

In addition, a need exists for the development of safer anti-CTLA4 antibodies that are only active in certain contexts, such as in the protease-rich tumor microenvironment.

[0005] All references cited herein, including patent applications, patent publications, non-patent literature, and UniProtKB/Swiss-Prot/GenBank Accession numbers are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0006] To meet the above and other needs, disclosed herein are antibodies (*e.g.*, cross-reactive antibodies), and antigen binding fragments thereof, that bind to human CTLA4. The anti-CTLA4 antibodies, or antigen binding fragments thereof, of the present disclosure possessed at least one (*e.g.*, one, some, or all) of the following functional properties: (a) bind to human, cynomolgus monkey, mouse, rat, and/or dog CTLA4 with a K_D of 500 nM or less; (b) have antagonist activity on human CTLA4; (c) do not bind to human PD-1, PD-L1, PD-L2, LAG3, TIM3, B7-H3, CD95, CD120a, OX40, CD40, BTLA, VISTA, ICOS, and/or B7-H4 at concentration up to 100 nM; (d) are cross-reactive with monkey, mouse, rat, and/or dog CTLA4; (e) induces ADCC effects (*e.g.*, on Tregs); (f) activates human PBMCs (*e.g.*, stimulates secretion of IL-2 and/or IFN γ); (g) are capable of inhibiting tumor cell growth and establishing immune memory against tumor cells; (h) have therapeutic effect on a cancer; and (i) block binding of human CTLA4 to human CD80 and/or human CD86 (*see* Examples 1-5 below).

[0007] Disclosed herein are precision/context-dependent activatable antibodies that bind to human CTLA4 when in active form but not in inactive form, *i.e.*, the activatable antibodies bound to CTLA4 (were active) only after cleavage of the cleavable moiety (CM) to remove the masking moiety (MM). In some examples, the discovered masking moieties (MMs) described herein were capable of efficiently masking antibody activity and/or reducing or completely inhibiting antigen binding, while in some examples being devoid of the chemically labile residues methionine and/or tryptophan. Furthermore, the activatable antibodies identified and described herein are as efficient at treating multiple cancer types as their parental antibody, while having significantly reduced cytotoxicity in susceptible animals (NOD mice).

[0008] Accordingly, in one example, provided herein is an anti-CTLA4 antibody (e.g., human antibodies) that binds human CTLA4 and is cross-reactive with a CTLA4 polypeptide from at least one non-human animal selected from the group consisting of cynomolgus monkey, mouse, rat, and dog. In some examples, the antibody binds to cynomolgus monkey CTLA4 and mouse CTLA4. In some examples that may be combined with any of the preceding examples, the antibody binds to human CTLA4, cynomolgus monkey CTLA4, mouse CTLA4, rat CTLA4, and/or dog CTLA4 with a dissociation constant (K_D) of about 350 nM or less (e.g., about 300 nM or less, about 200 nM or less, about 100 nM or less, about 50 nM or less, or about 10 nM or less). In some examples, the K_D is measured by surface plasmon resonance (SPR). In some examples, binding of the antibody to CTLA4 induces antibody-dependent cell cytotoxicity (ADCC) against a CTLA4-expressing cell. In some examples, binding of the antibody to CTLA4 induces ADCC against a Treg cell. In some examples, binding of the anti-CTLA4 antibody described herein induces antibody-dependent cell cytotoxicity (ADCC) against a CTLA4-expressing human cell or a human Treg cell, wherein the ADCC activity of the anti-CTLA4 antibody is higher than the ADCC activity of ipilimumab in vitro, and wherein both antibodies comprise wild type human IgG1 Fc region. In some examples, binding of the anti-CTLA4 antibody described herein induces antibody-dependent cell cytotoxicity (ADCC) against a CTLA4-expressing human cell or a human Treg cell, wherein the ADCC activity of the anti-CTLA4 antibody is two times or higher than the ADCC activity of ipilimumab in vitro, and wherein both antibodies comprise wild type human IgG1 Fc region. In some examples, the EC50 of the anti-CTLA4 antibody ADCC activity is 50% or less than the EC50 of ipilimumab ADCC activity in vitro. Assays for measuring ADCC activities are described in Examples 3 and 15. In some examples, the anti-CTLA4 antibody depletes Treg cells selectively in tumor microenvironment (e.g., reducing percentage of Treg cells in tumor infiltrating lymphocytes), as compared to PBMC or spleen in a mouse cancer model. See, e.g., Example 18.

[0009] In some examples that may be combined with any of the preceding examples, the antibody specifically binds to an epitope comprising amino acid residues at a ligand binding site of human CTLA4, such as CD80 and/or CD86 binding site of human CTLA4. In some examples, the antibody specifically binds to an epitope similar to a ligand binding site of human CTLA4, such as CD80 and/or CD86 binding site of human CTLA4. In

some examples, the antibody specifically binds to an epitope comprising amino acid residues Y105 and L106 of human CTLA4, wherein the numbering of the amino acid residues is according to SEQ ID NO: 207. In some examples, the antibody does not bind to residue I108 of human CTLA4, wherein the numbering of the amino acid residues is according to SEQ ID NO: 207. In some examples, the anti-CTLA4 antibody blocks binding of CD80 and/or CD86 to human CTLA4. In some examples, the anti-CTLA4 antibody has an IC50 higher than the IC50 of ipilimumab for blocking binding of CD80 and/or CD86 to human CTLA4. In some examples, the anti-CTLA4 antibody has an IC50 that is 3.5 times or higher (including 3.9 times or higher) than the IC50 of ipilimumab for blocking binding of CD80 and/or CD86 to human CTLA4 in an assay that CD86 or CD80 is plate bound and CTLA4 is in solution or CTLA4 displayed on cell surface. See Example 13, Table 23, FIGs 57A-57D and 58. Assays for testing antibody's blocking activities (ligand competition) and IC50 are described in Examples 3 and 13.

[0010] In some examples that may be combined with any of the preceding examples, the antibody comprises a heavy chain variable region and a light chain variable region, a) wherein the heavy chain variable region comprises an HVR-H1, an HVR-H2, and an HVR-H3, wherein the HVR-H1 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (I): X1TFSX2YX3IHWV (SEQ ID NO: 1), wherein X1 is F or Y, X2 is D or G, and X3 is A, G, or W; Formula (II): YSIX1SGX2X3WX4WI (SEQ ID NO: 2), wherein X1 is S or T, X2 is H or Y, X3 is H or Y, and X4 is A, D, or S; and Formula (III): FSLSTGGVAVX1WI (SEQ ID NO: 3), wherein X1 is G or S; wherein the HVR-H2 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (IV): IGX1IX2HSGSTYYX3SLKSRV (SEQ ID NO: 4), wherein X1 is D or E, X2 is S or Y, and X3 is P or Q; Formula (V): IGX1ISPSX2GX3TX4YAQKFQGRV (SEQ ID NO: 5), wherein X1 is I or W, X2 is G or S, X3 is G or S, and X4 is K or N; and Formula (VI): VSX1ISGX2GX3X4TYYADSVKGRF (SEQ ID NO: 6), wherein X1 is A, G, or S, X2 is S or Y, X3 is G or S, and X4 is S or T; and wherein the HVR-H3 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (VII): ARX1X2X3X4FDX5 (SEQ ID NO: 7), wherein X1 is G, R, or S, X2 is A, I, or Y, X3 is D, V, or Y, X4 is A, E, or Y, and X5 is I or Y; Formula (VIII): ARX1GX2GYFDX3 (SEQ ID NO: 8), wherein X1 is D or L, X2 is F or Y, and X3 is V

or Y; Formula (IX): ARX1X2X3X4AX5X6FDY (SEQ ID NO: 9), wherein X1 is L or R, X2 is I or P, X3 is A or Y, X4 is S or T, X5 is T or Y, and X6 is A or Y; Formula (X): ARDX1X2X3GSSGYX4GFDX5 (SEQ ID NO: 10), wherein X1 is I or V, X2 is A or H, X3 is P or S, X4 is D or Y, and X5 is F or V; and b) wherein the light chain variable region comprises an HVR-L1, an HVR-L2, and an HVR-L3, wherein the HVR-L1 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (XI): RASQX1X2X3SX4LX5 (SEQ ID NO: 11), wherein X1 is G or S, X2 is I or V, X3 is G or S, X4 is S or Y, and X5 is A or N; Formula (XII): RASQX1VX2X3RX4LA (SEQ ID NO: 12), wherein X1 is S or T, X2 is F, R, or S, X3 is G or S, and X4 is F or Y; and Formula (XIII): RASX1SVDFX2GX3SFLX4 (SEQ ID NO: 13), wherein X1 is E or Q, X2 is D, F, H, or Y, X3 is F, I, or K, and X4 is A, D, or H; wherein the HVR-L2 comprises an amino acid sequence according to Formula (XIV): X1ASX2X3X4X5GX6 (SEQ ID NO: 14), wherein X1 is A or D, X2 is N, S, or T, X3 is L or R, X4 is A, E, or Q, X5 is S or T, and X6 is I or V; and wherein the HVR-L3 comprises an amino acid sequence according to a formula selected from the group consisting of: Formula (XV): YCX1X2X3X4X5X6PX7T (SEQ ID NO: 15), wherein X1 is E, Q, or V, X2 is H or Q, X3 is A, G, H, R, or S, X4 is D, L, S, or Y, X5 is E, G, P, Q, or S, X6 is L, T, V, or W, and X7 is F, L, P, W, or Y; Formula (XVI): YCQX1X2X3WPPWT (SEQ ID NO: 16), wherein X1 is S or Y, X2 is D or Y, and X3 is Q or Y; and Formula (XVII): YCQX1YX2SSPPX3YT (SEQ ID NO: 17), wherein X1 is H or Q, X2 is T or V, and X3 is E or V. In some examples, the HVR-H1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 18-29, the HVR-H2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 30-39, the HVR-H3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 40-52, the HVR-L1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 53-65, the HVR-L2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 66-69, and the HVR-L3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 70-81. In some examples, the antibody comprises: a) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 18, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 30, an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 40, an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 53, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 66, and an HVR-L3 comprising the amino

NO: 69, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 77; i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 26, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 37, an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 48, an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 61, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 66, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 78; j) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 27, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 32, an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 49, an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 62, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 67, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 79; k) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 28, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 37, an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 50, an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 63, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 67, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 80; l) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 18, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 38, an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 51, an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 64, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 67, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 81; or m) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 29, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 39, an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 52, an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 65, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 68, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 77. In some examples that may be combined with any of the preceding examples, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 82-94, and/or the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 95-107. In some examples that may be combined with any of the preceding examples, the antibody comprises: a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 82 or a variant thereof having at least about 90% (*e.g.*, at least about 92%, 95%, 98%, 99% or more) sequence identity to the amino acid sequence of SEQ ID

to the amino acid sequence of SEQ ID NO: 106; or m) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 94 or a variant thereof having at least about 90% (*e.g.*, at least about 92%, 95%, 98%, 99% or more) sequence identity to the amino acid sequence of SEQ ID NO: 94, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 107 or a variant thereof having at least about 90% (*e.g.*, at least about 92%, 95%, 98%, 99% or more) sequence identity to the amino acid sequence of SEQ ID NO: 107.

[0011] In some examples, the anti-CTLA4 antibody described herein comprises a heavy chain variable region and a light chain variable region, wherein one, two, three, four, five, or six HVRs of the antibody comprise a HVR sequence shown in Table A. In some examples, the anti-CTLA4 antibody comprises a heavy chain variable region comprising an HVR-H1, an HVR-H2, and an HVR-H3, wherein the HVR-H1 comprises the amino acid sequence of SEQ ID NO: 23, or the HVR-H2 comprises the amino acid sequence of SEQ ID NO: 35, or the HVR-H3 comprises the amino acid sequence of SEQ ID NO: 45. In some examples, the anti-CTLA4 antibody comprises a light chain variable region comprising an HVR-L1, an HVR-L2, and an HVR-L3, wherein the HVR-L1 comprises the amino acid sequence of SEQ ID NO: 58, or the HVR-L2 comprises the amino acid sequence of SEQ ID NO: 66, or the HVR-L3 comprises the amino acid sequence of SEQ ID NO: 75. In some examples, the HVR-H2 of the antibody comprises the amino acid sequence of SEQ ID NO: 35. In some examples, the anti-CTLA4 antibody comprises (a) a heavy chain variable region comprising an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 23, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 35, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 45, and/or a light chain variable region comprising an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 58, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 66, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 75. In some examples, one, two, three, four, five or six of the HVRs of the antibody may comprise one, two or three conservative amino acid substitutions in the HVRs. In some examples, the anti-CTLA4 antibody comprises (b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 87 or an amino acid sequence having at least 90% (*e.g.*, 91%, 92%, 93%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of SEQ ID NO: 87, and/or a light chain

variable region comprising the amino acid sequence of SEQ ID NO: 100 or an amino acid sequence having at least 90% (e.g., 91%, 92%, 93%, 95%, 96%, 97%, 98%, or 99%) sequence identity to the amino acid sequence of SEQ ID NO: 100.

[0012] In some examples that may be combined with any of the preceding examples, the antibody is an antibody fragment. In some examples, the fragment is a Fab, Fab', Fab'-SH, F(ab')₂, Fv or scFv fragment. In some examples that may be combined with any of the preceding examples, the antibody comprises an IgG1, IgG2, IgG3, or IgG4 Fc region (such as human IgG1, IgG2, IgG3, or IgG4 Fc region). In some examples, the antibody comprising a human IgG1 or a variant that has enhanced ADCC activity. In some examples, the antibody comprises a human IgG1 with reduced fucosylation (or non-fucosylated). In some examples, the antibody is a human antibody.

[0013] Other examples of the present disclosure relate to an antibody that competes or cross-competes for binding to human CTLA4 with any of the antibodies described herein. Also provided herein are antibodies that bind to the same epitope and/or essentially the same epitope as any of the antibodies described herein.

[0014] Other examples of the present disclosure relate to an activatable antibody comprising: a) a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM comprises an amino acid sequence according to Formula (XVIII): X_mCX_nCZ_o (SEQ ID NO: 134), wherein m is from 2-10, n is from 3-10, and o is from 1-10, wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y, and wherein each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P; wherein the MM inhibits the binding of the activatable antibody to human CTLA4 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; and wherein the TBM comprises an antibody heavy chain variable region (VH); and b) a second polypeptide comprising an antibody light chain variable region (VL); and wherein the activatable antibody binds to human CTLA4 via the VH and VL when the CM is cleaved. In some examples, m is from 3-10. In one aspect, the present disclosure refers to an activatable antibody comprising: a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 189-193, 195-196, and 213-216; wherein the CM comprises at least a first cleavage site; wherein: a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH); b) the TBM comprises an antibody heavy chain variable region (VH), and the activatable antibody further comprises a second polypeptide comprising an antibody light chain variable region (VL); c) the TBM comprises from the N-terminus to the C-terminus, an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH); or d) the TBM comprises from the N-terminus to the C-terminus, an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL); wherein the VH comprises an HVR-H1 comprising the amino acid sequence YSISSGYHWSWI (SEQ ID NO: 23), an HVR-H2 comprising the amino acid sequence LARIDWDDDKYYSTSLKSRL (SEQ ID NO: 35), and an HVR-H3 comprising the amino acid sequence ARSYVYFDY (SEQ ID NO: 45); wherein the VL comprises an HVR-L1 comprising the amino acid sequence RASQSVRGRFLA (SEQ ID NO: 58), an HVR-L2 comprising the amino acid sequence DASNRATGI (SEQ ID NO: 66), and an HVR-L3 comprising the amino acid sequence YCQQSSSWPPT (SEQ ID NO: 75); and wherein the activatable antibody binds to human CTLA4 via the VH and VL when the CM is cleaved.

[0015] Other examples of the present disclosure relate to an activatable antibody comprising: a) a polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM

comprises an amino acid sequence according to Formula (XVIII): $X_mCX_nCZ_o$ (SEQ ID NO: 134), wherein m is from 2-10, n is from 3-10, and o is from 1-10, wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y, and wherein each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P; wherein the MM inhibits the binding of the activatable antibody to human CTLA4 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; and wherein the TBM comprises an antibody light chain variable region (VL); and b) a second polypeptide comprising an antibody heavy chain variable region (VH); and wherein the activatable antibody binds to human CTLA4 via the VH and VL when the CM is cleaved. In some examples, m is from 3-10. In one aspect, the present disclosure refers to an activatable antibody comprising: (a) a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and an antibody light chain variable region (VL); and (b) a second polypeptide comprising the antibody heavy chain variable region (VH); wherein the MM comprises the amino acid sequence PNPSSDCVPYYYACAY (SEQ ID NO: 144); wherein the CM comprises at least a first cleavage site; wherein the VH comprises the amino acid sequence of SEQ ID NO: 87, and wherein the VL comprises the amino acid sequence of SEQ ID NO: 100; and wherein the activatable antibody binds to human CTLA4 via the VH and VL when the CM is cleaved.

[0016] Other examples of the present disclosure relate to an activatable antibody comprising: a polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM comprises an amino acid sequence according to Formula (XVIII): $X_mCX_nCZ_o$ (SEQ ID NO: 134), wherein m is from 2-10, n is from 3-10, and o is from 1-10, wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y, and wherein each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P; wherein the MM inhibits the binding of the activatable antibody to human CTLA4 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; wherein the TBM comprises from the N-terminus to the C-terminus, an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH); and wherein the activatable antibody binds to human CTLA4 via the VH and VL when the CM is cleaved. In some examples, m is from 3-10.

[0017] Other examples of the present disclosure relate to an activatable antibody comprising: a polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), wherein the MM comprises an amino acid sequence according to Formula (XVIII): $X_mCX_nCZ_o$ (SEQ ID NO: 134), wherein m is from 2-10, n is from 3-10, and o is from 1-10, wherein each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y, and wherein each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P; wherein the MM inhibits

the binding of the activatable antibody to human CTLA4 when the CM is not cleaved; wherein the CM comprises at least a first cleavage site; wherein the TBM comprises from the N-terminus to the C-terminus, an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL); and wherein the activatable antibody binds to human CTLA4 via the VH and VL when the CM is cleaved.

[0018] In some examples according to any one of the activatable antibodies described above, m is 2, 3, 4, 5, or 6. In some examples, m is 6. In some examples, n is from 6-8. In some examples, n is 6. In some examples, o is from 1-2. In some examples, o is 2. In some examples that may be combined with any of the preceding examples, each X is not M, W, or C. In some examples that may be combined with any of the preceding examples, each X in X_m of Formula (XVIII) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P. In some examples that may be combined with any of the preceding examples, each X in X_n of Formula (XVIII) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P. In some examples, the MM comprises an amino acid sequence selected from the group consisting of X_m CPDHPYPCXX (SEQ ID NO:181), X_m CDAFYPCXX (SEQ ID NO:182), X_m CDSHYPCXX (SEQ ID NO:183), and X_m CVPYYYACXX (SEQ ID NO:184), where m is from 2-10, and where each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y. In some examples, each X is not M, W, or C. In some examples, each X is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P. In some examples that may be combined with any of the preceding examples, the masking moiety (MM) comprises an amino acid sequence selected from SEQ ID NOS: 141-147. In some examples that may be combined with any of the preceding examples, the MM further comprises, at its N-terminus, an additional amino acid sequence. In some examples, the additional amino acid sequence comprises the amino acid sequence of SEQ ID NO: 148.

[0019] In some examples that may be combined with any of the preceding examples, the first cleavage site is a protease cleavage site for a protease selected from the group consisting of urokinase-type plasminogen activator (uPA), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, Tobacco Etch Virus (TEV) protease,

plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE. In some examples that may be combined with any of the preceding examples, the CM further comprises a first linker (L₁) C-terminal to the first cleavage site. In some examples, the L₁ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 156-163. In some examples that may be combined with any of the preceding examples, the CM further comprises a second cleavage site. In some examples, the second cleavage site is C-terminal to the L₁. In some examples, the second cleavage site is a protease cleavage site for a protease selected from the group consisting of urokinase-type plasminogen activator (uPA), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, Tobacco Etch Virus (TEV) protease, plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE. In some examples, the first and second cleavage sites are different. In some examples that may be combined with any of the preceding examples, the CM further comprises a second linker (L₂) C-terminal to the second cleavage site. In some examples, the L₂ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 156-163. In some examples that may be combined with any of the preceding examples, the CM further comprises a third linker (L₃) N-terminal to the first cleavage site. In some examples that may be combined with any of the preceding examples, the CM comprises at least a first protease cleavage site and is cleaved with one or more proteases selected from the group consisting of urokinase-type plasminogen activator (uPA), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, Tobacco Etch Virus (TEV) protease, plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE.

[0020] In some examples that may be combined with any of the preceding examples, the activatable antibody comprises a masking moiety (MM) and cleavable

moiety (CM) comprising an amino acid sequence according to Formula (XXIX):

EVGSYX1X2X3X4X5X6CX7X8X9X10X11X12CX13X14SGRSAGGGGTENLYFQGS GGS (SEQ ID NO: 164), wherein X1 is A, D, I, N, P, or Y, X2 is A, F, N, S, or V, X3 is A, H, L, P, S, V, or Y, X4 is A, H, S, or Y, X5 is A, D, P, S, V, or Y, X6 is A, D, L, S, or Y, X7 is D, P, or V, X8 is A, D, H, P, S, or T, X9 is A, D, F, H, P, or Y, X10 is L, P, or Y, X11 is F, P, or Y, X12 is A, P, S, or Y, X13 is A, D, N, S, T, or Y, and X14 is A, S, or Y. In some examples that may be combined with any of the preceding examples, the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 165-179.

[0021] In some examples that may be combined with any of the preceding examples, the VL comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 58, an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 66, and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 75. In some examples that may be combined with any of the preceding examples, the VL comprises the amino acid sequence of SEQ ID NO: 100, or a variant thereof having at least about 90% (*e.g.*, at least about 92%, 95%, 98%, 99% or more) sequence identity to the amino acid sequence of SEQ ID NO: 100. In some examples that may be combined with any of the preceding examples, the VH comprises an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 23, an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 35, and an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 45. In some examples that may be combined with any of the preceding examples, the VH comprises the amino acid sequence of SEQ ID NO: 87, or a variant thereof having at least about 90% (*e.g.*, at least about 92%, 95%, 98%, 99% or more) sequence identity to the amino acid sequence of SEQ ID NO: 87.

[0022] Other examples of the present disclosure relate to a pharmaceutical composition comprising any of the antibodies and/or activatable antibodies described herein and a pharmaceutically acceptable carrier. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the activatable antibody as disclosed herein and a pharmaceutically acceptable carrier.

[0023] Other examples of the present disclosure relate to a polynucleotide encoding any of the antibodies and/or activatable antibodies described herein. In some examples, the polynucleotide comprises a sequence selected from SEQ ID NOS: 108-133.

[0024] Other examples of the present disclosure relate to a vector comprising any of the polynucleotides described herein. In some examples, the vector is an expression vector and/or a display vector.

[0025] Other examples of the present disclosure relate to a host cell comprising any of the polynucleotides and/or vectors described herein. In some examples, the host cell is a eukaryotic cell. In some examples, the host cell is a Chinese Hamster Ovary (CHO) cell.

[0026] Other examples of the present disclosure relate to a method of making an antibody or activatable antibody comprising culturing any of the host cells described herein under conditions suitable for producing the antibody or activatable antibody. In some examples, the method further comprises recovering the antibody or activatable antibody produced by the cell.

[0027] Other examples of the present disclosure relate to a method of treating or delaying progression of cancer in a subject in need thereof comprising administering to the subject an effective amount of any of the antibodies, activatable antibodies, and/or pharmaceutical compositions described herein. In one aspect, the present disclosure refers to use of the activatable antibody as disclosed herein in the manufacture of a medicament for treating or delaying progression of cancer in a subject in need thereof. In some examples, the cancer is liver cancer, a cancer of the digestive system (e.g., colon cancer, colorectal cancer), lung cancer, bone cancer, heart cancer, brain cancer, kidney cancer, bladder cancer, a hematological cancer (e.g., leukemia), skin cancer, breast cancer, thyroid cancer, pancreatic cancer, a head and/or neck cancer, an eye-related cancer, a male reproductive system cancer (e.g., prostate cancer, testicular cancer), or a female reproductive system cancer (e.g., uterine cancer, cervical cancer). Other examples of the present disclosure relate to a method of reducing size of a solid tumor in a subject in need thereof, wherein the solid tumor has a size of about 400-1000 mm³, the method comprises administering to the subject an effective amount of any of the antibodies, activatable antibodies, and/or pharmaceutical compositions described herein. In one aspect, the present disclosure refers to use of the activatable antibody as disclosed herein in the manufacture of a medicament for reducing size of a solid tumor in a subject in need thereof, wherein the solid tumor has a size of about 400-1000 mm³. In some examples, the solid tumor has a size of about 400-800 mm³. In some examples, the method further comprises administering to the subject an effective amount of at least one additional therapeutic agent. In some examples, the at least one additional therapeutic agent is selected from the group consisting of viral gene therapy, immune checkpoint inhibitors, target therapies, radiation therapies, vaccination therapies, and chemotherapies. In some examples, the at least one additional therapeutic agent is selected from the group consisting of pomalyst, revlimid, lenalidomide, pomalidomide, thalidomide, a DNA-alkylating platinum-

containing derivative, cisplatin, 5-fluorouracil, cyclophosphamide, an anti-CD137 antibody, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-CD20 antibody, an anti-CD40 antibody, an anti-DR5 antibody, an anti-CD1d antibody, an anti-TIM3 antibody, an anti-SLAMF7 antibody, an anti-KIR receptor antibody, an anti-OX40 antibody, an anti-HER2 antibody, an anti-ErbB-2 antibody, an anti-EGFR antibody, cetuximab, rituximab, trastuzumab, pembrolizumab, radiotherapy, single dose radiation, fractionated radiation, focal radiation, whole organ radiation, IL-12, IFN α , GM-CSF, a chimeric antigen receptor, adoptively transferred T cells, an anti-cancer vaccine, and an oncolytic virus. In some examples, the method comprises administering to the subject an effective amount of the anti-CTLA4 antibody, the activatable antibody or the pharmaceutical composition described herein prior to a surgery or after a surgery to remove the tumor in the subject. In some examples, the anti-CD137 antibody comprises an antibody heavy chain variable region comprising an HVR-H1 comprising the amino acid sequence FSLSTGGVGVGWI (SEQ ID NO: 223), an HVR-H2 comprising the amino acid sequence LALIDWADDKYYSPSLKSRL (SEQ ID NO: 224), and an HVR-H3 comprising the amino acid sequence ARGGS DTVIGDWFAY (SEQ ID NO: 225), and an antibody light chain variable region comprising an HVR-L1 comprising the amino acid sequence RASQSIGSYLA (SEQ ID NO: 226), an HVR-L2 comprising the amino acid sequence DASNLETGV (SEQ ID NO: 227), and an HVR-L3 comprising the amino acid sequence YCQQGYLWT (SEQ ID NO: 228). In some examples, the anti-CD137 antibody comprises an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 229 or a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) to the sequence of SEQ ID NO: 229; and/or an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 230 or a sequence having at least 90% (e.g., 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%) to the sequence of SEQ ID NO: 230.

EVQLVESGGGLVQP GGSRLSCAASG FSLSTGGVGVGWIRQAPGKGLEWLALIDWAD
DKYYSPSLKSRLTISRDN SKNTLYLQLNSLRAEDTAVYYCARGGS DTVIGDWFAYWG
QGTLVTVSS (SEQ ID NO: 229)

DIQLTQSPSSLSASVGDRVTITCRASQSIGSYLAWYQQKPKAPKLLIYDASNLETGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQGYLWTFGQGTKVEIK (SEQ ID NO: 230)

[0028] It is to be understood that one, some, or all of the properties of the various examples described above and herein may be combined to form other examples of the present disclosure. These and other examples of the present disclosure will become apparent to one of skill in the art. These and other examples of the present disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **FIGS. 1A-B** show antibody binding to CTLA4, as determined by ELISA. **FIG. 1A** shows binding of the indicated antibodies to human CTLA4. **FIG. 1B** shows binding of the indicated antibodies to canine CTLA4.

[0030] **FIG. 2** shows species cross-reactivity of the indicated antibodies, isotype control, or vehicle (PBSA) to HEK293F cells transiently overexpressing empty vector (pIRES), or mouse or human CTLA4, as determined by flow cytometry.

[0031] **FIG. 3** shows binding of antibody TY21580, or isotype control, to activated human, monkey, and mouse T cells, as determined by flow cytometry.

[0032] **FIGS. 4A-C** show antibody specificity for CTLA4, as determined by flow cytometry. **FIG. 4A** shows binding of the indicated antibodies, or isotype control, to HEK293F cells transiently overexpressing human PD-1, CTLA4, LAG3, TIM3, B7-H3, or empty vector (293F). **FIG. 4B** shows binding of the indicated antibodies, isotype control, or vehicle (PBSA) to HEK293F cells transiently overexpressing human CD95, CD120a, OX40, CD40, CTLA4, or empty vector (pIRES). **FIG. 4C** shows binding of the indicated antibodies, isotype control, or vehicle (PBSA) to HEK293F cells transiently overexpressing human TIM3, CTLA4, PD-L1, LAG3, BTLA, VISTA, PD-L2, ICOS, B7-H4, PD-1, B7-H3, or empty vector (pIRES).

[0033] **FIGS. 5A-D** show the blocking capabilities of the antibodies, as determined by ELISA. **FIG. 5A** shows the ability of antibodies TY21687, TY21689, TY21680, and TY21691 to block human CD80 binding to human CTLA4. **FIG. 5B** shows the ability of antibodies TAC2114, TY21585, TY21587, TY21588, TY21589, TY21580, and TY21591 to block human CD80 binding to human CTLA4. **FIG. 5C** shows the ability of antibodies TY21687, TY21689, TY21680, and TY21691 to block human CD86 binding to human CTLA4. **FIG. 5D** shows the ability of antibodies TAC2114, TY21585, TY21587, TY21588, TY21589, TY21580, and TY21591 to block human CD86 binding to human CTLA4.

CLAIMS

What is claimed is:

1. An activatable antibody comprising:

a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM),

wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 189-193, 195-196, and 213-216;

wherein the CM comprises at least a first cleavage site;

wherein:

a) the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH);

b) the TBM comprises an antibody heavy chain variable region (VH), and the activatable antibody further comprises a second polypeptide comprising an antibody light chain variable region (VL);

c) the TBM comprises from the N-terminus to the C-terminus, an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH); or

d) the TBM comprises from the N-terminus to the C-terminus, an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL);

wherein the VH comprises an HVR-H1 comprising the amino acid sequence YSISGYPHWSWI (SEQ ID NO: 23), an HVR-H2 comprising the amino acid sequence LARIDWDDDKYYSTSLKSRL (SEQ ID NO: 35), and an HVR-H3 comprising the amino acid sequence ARSYVYFDY (SEQ ID NO: 45);

wherein the VL comprises an HVR-L1 comprising the amino acid sequence RASQSVRGRFLA (SEQ ID NO: 58), an HVR-L2 comprising the amino acid sequence DASNRATGI (SEQ ID NO: 66), and an HVR-L3 comprising the amino acid sequence YCQQSSSWPPT (SEQ ID NO: 75); and

wherein the activatable antibody binds to human CTLA4 via the VH and VL when the CM is cleaved.

2. The activatable antibody of claim 1, wherein the TBM comprises an antibody light chain variable region (VL), and the activatable antibody further comprises a second polypeptide comprising an antibody heavy chain variable region (VH).
3. The activatable antibody of claim 1, wherein the first cleavage site is a protease cleavage site for a protease selected from the group consisting of urokinase-type plasminogen activator (uPA), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, Tobacco Etch Virus (TEV) protease, plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE.
4. The activatable antibody of claim 1, wherein the CM further comprises a first linker (L₁) C-terminal to the first cleavage site.
5. The activatable antibody of claim 4, wherein the L₁ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 156-163.
6. The activatable antibody of claim 1, wherein the CM further comprises a second cleavage site.
7. The activatable antibody of claim 6, wherein the CM further comprises a first linker (L₁) C-terminal to the first cleavage site, wherein the second cleavage site is C-terminal to the L₁.
8. The activatable antibody of claim 6, wherein the second cleavage site is a protease cleavage site for a protease selected from the group consisting of urokinase-type plasminogen

activator (uPA), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, Tobacco Etch Virus (TEV) protease, plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE.

9. The activatable antibody of claim 6, wherein the first and second cleavage sites are different.
10. The activatable antibody of claim 6, wherein the CM further comprises a second linker (L₂) C-terminal to the second cleavage site.
11. The activatable antibody of claim 10, wherein the L₂ comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 156-163.
12. The activatable antibody of claim 1, wherein the CM further comprises a linker (L₃) N-terminal to the first cleavage site.
13. The activatable antibody of claim 1, wherein the CM comprises at least a first protease cleavage site and is cleaved with one or more proteases selected from the group consisting of urokinase-type plasminogen activator (uPA), matrix metalloproteinase-1 (MMP-1), MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, Tobacco Etch Virus (TEV) protease, plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE.
14. The activatable antibody of claim 1, wherein the activatable antibody comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 165-179 and 198-202.
15. Use of the activatable antibody of claim 1 in the manufacture of a medicament for treating or delaying progression of cancer in a subject in need thereof.

16. Use of the activatable antibody of claim 1 in the manufacture of a medicament for reducing size of a solid tumor in a subject in need thereof, wherein the solid tumor has a size of about 400-1000 mm³.
17. The use of claim 16, wherein the subject is to be further administered an effective amount of at least one additional therapeutic agent, wherein the at least one additional therapeutic agent is selected from the group consisting of viral gene therapy, immune checkpoint inhibitors, target therapies, radiation therapies, vaccination therapies, and chemotherapies.
18. The activatable antibody of claim 1, wherein the VH comprises the amino acid sequence of SEQ ID NO: 87, and the VL comprises the amino acid sequence of SEQ ID NO: 100.
19. The activatable antibody of claim 1, wherein the MM comprises the amino acid sequence PNPSSDCVPYYYACAY (SEQ ID NO: 144).
20. The activatable antibody of claim 18, wherein the MM comprises the amino acid sequence PNPSSDCVPYYYACAY (SEQ ID NO: 144).
21. The activatable antibody of claim 1, wherein the activatable antibody comprises the amino acid sequence EVGSYPNPSSDCVPYYYACAY (SEQ ID NO: 192).
22. The activatable antibody of claim 18, wherein the activatable antibody comprises the amino acid sequence EVGSYPNPSSDCVPYYYACAY (SEQ ID NO: 192).
23. An activatable antibody comprising:
- (a) a first polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and an antibody light chain variable region (VL); and
 - (b) a second polypeptide comprising the antibody heavy chain variable region (VH);
- wherein the MM comprises the amino acid sequence PNPSSDCVPYYYACAY (SEQ ID NO: 144);
- wherein the CM comprises at least a first cleavage site;
- wherein the VH comprises the amino acid sequence of SEQ ID NO: 87, and
- wherein the VL comprises the amino acid sequence of SEQ ID NO: 100; and

wherein the activatable antibody binds to human CTLA4 via the VH and VL when the CM is cleaved.

24. The activatable antibody of claim 23, wherein the first polypeptide comprises the amino acid sequence EVGSYPNPSSDCVPYYYACAYSGRSAGGGGTPLGLAGSGGS (SEQ ID NO: 200).
25. The activatable antibody of claim 1, wherein the activatable antibody comprises a human IgG1 Fc region comprising one or more mutations that increase antibody-dependent cellular cytotoxicity (ADCC) activity.
26. The activatable antibody of claim 23, wherein the activatable antibody comprises a human IgG1 Fc region comprising one or more mutations that increase antibody-dependent cellular cytotoxicity (ADCC) activity.
27. Use of the activatable antibody of claim 18 in the manufacture of a medicament for treating or delaying progression of cancer in a subject in need thereof.
28. Use of the activatable antibody of claim 19 in the manufacture of a medicament for treating or delaying progression of cancer in a subject in need thereof.
29. Use of the activatable antibody of claim 20 in the manufacture of a medicament for treating or delaying progression of cancer in a subject in need thereof.
30. Use of the activatable antibody of claim 21 in the manufacture of a medicament for treating or delaying progression of cancer in a subject in need thereof.
31. Use of the activatable antibody of claim 23 in the manufacture of a medicament for treating or delaying progression of cancer in a subject in need thereof.
32. Use of the activatable antibody of claim 24 in the manufacture of a medicament for treating or delaying progression of cancer in a subject in need thereof.
33. Use of the activatable antibody of claim 25 in the manufacture of a medicament for treating or delaying progression of cancer in a subject in need thereof.

34. A pharmaceutical composition comprising the activatable antibody of claim 1 and a pharmaceutically acceptable carrier.
35. A pharmaceutical composition comprising the activatable antibody of claim 18 and a pharmaceutically acceptable carrier.
36. A pharmaceutical composition comprising the activatable antibody of claim 21 and a pharmaceutically acceptable carrier.
37. A pharmaceutical composition comprising the activatable antibody of claim 22 and a pharmaceutically acceptable carrier.
38. A pharmaceutical composition comprising the activatable antibody of claim 23 and a pharmaceutically acceptable carrier.
39. A pharmaceutical composition comprising the activatable antibody of claim 24 and a pharmaceutically acceptable carrier.
40. A pharmaceutical composition comprising the activatable antibody of claim 25 and a pharmaceutically acceptable carrier.