunoglobulin molecules shown herein, and nucleic acid molecules encoding the light chain immunoglobulin molecules shown herein.

[00167] The term “isolated protein” referred to herein means a protein of cDNA, recombinant RNA, or synthetic origin or some combination thereof, which by virtue of its origin, or source of derivation, the “isolated protein” (1) is not associated with proteins found in nature, (2) is free of other proteins from the same source, *e.g.*, free of murine proteins, (3) is expressed by a cell from a different species, or (4) does not occur in nature.

[00168] The term “polypeptide” is used herein as a generic term to refer to native protein, fragments, or analogs of a polypeptide sequence. Hence, native protein fragments, and analogs are species of the polypeptide genus. Polypeptides in accordance with the invention comprise the heavy chain immunoglobulin molecules shown herein, and the light chain immunoglobulin molecules shown herein, as well as antibody molecules formed by combinations comprising the heavy chain immunoglobulin molecules with light chain immunoglobulin molecules, such as kappa light chain immunoglobulin molecules, and vice versa, as well as fragments and analogs thereof.

[00169] The term “naturally-occurring” as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

[00170] The term “operably linked” as used herein refers to positions of components so described are in a relationship permitting them to function in their intended manner. A control sequence “operably linked” to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

[00171] The term “control sequence” as used herein refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the

host organism in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term “control sequences” is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. The term “polynucleotide” as referred to herein means nucleotides of at least 10 bases in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA.

[00172] The term oligonucleotide referred to herein includes naturally occurring, and modified nucleotides linked together by naturally occurring, and non-naturally occurring oligonucleotide linkages. Oligonucleotides are a polynucleotide subset generally comprising a length of 200 bases or fewer. In some embodiments, oligonucleotides are 10 to 60 bases in length and in some embodiments, 12, 13, 14, 15, 16, 17, 18, 19, or 20 to 40 bases in length. Oligonucleotides are usually single stranded, *e.g.*, for probes, although oligonucleotides may be double stranded, *e.g.*, for use in the construction of a gene mutant. Oligonucleotides of the invention are either sense or antisense oligonucleotides.

[00173] The term “naturally occurring nucleotides” referred to herein includes deoxyribonucleotides and ribonucleotides. The term “modified nucleotides” referred to herein includes nucleotides with modified or substituted sugar groups and the like. The term “oligonucleotide linkages” referred to herein includes oligonucleotide linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoromidate, and the like. *See e.g.*, LaPlanche *et al.* Nucl. Acids Res. 14:9081 (1986); Stec *et al.* J. Am. Chem. Soc. 106:6077 (1984), Stein *et al.* Nucl. Acids Res. 16:3209 (1988), Zon *et al.* Anti Cancer Drug Design 6:539 (1991); Zon *et al.* Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); Stec *et al.* U.S. Patent No. 5,151,510; Uhlmann and Peyman Chemical Reviews 90:543 (1990). An oligonucleotide can include a label for detection, if desired.

[00174] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. *See Immunology - A Synthesis* (2nd Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland7 Mass. (1991)). Stereoisomers (*e.g.*, D-amino acids) of the twenty conventional amino acids, unnatural amino acids such as α -, α -

disubstituted amino acids, N-alkyl amino acids, lactic acid, and other unconventional amino acids may also be suitable components for polypeptides of the present invention. Examples of unconventional amino acids include: 4-hydroxyproline, γ -carboxyglutamate, ϵ -N,N,N-trimethyllysine, ϵ -N-acetyllysine, O-phosphoserine, N-acetylserine, N-formylmethionine, 3-methylhistidine, 5-hydroxylysine, σ -N-methylarginine, and other similar amino acids and imino acids (*e.g.*, 4-hydroxyproline). In the polypeptide notation used herein, the left-hand direction is the amino terminal direction and the right-hand direction is the carboxy-terminal direction, in accordance with standard usage and convention.

[00175] Similarly, unless specified otherwise, the left-hand end of single-stranded polynucleotide sequences is the 5' end the left-hand direction of double-stranded polynucleotide sequences is referred to as the 5' direction. The direction of 5' to 3' addition of nascent RNA transcripts is referred to as the transcription direction sequence regions on the DNA strand having the same sequence as the RNA and that are 5' to the 5' end of the RNA transcript are referred to as "upstream sequences", sequence regions on the DNA strand having the same sequence as the RNA and that are 3' to the 3' end of the RNA transcript are referred to as "downstream sequences".

[00176] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 80 percent sequence identity, in some embodiments, at least 90 percent sequence identity, in some embodiments, at least 95 percent sequence identity, and in some embodiments, at least 99 percent sequence identity.

[00177] In some embodiments, residue positions that are not identical differ by conservative amino acid substitutions.

[00178] As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present invention, providing that the variations in the amino acid sequence maintain at least 75%, in some embodiments, at least 80%, 90%, 95%, and in some embodiments, 99%. In particular, conservative amino acid replacements are contemplated. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Genetically encoded amino acids are generally divided into families: (1) acidic amino acids are aspartate, glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine,

methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. The hydrophilic amino acids include arginine, asparagine, aspartate, glutamine, glutamate, histidine, lysine, serine, and threonine. The hydrophobic amino acids include alanine, cysteine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, tyrosine and valine. Other families of amino acids include (i) serine and threonine, which are the aliphatic-hydroxy family; (ii) asparagine and glutamine, which are the amide containing family; (iii) alanine, valine, leucine and isoleucine, which are the aliphatic family; and (iv) phenylalanine, tryptophan, and tyrosine, which are the aromatic family. For example, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid will not have a major effect on the binding or properties of the resulting molecule, especially if the replacement does not involve an amino acid within a framework site. Whether an amino acid change results in a functional peptide can readily be determined by assaying the specific activity of the polypeptide derivative. Assays are described in detail herein. Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art. In some embodiments, amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains. Structural and functional domains can be identified by comparison of the nucleotide and/or amino acid sequence data to public or proprietary sequence databases. In some embodiments, computerized comparison methods are used to identify sequence motifs or predicted protein conformation domains that occur in other proteins of known structure and/or function. Methods to identify protein sequences that fold into a known three-dimensional structure are known. Bowie *et al.* Science 253:164 (1991). Thus, the foregoing examples demonstrate that those of skill in the art can recognize sequence motifs and structural conformations that may be used to define structural and functional domains in accordance with the invention.

[00179] In some embodiments, amino acid substitutions are those that: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for forming protein complexes, (4) alter binding affinities, and (4) confer or modify other physicochemical or functional properties of such analogs. Analog can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (in some embodiments, conservative amino acid

substitutions) may be made in the naturally-occurring sequence (in some embodiments, in the portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence (*e.g.*, a replacement amino acid should not tend to break a helix that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence). Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton et al. *Nature* 354:105 (1991).

[00180] The term “polypeptide fragment” as used herein refers to a polypeptide that has an amino terminal and/or carboxy-terminal deletion and/or one or more internal deletion(s), but where the remaining amino acid sequence is identical to the corresponding positions in the naturally-occurring sequence deduced, for example, from a full length cDNA sequence. Fragments typically are at least 5, 6, 8 or 10 amino acids long, in some embodiments, at least 14 amino acids long, in some embodiments, at least 20 amino acids long, usually at least 50 amino acids long, and in some embodiments, at least 70 amino acids long. The term “analog” as used herein refers to polypeptides that are comprised of a segment of at least 25 amino acids that has substantial identity to a portion of a deduced amino acid sequence and that has specific binding to EGFR, under suitable binding conditions. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally-occurring sequence. Analog typically are at least 20 amino acids long, in some embodiments, at least 50 amino acids long or longer, and can often be as long as a full-length naturally-occurring polypeptide.

[00181] The term “agent” is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials.

[00182] As used herein, the terms “label” or “labeled” refers to incorporation of a detectable marker, *e.g.*, by incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (*e.g.*, streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods). In certain situations, the label or marker can also be therapeutic. Various methods of labeling polypeptides and glycoproteins are known in the art and may

be used. Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (*e.g.*, ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I), fluorescent labels (*e.g.*, FITC, rhodamine, lanthanide phosphors), enzymatic labels (*e.g.*, horseradish peroxidase, p-galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance. The term “pharmaceutical agent or drug” as used herein refers to a chemical compound or composition capable of inducing a desired therapeutic effect when properly administered to a patient.

[00183] Other chemistry terms herein are used according to conventional usage in the art, as exemplified by The McGraw-Hill Dictionary of Chemical Terms (Parker, S., Ed., McGraw-Hill, San Francisco (1985)).

[00184] As used herein, “substantially pure” means an object species is the predominant species present (*i.e.*, on a molar basis it is more abundant than any other individual species in the composition), and in some embodiments, a substantially purified fraction is a composition wherein the object species comprises at least about 50 percent (on a molar basis) of all macromolecular species present.

[00185] Generally, a substantially pure composition will comprise more than about 80 percent of all macromolecular species present in the composition, in some embodiments, more than about 85%, 90%, 95%, and 99%. In some embodiments, the object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) wherein the composition consists essentially of a single macromolecular species.

[00186] The term patient includes human and veterinary subjects.

Antibodies

[00187] Activatable antibodies of the invention specifically bind human epidermal growth factor receptor (EGFR). Also included in the invention are activatable antibodies that bind to the same epitope as the activatable anti-EGFR antibodies described herein.

[00188] Those skilled in the art will recognize that it is possible to determine, without undue experimentation, if a monoclonal antibody (*e.g.*, a murine monoclonal or humanized antibody) has the same specificity as a monoclonal antibody used in the methods described

herein by ascertaining whether the former prevents the latter from binding to EGFR. If the monoclonal antibody being tested competes with the monoclonal antibody of the invention, as shown by a decrease in binding by the monoclonal antibody of the invention, then the two monoclonal antibodies bind to the same, or a closely related, epitope. An alternative method for determining whether a monoclonal antibody has the specificity of a monoclonal antibody of the invention is to pre-incubate the monoclonal antibody of the invention with EGFR and then add the monoclonal antibody being tested to determine if the monoclonal antibody being tested is inhibited in its ability to bind EGFR. If the monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or functionally equivalent, epitopic specificity as the monoclonal antibody of the invention.

Use of activatable anti-EGFR antibodies

[00189] The invention also provides a method of manufacturing activatable antibodies that in an activated state binds Epidermal Growth Factor Receptor (EGFR) by (a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody under conditions that lead to expression of the activatable antibody, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM), and an antibody or an antigen binding fragment thereof (AB) that specifically binds EGFR, (i) wherein the CM is a polypeptide that functions as a substrate for a protease; and (ii) wherein the CM is positioned in the activatable antibody such that, in an uncleaved state, the MM interferes with specific binding of the AB to EGFR and, when the activatable antibody is in a cleaved state, the MM does not interfere or compete with specific binding of the AB to EGFR; and (b) recovering the activatable antibody.

[00190] The invention provides methods of treating, preventing and/or delaying the onset or progression of, or alleviating a symptom of an indication, *e.g.*, disease or disorder, associated with aberrant expression and/or activity of EGFR, *e.g.*, an EGFR-related disorder or EGFR-related disease, in a subject using a therapeutic molecule, *e.g.*, activatable antibodies that bind EGFR and/or conjugated activatable antibodies that bind EGFR, particularly activatable antibodies and/or conjugated activatable antibodies that bind and neutralize or otherwise inhibit at least one biological activity of EGFR and/or EGFR-mediated signaling.

[00191] In some embodiments, the indication, *e.g.*, disease or disorder, associated with aberrant expression and/or activity of EGFR is a cancer. In some embodiments, the

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

[00192] In some embodiments, the indication, *e.g.*, disease or disorder, associated with aberrant expression and/or activity of EGFR is an inflammatory disorder and/or an autoimmune disease. In some embodiments, the inflammatory and/or autoimmune disease is psoriasis.

[00193] The therapeutic molecule, *e.g.*, activatable anti-EGFR antibody and/or conjugated anti-EGFR activatable antibody, can be administered at any stage of the disease. For example, a therapeutic molecule can be administered to a patient suffering cancer of any stage, from early to metastatic. For example, therapeutic molecule can be administered to a patient suffering from an inflammatory disorder and/or autoimmune disease of any stage, from early onset to an advanced stage. It is to be understood that the terms subject and patient are used interchangeably herein.

[00194] The therapeutic molecules, *e.g.* activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies, are also useful in other therapeutic indications and treatment regimens. For example, the therapeutic molecules of the embodiments provided herein can be used in a treatment regimen that includes neoadjuvant therapy.

[00195] In some embodiments, the therapeutic molecules, *e.g.* activatable anti-EGFR antibodies and/or conjugated activatable anti-EGFR antibodies, are administered during

and/or after treatment in combination with one or more additional agents such as, by way of non-limiting example, a chemotherapeutic agent. In some embodiments, the therapeutic molecule and the additional agent(s) are administered simultaneously. For example, the therapeutic molecule and the additional agent(s) can be formulated in a single composition or administered as two or more separate compositions. In some embodiments, the therapeutic molecule and the additional agent(s) are administered sequentially.

[00196] The invention also provides methods of preventing, delaying the progression of, treating, alleviating a symptom of, or otherwise ameliorating an EGFR-related disorder, for example, cancer, in a subject by administering a therapeutically effective amount of an activatable anti-EGFR antibody described herein to a subject in need thereof.

[00197] The invention also provides methods of inhibiting angiogenesis in a subject by administering a therapeutically effective amount of an activatable anti-EGFR antibody described herein to a subject in need thereof.

[00198] In some embodiments, the subject is a mammal. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human mammal, such as a rodent, a non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal.

[00199] The activatable anti-EGFR antibody and therapeutic formulations thereof are administered to a subject suffering from or susceptible to a disease or disorder associated with aberrant EGFR expression and/or activity. A subject suffering from or susceptible to a disease or disorder associated with aberrant EGFR expression and/or activity is identified using any of a variety of methods known in the art. For example, subjects suffering from cancer or other neoplastic condition are identified using any of a variety of clinical and/or laboratory tests such as, physical examination and blood, urine and stool analysis to evaluate health status.

[00200] Administration of an activatable anti-EGFR antibody to a patient suffering from a disease or disorder associated with aberrant EGFR expression and/or activity is considered successful if any of a variety of laboratory or clinical objectives is achieved. For example, administration of an activatable anti-EGFR antibody to a patient suffering from a disease or disorder associated with aberrant EGFR expression and/or activity is considered successful if one or more of the symptoms associated with the disease or disorder is alleviated, reduced, inhibited or does not progress to a further, *i.e.*, worse, state.

Administration of an activatable anti-EGFR antibody to a patient suffering from a disease or

disorder associated with aberrant EGFR expression and/or activity is considered successful if the diabetes enters remission or does not progress to a further, *i.e.*, worse, state.

[00201] In some embodiments, the activatable anti-EGFR antibody is administered during and/or after treatment in combination with one or more additional agents such as, for example, a chemotherapeutic agent. In some embodiments, the activatable anti-EGFR antibody and the additional agent(s) are administered simultaneously. For example, the activatable anti-EGFR antibody and the additional agent(s) can be formulated in a single composition or administered as two or more separate compositions. In some embodiments, the activatable anti-EGFR antibody and the additional agent(s) are administered sequentially.

[00202] It will be appreciated that administration of therapeutic entities in accordance with the invention 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 invention, 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.

[00203] Therapeutic formulations of the invention, which include an activatable anti-EGFR antibody, are used to prevent, treat or otherwise ameliorate a disease or disorder associated with aberrant EGFR expression and/or activity. For example, therapeutic formulations of the invention, which include an activatable anti-EGFR antibody, are used to treat or otherwise ameliorate a cancer or other neoplastic condition.

[00204] Increased proteolysis is known to be a hallmark of cancer. (*See e.g.*, Affara NI, et al. "Delineating protease functions during cancer development." *Methods Mol Biol.* 539 (2009): 1-32). Progression, invasion and metastasis of tumors result from several interdependent processes in which proteases are implicated.

[00205] Efficaciousness of prevention, amelioration or treatment is determined in association with any known method for diagnosing or treating the disease or disorder associated with aberrant EGFR expression and/or activity. Prolonging the survival of a subject or otherwise delaying the progression of the disease or disorder associated with aberrant EGFR expression and/or activity in a subject indicates that the activatable antibody confers a clinical benefit.

[00206] Activatable anti-EGFR antibodies can be administered in the form of pharmaceutical compositions. Principles and considerations involved in preparing such compositions, as well as guidance in the choice of components are provided, for example, in Remington : 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.

[00207] Where activatable antibody fragments are used, the smallest fragment that specifically binds to the binding domain of the target protein is used. For example, based upon the variable-region sequences of an antibody, peptide molecules can be designed that retain the ability to bind the target protein sequence. Such peptides can be synthesized chemically and/or produced by recombinant DNA technology. (*See, e.g.*, Marasco et al., *Proc. Natl. Acad. Sci. USA*, 90: 7889-7893 (1993)). The formulation can also contain more than one active compound as necessary for the particular indication being treated, such as, 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.

[00208] The active ingredients can also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

[00209] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[00210] 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.

[00211] In some embodiments, the activatable antibody contains a detectable label. 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 a DNA probe 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 invention can be used to detect an analyte mRNA, protein, or genomic DNA in a biological sample *in vitro* as well as *in vivo*. For example, *in vitro* techniques for detection of an analyte mRNA include Northern hybridizations and *in situ* hybridizations. *In vitro* techniques for detection of an analyte protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, immunochemical staining, and immunofluorescence. *In vitro* techniques for detection of an analyte genomic DNA include Southern hybridizations. 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.

Diagnostic and Prophylactic Formulations

[00212] The anti-EGFR antibodies and/or activatable anti-EGFR antibodies of the invention are used in diagnostic and prophylactic formulations. In one embodiment, an anti-EGFR antibody and/or activatable anti-EGFR antibody is administered to patients that are at risk of developing one or more of the aforementioned cancer or other disorders. A patient's or organ's predisposition to one or more of the aforementioned disorders can be determined using genotypic, serological or biochemical markers.

[00213] In another embodiment of the invention, an anti-EGFR antibody and/or activatable anti-EGFR antibody is administered to human individuals diagnosed with a clinical indication associated with one or more of the aforementioned disorders. Upon diagnosis, an anti-EGFR antibody and/or activatable anti-EGFR antibody is administered to mitigate or reverse the effects of the clinical indication.

[00214] Antibodies and/or activatable antibodies of the invention are also useful in the detection of EGFR in patient samples and accordingly are useful as diagnostics. For example, the anti-EGFR antibodies and/or activatable anti-EGFR antibodies of the invention are used in *in vitro* assays, *e.g.*, ELISA, to detect EGFR levels in a patient sample.

[00215] In one embodiment, an anti-EGFR antibody and/or activatable anti-EGFR antibody of the invention is immobilized on a solid support (e.g., the well(s) of a microtiter plate). The immobilized antibody and/or activatable antibody serves as a capture antibody for any EGFR that may be present in a test sample. Prior to contacting the immobilized antibody 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.

[00216] 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 EGFR antigen in the test sample is determined by comparison with a standard curve developed from the standard samples.

[00217] It will be appreciated that based on the results obtained using the anti-EGFR antibodies of the invention in an *in vitro* diagnostic assay, it is possible to stage a disease in a subject based on expression levels of the EGFR antigen. 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.

[00218] Anti-EGFR antibodies and/or activatable anti-EGFR antibodies 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, activatable anti-EGFR 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 activatable anti-EGFR 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 anti-EGFR antibodies (*i.e.*, antibodies resulting from cleavage of an activatable anti-EGFR

antibody) in a given cell or tissue of a given host organism. Such accumulation of activated anti-EGFR 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 target to which the activated antibody binds.

[00219] 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. 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 anti-EGFR antibody and/or activatable anti-EGFR antibody. Suitable detectable labels are discussed in the context of the above screening methods and additional specific examples are provided below. Using an 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, activatable anti-EGFR antibodies 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 anti-EGFR antibodies in the disease tissue is enhanced relative to non-disease tissues.

[00220] In another example, activatable anti-EGFR antibodies can be used to detect the presence or absence of a cleaving agent in a sample. For example, where the activatable anti-EGFR antibodies contain a CM susceptible to cleavage by an enzyme, the activatable anti-EGFR antibodies can be used to detect (either qualitatively or quantitatively) the presence of an enzyme in the sample. In another example, where the activatable anti-EGFR antibodies contain a CM susceptible to cleavage by reducing agent, the activatable anti-EGFR antibodies can be used to detect (either qualitatively or quantitatively) the presence of reducing conditions in a sample. To facilitate analysis in these methods, the activatable antibodies 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 activatable anti-EGFR antibody 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 activatable anti-EGFR antibodies 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 activatable anti-EGFR antibodies prior to contacting with the sample *e.g.*, the presence of and/or an increase in detectable signal due to cleavage of the activatable antibody by the cleaving agent in the sample.

[00221] Such detection methods can be adapted to also provide for detection of the presence or absence of a target that is capable of binding the AB of the activatable anti-EGFR antibodies when cleaved. 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 (EGFR). The presence or absence of the cleaving agent can be detected by the presence of and/or an increase in detectable label of the activatable anti-EGFR antibodies 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.

[00222] Activatable anti-EGFR antibodies are also useful in *in situ* imaging for the validation of activatable antibody 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).

[00223] 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.

[00224] In these techniques, an activatable anti-EGFR antibody is labeled with a detectable label. The detectable label may be a fluorescent dye, (*e.g.* a fluorescein derivative, *e.g.*, Fluorescein Isothiocyanate (FITC), Rhodamine Isothiocyanate (TRITC), 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).

[00225] Detection of the label in a sample that has been incubated with the labeled, activatable anti-EGFR antibody indicates that the sample contains the target, *i.e.*, EGFR,

and contains a protease that is specific for the CM of the activatable anti-EGFR antibody. 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 activatable anti-EGFR antibody. In some embodiments, the presence of the target can be confirmed using an agent that is specific for the target, *e.g.*, another anti-EGFR antibody, or the detectable label can be competed with unlabeled EGFR. In some embodiments, unlabeled activatable anti-EGFR antibody could be used, with detection by a labeled secondary antibody or more complex detection system.

[00226] 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, *i.e.*, EGFR, and contains a protease that is specific for the CM of the activatable anti-EGFR antibody.

[00227] 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 activatable anti-EGFR antibody.

[00228] In some embodiments, *in situ* imaging and/or *in vivo* imaging are useful in methods to identify which patients to treat. For example, in *in situ* imaging, the activatable anti-EGFR antibodies are used to screen patient samples to identify those patients having the appropriate protease(s) and target(s) at the appropriate location, *e.g.*, at a tumor site.

[00229] In some embodiments *in situ* imaging is used to identify or otherwise refine a patient population suitable for treatment with an anti-EGFR activatable antibody of the disclosure. For example, patients that test positive for both the target (*e.g.*, EGFR) and a protease that cleaves the substrate in the cleavable moiety (CM) of the anti-EGFR activatable antibody being tested (*e.g.*, accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an anti-EGFR activatable antibody comprising such a CM. Likewise, patients that test negative for either or both of the target (*e.g.*, EGFR) and the protease that cleaves the substrate in the CM in the activatable antibody being tested using these methods might be identified as suitable

candidates for another form of therapy (*i.e.*, not suitable for treatment with the anti-EGFR activatable antibody being tested). In some embodiments, such patients that test negative with respect to a first anti-EGFR activatable antibody can be tested with other anti-EGFR activatable antibodies comprising different CMs until a suitable anti-EGFR activatable antibody for treatment is identified (*e.g.*, an anti-EGFR activatable antibody comprising a CM that is cleaved by the patient at the site of disease).

[00230] In some embodiments *in vivo* imaging is used to identify or otherwise refine a patient population suitable for treatment with an anti-EGFR activatable antibody of the disclosure. For example, patients that test positive for both the target (*e.g.*, EGFR) and a protease that cleaves the substrate in the cleavable moiety (CM) of the anti-EGFR activatable antibody being tested (*e.g.*, accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an anti-EGFR activatable antibody comprising such a CM. Likewise, patients that test negative might be identified as suitable candidates for another form of therapy (*i.e.*, not suitable for treatment with the anti-EGFR activatable antibody being tested). In some embodiments, such patients that test negative with respect to a first anti-EGFR activatable antibody can be tested with other anti-EGFR activatable antibodies comprising different CMs until a suitable anti-EGFR activatable antibody for treatment is identified (*e.g.*, an anti-EGFR activatable antibody comprising a CM that is cleaved by the patient at the site of disease).

Pharmaceutical compositions

[00231] The activatable anti-EGFR antibodies of the invention (also referred to herein as “active compounds”), and derivatives, fragments, analogs and homologs thereof, can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the activatable antibody and a pharmaceutically acceptable carrier. 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. 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. Supplementary active compounds can also be incorporated into the compositions.

[00232] A pharmaceutical composition of the invention 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.

[00233] 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.

[00234] 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.

[00235] 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.

[00236] 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.

[00237] 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.

[00238] 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.

[00239] In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[00240] 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 invention 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.

[00241] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[00242] The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

Examples

Example 1. Materials and Methods

[00243] *Activatable anti-EGFR antibody expression and purification.* The cDNA coding for the heavy chain and the light chain of each of the activatable anti-EGFR antibodies were separately cloned into a modified pcDNA3.1 mammalian expression vector (Life Technologies). Expi293™ cells (Life Technologies) were transiently transfected with the plasmids for each activatable anti-EGFR antibody for 5-7 days using ExpiFectamine™ transfection reagent (Life Technologies) following the manufacturer's instructions. The activatable anti-EGFR antibodies were purified using a HiTrap Mab Select Sure protein A column (GE Healthcare) coupled to an AKTA purifier (GE Healthcare). The purity and the homogeneity of purified activatable anti-EGFR antibodies were analyzed by SDS-PAGE in reducing and non-reducing conditions and size exclusion chromatography using a Superdex 200, 10/300 GL column (GE Healthcare), respectively.

Example 2. *In vitro* activity of activatable anti-EGFR antibodies

[00244] This Example describes the ability of a masking moiety of an activatable antibody of the disclosure to reduce the ability of such an anti-EGFR activatable antibody to bind to EGFR compared to EGFR binding by EGFR antibody cetuximab.

[00245] The abilities of the following anti-EGFR antibody and activatable antibodies to bind to human EGFR in an *in vitro* binding assay were evaluated: cetuximab; a control anti-EGFR activatable antibody having the masked light chain amino acid sequence shown below in SEQ ID NO: 74 and the heavy chain sequence of SEQ ID NO: 2; and the following C225v5 activatable antibodies: the Des-1-3954-1204-c225v5 activatable antibody (heavy chain sequence of SEQ ID NO: 2 and masked light chain sequence of SEQ ID NO: 4); the Des-2-3954-1204-c225v5 activatable antibody (SEQ ID NO: 2 and SEQ ID NO: 6); the Des-3-3954-1204-c225v5 activatable antibody (SEQ ID NO: 2 and SEQ ID NO: 8); the Des-4-3954-1204-c225v5 activatable antibody (SEQ ID NO: 2 and SEQ ID NO: 10); and the Des-5-3954-1204-c225v5 activatable antibody (SEQ ID NO: 2 and SEQ ID NO: 12).

Control anti-EGFR activatable antibody:

[**Spacer** (SEQ ID NO: 75)][Mask (SEQ ID NO: 24)][Linker 1 (SEQ ID NO: 26)][**1204 Substrate** (SEQ ID NO: 28)][Linker 2 (SEQ ID NO: 30)][C225 (SEQ ID NO: 32)]

[**QGQSGQ**][CISPRGCPDGPYVMY][GSSGGSGGSGGSG][**LSGRSDNH**][GSSGT][QIL
 LTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKYASESISGIPSRFSG
 SGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQL
 KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADY
 EKHKVYACEVTHQGLSSPVTKSFNRGEC*] (SEQ ID NO: 74)

[00246] Briefly, 96-well plates were coated with 1 microgram per milliliter (ug/mL) EGFR overnight at 4°C. After blocking and washing, different concentrations of C225 antibody or of each of the activatable antibodies were added to the coated plates and incubated at room temperature for 1 h. The amounts of bound cetuximab or activatable antibody were detected by anti-human IgG (Fab)₂-HRP. The K_d for each antibody or activatable antibody was calculated using Prism 6 program.

[00247] The binding of the control anti-EGFR activatable antibody (heavy chain sequence of SEQ ID NO: 2 and masked light chain sequence of SEQ ID NO: 74) and each of the other C225v5 activatable antibodies to EGFR was reduced by more than 50-fold relative to cetuximab. The apparent K_d of cetuximab was approximately 0.12 nM as compared to a range of approximately 6.5 to 8.89 nM for the tested anti-EGFR activatable antibodies.

Other Embodiments

[00248] 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.

What is claimed is:

1. An activatable antibody that in an activated state binds Epidermal Growth Factor Receptor (EGFR) comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds to EGFR, wherein the AB comprises (i) a heavy chain amino acid sequence selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 34 and SEQ ID NO: 36 and a light chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 32;

wherein the light chain amino acid sequence is coupled to a spacer sequence and a masking moiety (MM) via a cleavable moiety (CM) to produce a masked light chain, wherein the masked light chain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, and SEQ ID NO: 12,

wherein the MM inhibits the binding of the AB of the activatable antibody in an uncleaved state to EGFR;

wherein the CM is a polypeptide that functions as a substrate for a protease; and

wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: Spacer Sequence-MM-Linking Peptide 1 (LP1)-CM-Linking Peptide 2 (LP2)-AB or AB-LP2-CM-LP1-MM-Spacer Sequence .

2. The activatable antibody of claim 1, wherein the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a F(ab')₂ fragment, a scFv, and a scAb.

3. The activatable antibody of claim 1, wherein the activatable antibody in an uncleaved state comprises a combination of a heavy chain and a masked light chain selected from the group consisting of:

- (a) a heavy chain comprising amino acid sequence of SEQ ID NO: 2 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 4;
- (b) a heavy chain comprising amino acid sequence of SEQ ID NO: 2 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 6;

- (c) a heavy chain comprising amino acid sequence of SEQ ID NO: 2 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 8;
- (d) a heavy chain comprising amino acid sequence of SEQ ID NO: 2 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 10;
- (e) a heavy chain comprising amino acid sequence of SEQ ID NO: 2 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 12;
- (f) a heavy chain comprising amino acid sequence of SEQ ID NO: 34 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 4;
- (g) a heavy chain comprising amino acid sequence of SEQ ID NO: 34 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 6;
- (h) a heavy chain comprising amino acid sequence of SEQ ID NO: 34 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 8;
- (i) a heavy chain comprising amino acid sequence of SEQ ID NO: 34 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 10;
- (j) a heavy chain comprising amino acid sequence of SEQ ID NO: 34 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 12;
- (k) a heavy chain comprising amino acid sequence of SEQ ID NO: 36 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 4;
- (l) a heavy chain comprising amino acid sequence of SEQ ID NO: 36 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 6;
- (m) a heavy chain comprising amino acid sequence of SEQ ID NO: 36 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 8;
- (n) a heavy chain comprising amino acid sequence of SEQ ID NO: 36 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 10;
and
- (o) a heavy chain comprising amino acid sequence of SEQ ID NO: 36 and a masked light chain comprising the amino acid sequence of SEQ ID NO: 12.

4. The activatable antibody of claim 1, wherein the AB has an equilibrium dissociation constant of about 100 nM or less for binding to EGFR.

5. The activatable antibody of claim 1, wherein the MM has an equilibrium dissociation constant for binding to the AB that is greater than the equilibrium dissociation constant of the AB to EGFR.
6. The activatable antibody of claim 1, wherein the protease is co-localized with EGFR in a tissue, and wherein the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease.
7. The activatable antibody of claim 1, wherein the AB is conjugated to an agent.
8. The activatable antibody of claim 7, wherein the agent is a toxin or fragment thereof.
9. The activatable antibody of claim 7, wherein the agent is selected from the group consisting of a dolastatin, an auristatin or derivative thereof, a maytansinoid or a derivative thereof, a duocarmycin or derivative thereof, a calicheamicin or derivative thereof.
10. The activatable antibody of claim 7, wherein the agent is auristatin E or a derivative thereof.
11. The activatable antibody of claim 7, wherein the agent is monomethyl auristatin E (MMAE).
12. The activatable antibody of claim 7, wherein the agent is monomethyl auristatin D (MMAD).
13. The activatable antibody of claim 7, wherein the agent is DM1 or DM4.
14. The activatable antibody of claim 7, wherein the agent is conjugated to the AB via a linker.
15. The activatable antibody of claim 14, wherein the linker is a cleavable linker.
16. The activatable antibody of claim 14, wherein the linker is a non-cleavable linker.

17. The activatable antibody of claim 1 comprising a detectable moiety.
18. The activatable antibody of claim 17, wherein the detectable moiety is a diagnostic agent.
19. An isolated nucleic acid molecule encoding the activatable antibody of claim 1.
20. A vector comprising the isolated nucleic acid molecule of claim 19.
21. A method of producing an activatable antibody by culturing a cell under conditions that lead to expression of the activatable antibody, wherein the cell comprises the vector of claim 20.
22. A method of manufacturing an activatable antibody that in an activated state binds Epidermal Growth Factor Receptor (EGFR), the method comprising:
 - (a) culturing a cell comprising a nucleic acid construct that encodes the activatable antibody of claim 1 under conditions that lead to expression of the activatable antibody, and
 - (b) recovering the activatable antibody.
23. A method of treating or delaying the progression of an EGFR-related disorder in a subject comprising administering a therapeutically effective amount of the activatable antibody of claim 1 to a subject in need thereof.
24. The method of claim 23, wherein the EGFR-related disorder is a cancer.
25. The method of claim 24, wherein the cancer is breast cancer, colorectal cancer, gastric cancer, glioblastoma, head and neck cancer, lung cancer, ovarian cancer, endometrial cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, or skin cancer.
26. The method of claim 23, wherein the EGFR-related disorder is psoriasis.

27. The method of claim 23, wherein the AB is conjugated to an agent.
28. The method of claim 27, wherein the agent is a toxin or fragment thereof.
29. The method of claim 28, wherein the agent is selected from the group consisting of a dolastatin, an auristatin or derivative thereof, a maytansinoid or a derivative thereof, a duocarmycin or derivative thereof, and a calicheamicin or derivative thereof.
30. A method of detecting presence or absence of a cleaving agent and Epidermal Growth Factor Receptor (EGFR) in a subject or a sample, the method comprising:
- (a) contacting a subject or sample with the anti-EGFR activatable antibody of claim 1, and
 - (b) measuring a level of activated anti-EGFR activatable antibody in the subject or sample,
- wherein a detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent and EGFR are present in the subject or sample, and wherein no detectable level of activated anti-EGFR activatable antibody in the subject or sample indicates that the cleaving agent, EGFR or both the cleaving agent and EGFR are absent in the subject or sample.
31. The method of claim 30, wherein the anti-EGFR activatable antibody comprises a detectable label.
32. The method of claim 31, wherein the detectable label comprises an imaging agent, a radioisotope, a contrasting agent, an enzyme, a fluorescent label, a chromophore, a dye, one or more metal ions, or a ligand-based label.
33. The method of claim 30, wherein the method is an *in vivo* method, an *in situ* method, an *ex vivo* method or *in vitro* method.
34. A method of identifying or refining a patient population for treatment of an EGFR-related disorder, the method comprising (i) contacting a sample from a subject with an anti-

EGFR activatable antibody of claim 1; (ii) measuring a level of activated anti-EGFR activatable antibody in the sample, wherein a detectable level of activated anti-EGFR activatable antibody in the sample indicates that the sample is positive for the presence of EGFR and a cleaving agent that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody of claim 1; (iii) identifying and selecting one or more subjects that test positive for the presence of EGFR and the cleaving agent thereby identifying or refining a patient population; and (iv) administering a therapeutically effective amount of the activatable antibody of claim 1 to the one or more subjects in the patient population that test positive for the presence of EGFR and the cleaving agent.

35. The method of claim 34, wherein the anti-EGFR activatable antibody comprises a detectable label.

36. The method of claim 34, wherein the detectable label comprises an imaging agent, a radioisotope, a contrasting agent, an enzyme, a fluorescent label, a chromophore, a dye, one or more metal ions, or a ligand-based label.

37. The method of claim 33, wherein the EGFR-related disorder is cancer.

38. The method of claim 37, wherein the cancer is breast cancer, colorectal cancer, gastric cancer, glioblastoma, head and neck cancer, lung cancer, ovarian cancer, endometrial cancer, pancreatic cancer, prostate cancer, renal cancer, sarcoma, or skin cancer.

39. The method of claim 34, wherein the EGFR-related disorder is psoriasis.

FIGURE 1

