(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 November 2016 (10.11.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/179285 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61P 35/00* (2006.01)
C07K 19/00 (2006.01)

(21) International Application Number:
PCT/US2016/030785

(22) International Filing Date:
4 May 2016 (04.05.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/156,835 4 May 2015 (04.05.2015) US
62/220,805 18 September 2015 (18.09.2015) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

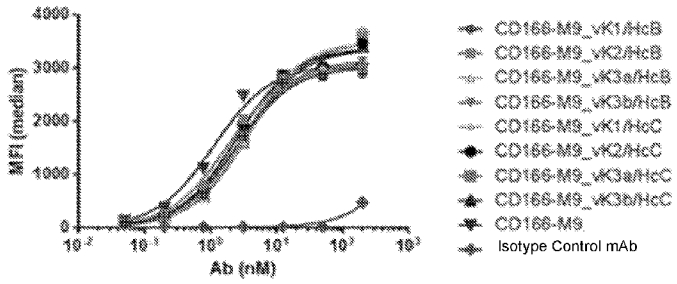
(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,

[Continued on next page]

(54) Title: ANTI-CD166 ANTIBODIES, ACTIVATABLE ANTI-CD166 ANTIBODIES, AND METHODS OF USE THEREOF

(57) Abstract: The invention relates generally to antibodies that bind CD166, activatable antibodies that specifically bind to CD166 and methods of making and using these anti-CD166 antibodies and anti-CD166 activatable antibodies in a variety of therapeutic, diagnostic and prophylactic indications.

FIGURE 1



Ab	Apparent Kd (nM); FACS
CD166-M9_vK1/HcB	2.39
CD166-M9_vK2/HcB	1.79
CD166-M9_vK3a/HcB	2.88
CD166-M9_vK3b/HcB	2.61
CD166-M9_vK1/HcC	3.26
CD166-M9_vK2/HcC	2.50
CD166-M9_vK3a/HcC	3.76
CD166-M9_vK3b/HcC	3.19
CD166-M9 chimera	1.12

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TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*

ANTI-CD166 ANTIBODIES, ACTIVATABLE ANTI-CD166 ANTIBODIES, AND METHODS OF USE THEREOF

Related Applications

[0001] This application claims the benefit of U.S. Provisional Application Nos. 62/156,835, filed May 4, 2015; and 62/220,805, filed September 18, 2015, the contents of each of which are incorporated herein by reference in their entirety.

Field of the Invention

[0002] The invention relates generally to antibodies that bind CD166, activatable antibodies that specifically bind to CD166 and methods of making and using these anti-CD166 antibodies and anti-CD166 activatable antibodies in a variety of therapeutic, diagnostic and prophylactic indications.

Background of the Invention

[0003] Antibody-based therapies have proven effective treatments for several diseases but in some cases, toxicities due to broad target expression have limited their therapeutic effectiveness. In addition, antibody-based therapeutics have exhibited other limitations such as rapid clearance from the circulation following administration.

[0004] 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.

[0005] 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

[0006] The disclosure provides antibodies or antigen-binding fragments thereof that specifically bind CD166, also known as cluster of differentiation 166, activated leukocyte cell adhesion molecule (ALCAM), and/or MEMD. The use of the term "CD166" is intended

to cover any variation thereof, such as, by way of non-limiting example, CD-166 and/or CD 166, and all variations are used herein interchangeably.

[0007] In some embodiments, the antibody includes an antibody or antigen-binding fragment thereof that specifically binds CD166. In some embodiments, the antibody or antigen-binding fragment thereof that binds CD166 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 antigen-binding fragment thereof that binds CD166 is a mouse, other rodent, chimeric, humanized or fully human monoclonal antibody.

[0008] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence comprising SEQ ID NO: 121. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence comprising SEQ ID NO: 122.

[0009] In some embodiments, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126. In some embodiments, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123-126. In some embodiments, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence comprising SEQ ID NO: 123.

[00010] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[00011] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123-126. In some

embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 121, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 122, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123.

[00012] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region 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: 119, 121, and 122. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising SEQ ID NO: 122. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising SEQ ID NO: 121.

[00013] In some embodiments, the antibody or antigen-binding fragment thereof comprises a light chain variable region 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: 120 and 123-126. In some embodiments, the antibody or antigen-binding fragment thereof comprises a light chain variable region 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: 123-126. In some embodiments, the antibody or antigen-binding fragment thereof comprises a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence SEQ ID NO: 123.

[00014] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region 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: 119, 121, and 122, and a light chain variable region 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: 120 and 123-126.

[00015] In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region 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: 121 or SEQ ID NO: 122, and a light chain variable region 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: 123-126. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence selected SEQ ID NO: 122, and a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence SEQ ID NO: 123. In some embodiments, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence selected SEQ ID NO: 121, and a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence SEQ ID NO: 123.

[00016] In some embodiments, the antibody or antigen-binding fragment thereof comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one complementarity determining region (CDR) sequence is selected from the group consisting of a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2

sequence comprising the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence comprising the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00017] In some embodiments, the antibody or antigen-binding fragment thereof comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one complementarity determining region (CDR) sequence is selected from the group consisting of a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence comprising the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence comprising the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132); and a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00018] In some embodiments, the antibody or antigen-binding fragment thereof comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to

a VH CDR2 sequence comprising the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence comprising the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence comprising the amino acid sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00019] In some embodiments, the antibody or antigen-binding fragment thereof comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence comprising the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence comprising the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence comprising the amino acid sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%

or more identical to a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00020] In some embodiments, the antibody or antigen-binding fragment thereof comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00021] In some embodiments, the antibody or antigen-binding fragment thereof comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00022] In some embodiments, the antibody or antigen-binding fragment thereof comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129);

the VL CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00023] In some embodiments, the antibody or antigen-binding fragment thereof comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); the VL CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence QMSNLAS (SEQ ID NO: 132); and the VL CDR3 sequence a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00024] In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 121 or SEQ ID NO: 122.

In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence comprising the amino acid sequence selected SEQ ID NO: 122. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence comprising the amino acid sequence selected SEQ ID NO: 121.

[00025] In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 123-126. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain amino acid sequence comprising the amino acid sequence SEQ ID NO: 123.

[00026] In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a nucleic acid sequence encoding a light chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[00027] In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 121, and 122, and a nucleic acid sequence encoding a light chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 123-126.

[00028] In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 122, and a nucleic acid sequence encoding a light chain amino acid sequence comprising the amino acid sequence SEQ ID NO: 123. In some embodiments, the antibody or antigen-

binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 121, and a nucleic acid sequence encoding a light chain amino acid sequence comprising the amino acid sequence SEQ ID NO: 123.

[00029] In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 121 or SEQ ID NO: 122. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 121 or SEQ ID NO: 122. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 122. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 121.

[00030] In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 123-126. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 123.

[00031] In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[00032] In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 121 or SEQ ID NO: 122, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 123-126. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 122, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 123. In some embodiments, the antibody or antigen-binding fragment thereof is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 121, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain amino acid sequence comprising the amino acid sequence of SEQ ID NO: 123.

[00033] In some embodiments, the antibody or antigen-binding fragment thereof is incorporated in a multispecific antibody or antigen-binding fragment thereof, where at least one arm of the multispecific antibody or antigen-binding fragment thereof specifically binds CD166. In some embodiments, the antibody or antigen-binding fragment thereof is incorporated in a bispecific antibody or antigen-binding fragment thereof, where at least one arm of the bispecific antibody or antigen-binding fragment thereof specifically binds CD166.

[00034] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 121 or SEQ ID NO: 122. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence comprising the amino acid sequence of SEQ ID NO: 122. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence comprising the amino acid sequence of SEQ ID NO: 121.

[00035] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 123-126. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a light chain variable region amino acid sequence comprising the amino acid sequence of SEQ ID NO: 123.

[00036] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[00037] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 123-126. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence comprising the amino acid sequence of SEQ ID NO: SEQ ID NO: 122, and a light chain variable region amino acid sequence comprising the amino acid sequence of SEQ ID NO: 123. , a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence comprising the amino acid sequence of SEQ ID NO: SEQ ID NO: 121, and a light chain variable region amino acid sequence comprising the amino acid sequence of SEQ ID NO: 123.

[00038] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 121 or SEQ ID NO: 122. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence that is at least 90%,

91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 122. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 121.

[00039] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 123-126. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 123.

[00040] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[00041] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment

thereof, comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 123-126.

[00042] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 122, and a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 123.

[00043] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 121, and a light chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising the amino acid sequence of SEQ ID NO: 123.

[00044] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also

referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence comprising the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence comprising the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00045] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence comprising the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence comprising the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132); and a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00046] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99% or more identical to a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence comprising the amino acid sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00047] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence comprising the amino acid sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more

identical to a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134)

[00048] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00049] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00050] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence

GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00051] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); the VL CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence QMSNLAS (SEQ ID NO: 132); and the VL CDR3 sequence a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00052] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain or a heavy chain variable region that comprises or is derived from an amino acid sequence selected from the group consisting of the heavy chain variable region amino acid sequences shown in Table 12. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a light chain or a light chain variable region that comprises or is derived from an amino acid sequence selected from the group consisting of the light chain variable region amino acid sequences shown in Table 12. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain or a heavy chain variable region that comprises or is derived from an amino acid sequence selected from the group consisting of the heavy chain variable region sequences shown in Table 12 and a light chain or a light chain variable region that comprises or is derived from an amino acid sequence selected from the group consisting of the light chain variable region amino acid sequences shown in Table 12.

[00053] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region 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 the heavy chain variable region sequences shown in Table 12. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises an 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 the light chain variable region sequences shown in Table 12. In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises an 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 the heavy chain variable region sequences shown in Table 12 and a light chain variable region 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 the light chain variable region sequences shown in Table 12.

[00054] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 13; a VH CDR2 sequence shown in Table 13; a VH CDR3 sequence shown in Table 13; a VL CDR1 sequence shown in Table 13; a VL CDR2 sequence shown in Table 13; and a VL CDR3 sequence shown in Table 13.

[00055] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence shown in Table 13; a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence shown in Table 13; a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence shown in Table 13; a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence shown in Table 13; a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence shown in Table 13; and a VL

CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence shown in Table 13.

[00056] In some embodiments at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 13.

[00057] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a light chain variable region that comprise a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the three light chain CDR sequences (VL CDR1, VL CDR2, VL CDR3) shown in a single row in Table 13.

[00058] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein the combination is a combination of the three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 13.

[00059] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 13.

[00060] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a heavy chain variable region that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein each CDR

sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 13.

[00061] In some embodiments, at least one arm of the multispecific antibody or antigen-binding fragment thereof, *e.g.*, a bispecific antibody or antigen-binding fragment thereof, comprises a light chain variable region that comprise a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of three light chain CDR sequences (VL CDR1, VL CDR2, VL CDR3) shown in a single row in Table 13.

[00062] Suitable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that binds to the same epitope on human CD166 and/or cynomolgus monkey CD166 as an anti-CD166 antibody comprising a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[00063] Suitable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that binds to the same epitope on human CD166 and/or cynomolgus monkey CD166 as an anti-CD166 antibody comprising the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00064] Suitable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that binds to the same epitope on human CD166 and/or cynomolgus monkey CD166 as an anti-CD166 antibody comprising the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the

VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00065] Suitable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that cross-competes for binding to human CD166 and/or cynomolgus monkey CD166 to an anti-CD166 antibody comprising a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[00066] Suitable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that cross-competes for binding to human CD166 and/or cynomolgus monkey CD166 to an anti-CD166 antibody comprising a heavy chain variable region amino acid sequence comprising SEQ ID NO: 122, and a light chain variable region amino acid sequence comprising SEQ ID NO: 123. Suitable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that cross-competes for binding to human CD166 and/or cynomolgus monkey CD166 to an anti-CD166 antibody comprising a heavy chain variable region amino acid sequence comprising SEQ ID NO: 121, and a light chain variable region amino acid sequence comprising SEQ ID NO: 123.

[00067] Suitable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that cross-competes for binding to human CD166 and/or cynomolgus monkey CD166 to an anti-CD166 antibody comprising the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00068] Suitable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that cross-competes for binding to human CD166 and/or cynomolgus monkey CD166 to an anti-CD166 antibody comprising the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[00069] The disclosure also provides activatable antibodies that include an antibody or antigen-binding fragment thereof that specifically binds CD166 coupled to a masking moiety (MM), such that coupling of the MM reduces the ability of the antibody or antigen-binding fragment thereof to bind CD166. In some embodiments, the MM is coupled via a sequence that includes a substrate for a protease, for example, a protease that is active in diseased tissue and/or a protease that is co-localized with CD166 at a treatment site in a subject. The activatable anti-CD166 antibodies provided herein, also referred to herein interchangeably as anti-CD166 activatable antibodies or CD166 activatable antibodies, are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *e.g.*, healthy tissue or other tissue not targeted for treatment and/or diagnosis, and, when activated, exhibit binding to CD166 that is at least comparable to the corresponding, unmodified antibody, also referred to herein as the parental antibody.

[00070] The invention also provides methods of treating, preventing and/or delaying the onset or progression of, or alleviating a symptom associated with aberrant expression and/or activity of CD166 in a subject using activatable antibodies that bind CD166, particularly activatable antibodies that bind and neutralize or otherwise inhibit at least one biological activity of CD166 and/or CD166-mediated signaling.

[00071] The invention also provides methods of treating, preventing and/or delaying the onset or progression of, or alleviating a symptom associated with the presence, growth, proliferation, metastasis, and/or activity of cells which are expressing CD166 or aberrantly expressing CD166 in a subject using activatable antibodies that bind CD166, particularly activatable antibodies that bind, target, neutralize, kill, or otherwise inhibit at least one biological activity of cells which are expressing or aberrantly expressing CD166.

[00072] The invention also provides methods of treating, preventing and/or delaying the onset or progression of, or alleviating a symptom associated with the presence, growth, proliferation, metastasis, and/or activity of cells which are expressing CD166 in a subject using activatable antibodies that bind CD166, particularly activatable antibodies that bind, target, neutralize, kill, or otherwise inhibit at least one biological activity of cells which are expressing CD166.

[00073] The invention also provides methods of treating, preventing and/or delaying the onset or progression of, or alleviating a symptom associated with the presence, growth, proliferation, metastasis, and/or activity of cells which are aberrantly expressing CD166 in a subject using activatable antibodies that bind CD166, particularly activatable antibodies that bind, target, neutralize, kill, or otherwise inhibit at least one biological activity of cells which are aberrantly expressing CD166.

[00074] The activatable antibodies in an activated state bind CD166 and include (i) an antibody or an antigen binding fragment thereof (AB) that specifically binds to CD166; (ii) a masking moiety (MM) that, when the activatable antibody is in an uncleaved state, inhibits the binding of the AB to CD166; and (c) a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

[00075] 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.

[00076] In some embodiments, the activatable antibody comprises a linking peptide between the MM and the CM.

[00077] In some embodiments, the activatable antibody comprises a linking peptide between the CM and the AB.

[00078] In some embodiments, the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM. In some embodiments, the two linking peptides need not be identical to each other.

[00079] In some embodiments, at least one of LP1 or LP2 comprises an amino acid sequence selected from the group consisting of $(GS)_n$, $(GGS)_n$, $(GSGGS)_n$ (SEQ ID NO: 1) and $(GGGS)_n$ (SEQ ID NO: 2), where n is an integer of at least one.

[00080] In some embodiments, at least one of LP1 or LP2 comprises an amino acid sequence selected from the group consisting of GGSG (SEQ ID NO: 3), GGSGG (SEQ ID NO: 4), GSGSG (SEQ ID NO: 5), GSGGG (SEQ ID NO: 6), GGGSG (SEQ ID NO: 7), and GSSSG (SEQ ID NO: 8).

[00081] In some embodiments, LP1 comprises the amino acid sequence GSSGGSGGSGGSG (SEQ ID NO: 9), GSSGGSGGSGG (SEQ ID NO: 10), GSSGGSGGSGGS (SEQ ID NO: 11), GSSGGSGGSGGSGGGS (SEQ ID NO: 12), GSSGGSGGSG (SEQ ID NO: 13), or GSSGGSGGSGS (SEQ ID NO: 14).

[00082] In some embodiments, LP2 comprises the amino acid sequence GSS, GGS, GGGS (SEQ ID NO: 15), GSSGT (SEQ ID NO: 16) or GSSG (SEQ ID NO: 17).

[00083] In some embodiments, the AB has a dissociation constant of about 100 nM or less for binding to CD166.

[00084] In some embodiments, the AB has a dissociation constant of about 100 nM or less for binding to mammalian CD166. In some embodiments, the AB has a dissociation constant of about 10 nM or less for binding to mammalian CD166. In some embodiments, the AB has a dissociation constant of about 5 nM or less for binding to CD166. In some embodiments, the AB has a dissociation constant of about 1 nM or less for binding to CD166. In some embodiments, the AB has a dissociation constant of about 0.5 nM or less for binding to CD166. In some embodiments, the AB has a dissociation constant of about 0.1 nM or less for binding to CD166. In some embodiments, the AB has a dissociation constant of 0.01 nM to 100 nM, 0.01 nM to 10 nM, 0.01 nM to 5 nM, 0.01 nM to 1 nM, 0.01 to 0.5 nM, 0.01 nm to 0.1 nM, 0.01 nm to 0.05 nM, 0.05 nM to 100 nM, 0.05 nM to 10 nM, 0.05 nM to 5 nM, 0.05 nM to 1 nM, 0.05 to 0.5 nM, 0.05 nm to 0.1 nM, 0.1 nM to 100 nM, 0.1 nM to 10 nM, 0.1 nM to 5 nM, 0.1 nM to 1 nM, 0.1 to 0.5 nM, 0.5 nM to 100 nM, 0.5 nM to 10 nM, 0.5 nM to 5 nM, 0.5 nM to 1 nM, 1 nM to 100 nM, 1 nM to 10 nM, 1 nM to 5 nM, 5 nM to 100 nM, 5 nM to 10 nM, or 10 nM to 100 nM, for binding to mammalian CD166.

[00085] In some embodiments, the activatable antibody includes an antibody or antigen-binding fragment thereof (AB) that specifically binds CD166. In some embodiments, the antibody or antigen-binding fragment thereof that binds CD166 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 antigen-binding fragment thereof that

binds CD166 is a mouse, other rodent, chimeric, humanized or fully human monoclonal antibody.

[00086] In some embodiments, the activatable antibody in an uncleaved state specifically binds to mammalian CD166 with a dissociation constant less than or equal to 1 nM, less than or equal to 5 nM, less than or equal to 10 nM, less than or equal to 15 nM, less than or equal to 20 nM, less than or equal to 25 nM, less than or equal to 50 nM, less than or equal to 100 nM, less than or equal to 150 nM, less than or equal to 250 nM, less than or equal to 500 nM, less than or equal to 750 nM, less than or equal to 1000 nM, and 122. /or less than or equal to 2000 nM.

[00087] In some embodiments, the activatable antibody in an uncleaved state specifically binds to mammalian CD166 with a dissociation constant greater than or equal to 1 nM, greater than or equal to 5 nM, greater than or equal to 10 nM, greater than or equal to 15 nM, greater than or equal to 20 nM, greater than or equal to 25 nM, greater than or equal to 50 nM, greater than or equal to 100 nM, greater than or equal to 150 nM, greater than or equal to 250 nM, greater than or equal to 500 nM, greater than or equal to 750 nM, greater than or equal to 1000 nM, and 122. /or greater than or equal to 2000 nM.

[00088] In some embodiments, the activatable antibody in an uncleaved state specifically binds to the mammalian CD166 with a dissociation constant in the range of 1 nM to 2000 nM, 1 nM to 1000 nM, 1 nM to 750 nM, 1 nM to 500 nM, 1 nM to 250 nM, 1 nM to 150 nM, 1 nM to 100 nM, 1 nM to 50 nM, 1 nM to 25 nM, 1 nM to 15 nM, 1 nM to 10 nM, 1 nM to 5 nM, 5 nM to 2000 nM, 5 nM to 1000 nM, 5 nM to 750 nM, 5 nM to 500 nM, 5 nM to 250 nM, 5 nM to 150 nM, 5 nM to 100 nM, 5 nM to 50 nM, 5 nM to 25 nM, 5 nM to 15 nM, 5 nM to 10 nM, 10 nM to 2000 nM, 10 nM to 1000 nM, 10 nM to 750 nM, 10 nM to 500 nM, 10 nM to 250 nM, 10 nM to 150 nM, 10 nM to 100 nM, 10 nM to 50 nM, 10 nM to 25 nM, 10 nM to 15 nM, 15 nM to 2000 nM, 15 nM to 1000 nM, 15 nM to 750 nM, 15 nM to 500 nM, 15 nM to 250 nM, 15 nM to 150 nM, 15 nM to 100 nM, 15 nM to 50 nM, 15 nM to 25 nM, 25 nM to 2000 nM, 25 nM to 1000 nM, 25 nM to 750 nM, 25 nM to 500 nM, 25 nM to 250 nM, 25 nM to 150 nM, 25 nM to 100 nM, 25 nM to 50 nM, 50 nM to 2000 nM, 50 nM to 1000 nM, 50 nM to 750 nM, 50 nM to 500 nM, 50 nM to 250 nM, 50 nM to 150 nM, 50 nM to 100 nM, 100 nM to 2000 nM, 100 nM to 1000 nM, 100 nM to 750 nM, 100 nM to 500 nM, 100 nM to 250 nM, 100 nM to 150 nM, 150 nM to 2000 nM, 150 nM to 1000 nM, 150 nM to 750 nM, 150 nM to 500 nM, 150 nM to 250 nM, 250 nM to 2000 nM, 250 nM to 1000 nM, 250 nM to 750 nM, 250 nM to 500 nM, 500 nM to

2000 nM, 500 nM to 1000 nM, 500 nM to 750 nM, 500 nM to 500 nM, 500 nM to 250 nM, 500 nM to 150 nM, 500 nM to 100 nM, 500 nM to 50 nM, 750 nM to 2000 nM, 750 nM to 1000 nM, or 1000 nM to 2000 nM.

[00089] In some embodiments, the activatable antibody in an activated state specifically binds to mammalian CD166 with a dissociation constant is less than or equal to 0.01 nM, 0.05 nM, 0.1 nM, 0.5 nM, 1 nM, 5 nM, or 10 nM.

[00090] In some embodiments, the activatable antibody in an activated state specifically binds to mammalian CD166 with a dissociation constant is greater than or equal to 0.01 nM, 0.05 nM, 0.1 nM, 0.5 nM, 1 nM, 5 nM, or 10 nM.

[00091] In some embodiments, the activatable antibody in an activated state specifically binds to the mammalian CD166 with a dissociation constant in the range of 0.01 nM to 100 nM, 0.01 nM to 10 nM, 0.01 nM to 5 nM, 0.01 nM to 1 nM, 0.01 to 0.5 nM, 0.01 nm to 0.1 nM, 0.01 nm to 0.05 nM, 0.05 nM to 100 nM, 0.05 nM to 10 nM, 0.05 nM to 5 nM, 0.05 nM to 1 nM, 0.05 to 0.5 nM, 0.05 nm to 0.1 nM, 0.1 nM to 100 nM, 0.1 nM to 10 nM, 0.1 nM to 5 nM, 0.1 nM to 1 nM, 0.1 to 0.5 nM, 0.5 nM to 100 nM, 0.5 nM to 10 nM, 0.5 nM to 5 nM, 0.5 nM to 1 nM, 1 nM to 100 nM, 1 nM to 10 nM, 1 nM to 5 nM, 5 nM to 100 nM, 5 nM to 10 nM, or 10 nM to 100 nM.

[00092] In some embodiments, the mammalian CD166 is selected from the group consisting of a human CD166 and a cynomolgus monkey CD166. In some embodiments, the AB specifically binds to human CD166 or cynomolgus monkey CD166 with a dissociation constant of less than 1 nM. In some embodiments, the mammalian CD166 is a human CD166. In some embodiments, the mammalian CD166 is a cynomolgus CD166.

[00093] In some embodiments, the AB has one or more of the following characteristics: (a) the AB specifically binds to human CD166; and (b) the AB specifically binds to human CD166 and cynomolgus monkey CD166.

[00094] In some embodiments, the AB has one or more of the following characteristics: (a) the AB specifically binds human CD166 and cynomolgus monkey CD166; (b) the AB inhibits binding of mammalian CD6 to mammalian CD166; (c) the AB inhibits binding of human CD6 to human CD166; and (d) the AB inhibits binding of cynomolgus monkey CD6 to cynomolgus monkey CD166.

[00095] In some embodiments, the AB blocks the ability of a natural ligand or receptor to bind to the mammalian CD166 with an EC50 less than or equal to 5 nM, less than or equal to 10 nM, less than or equal to 50 nM, less than or equal to 100 nM, less than

or equal to 500 nM, and/or less than or equal to 1000 nM. In some embodiments, the AB blocks the ability of mammalian CD6 to bind to the mammalian CD166 with an EC50 less than or equal to 5 nM, less than or equal to 10 nM, less than or equal to 50 nM, less than or equal to 100 nM, less than or equal to 500 nM, and/or less than or equal to 1000 nM. In some embodiments, the natural ligand or receptor of CD166 is CD6.

[00096] In some embodiments, the AB blocks the ability of a natural ligand to bind to the mammalian CD166 with an EC50 of 5 nM to 1000 nM, 5 nM to 500 nM, 5 nM to 100 nM 5 nM to 50 nM, 5 nM to 10 nM, 10 nM to 1000 nM, 10 nM to 500 nM, 10 nM to 100 nM 10 nM to 50 nM, 50 nM to 1000 nM, 50 nM to 500 nM, 50 nM to 100 nM, 100 nM to 1000 nM, 100 nM to 500 nM, 500 nM to 1000 nM. In some embodiments, the AB blocks the ability of mammalian CD6 to bind to the mammalian CD166 with an EC50 of 5 nM to 1000 nM, 5 nM to 500 nM, 5 nM to 100 nM 5 nM to 50 nM, 5 nM to 10 nM, 10 nM to 1000 nM, 10 nM to 500 nM, 10 nM to 100 nM 10 nM to 50 nM, 50 nM to 1000 nM, 50 nM to 500 nM, 50 nM to 100 nM, 100 nM to 1000 nM, 100 nM to 500 nM, 500 nM to 1000 nM. In some embodiments, the natural ligand or receptor of CD166 is CD6.

[00097] In some embodiments, the AB of the present disclosure inhibits or reduces the growth, proliferation, and/or metastasis of cells expressing mammalian CD166. Without intending to be bound by any theory, the AB of the present disclosure may inhibit or reduce the growth, proliferation, and/or metastasis of cells expressing mammalian CD166 by specifically binding to CD166 and inhibiting, blocking, and/or preventing the binding of a natural ligand or receptor to mammalian CD166. In some embodiments, the natural ligand or receptor of mammalian CD166 is mammalian CD6.

[00098] In some embodiments, the activatable antibody comprises a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122. In some embodiments, the activatable antibody comprises a heavy chain variable region amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122.

[00099] In some embodiments, the activatable antibody comprises a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126. In some embodiments, the activatable antibody comprises a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123-126.

[000100] In some embodiments, the activatable antibody comprises a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[000101] In some embodiments, the activatable antibody comprises a heavy chain variable region amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123-126.

[000102] In some embodiments, the activatable antibody comprises a heavy chain variable region 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: 119, 121, and 122. In some embodiments, the activatable antibody comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122.

[000103] In some embodiments, the activatable antibody comprises a light chain variable region 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: 120 and 123-126. In some embodiments, the activatable antibody comprises a light chain variable region 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: 123-126.

[000104] In some embodiments, the activatable antibody comprises a heavy chain variable region 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: 119, 121, and 122, and a light chain variable region 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: 120 and 123-126.

[000105] In some embodiments, the activatable antibody comprises a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to an amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region 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: 123-126.

[000106] In some embodiments, the activatable antibody comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence comprising the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence comprising the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[000107] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence comprising the amino acid

sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[000108] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[000109] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the VH CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to

the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[000110] In some embodiments, the AB of the activatable anti-CD166 antibody comprises a heavy chain variable region amino acid sequence selected from the group consisting of the heavy chain variable region sequences shown in Table 12. In some embodiments, the AB of the activatable anti-CD166 antibody comprises a light chain variable region amino acid sequence selected from the group consisting of the light chain variable region sequences shown in Table 12. In some embodiments, the AB of the activatable anti-CD166 antibody comprises a heavy chain variable region amino acid sequence selected from the group consisting of the heavy chain variable region sequences shown in Table 12 and a light chain variable region amino acid sequence selected from the group consisting of the light chain variable region sequences shown in Table 12.

[000111] In some embodiments, the AB of the activatable anti-CD166 antibody comprises a heavy chain variable region 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 the heavy chain variable region sequences shown in Table 12. In some embodiments, the AB of the activatable anti-CD166 antibody comprises a light chain variable region 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 the light chain variable region sequences shown in Table 12. In some embodiments, the AB of the activatable anti-CD166 antibody comprises a heavy chain variable region 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 the heavy chain variable region sequences shown in Table 12 and a light chain variable region 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 the light chain variable region sequences shown in Table 12.

[000112] In some embodiments, the activatable antibody comprises a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain

complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 13; a VH CDR2 sequence shown in Table 13; a VH CDR3 sequence shown in Table 13; a VL CDR1 sequence shown in Table 13; a VL CDR2 sequence shown in Table 13; and a VL CDR3 sequence shown in Table 13.

[000113] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence shown in Table 13; a VH CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence shown in Table 13; a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence shown in Table 13; a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence shown in Table 13; a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence shown in Table 13; and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence shown in Table 13.

[000114] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 13.

[000115] In some embodiments, the activatable antibody comprises a heavy chain variable region that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein the combination is a combination of the three

heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 13.

[000116] In some embodiments, the activatable antibody comprises a light chain variable region that comprise a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the three light chain CDR sequences (VL CDR1, VL CDR2, VL CDR3) shown in a single row in Table 13.

[000117] In some embodiments, the activatable antibody comprises a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 13.

[000118] In some embodiments, the activatable antibody comprises a heavy chain variable region that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 13.

[000119] In some embodiments, the activatable antibody comprises a light chain variable region that comprise a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of three light chain CDR sequences (VL CDR1, VL CDR2, VL CDR3) shown in a single row in Table 13.

[000120] In some embodiments, the activatable antibody includes may include one or more polypeptides that include the combination of sequences in a given row of Table A or any combination of a mask sequence (MM), a substrate sequence (CM), a light chain variable domain sequence or light chain variable domain CDR sequences, and a heavy chain variable domain sequence or heavy chain variable domain CDR sequences of Table B.

[000121] In some embodiments, the antibody drug conjugates (ADCs) and activatable antibody drug conjugates (AADCs) can include one or more polypeptides that include the combination of a light chain sequence or a light chain variable domain sequence, and a heavy chain sequence or a heavy chain variable domain sequences, a linker, and a toxin in a given row of Table C or any combination of a light chain sequence or a light chain variable domain sequence, and a heavy chain sequence or a heavy chain variable domain sequence, a linker, and a toxin of Table C.

[000122] In some embodiments, the MM has a dissociation constant for binding to the AB which is greater than the dissociation constant of the AB to CD166.

[000123] In some embodiments, the MM has a dissociation constant for binding to the AB which is no more than the dissociation constant of the AB to CD166.

[000124] In some embodiments, the MM has a dissociation constant for binding to the AB which is less than the dissociation constant of the AB to CD166.

[000125] In some embodiments, the dissociation constant (K_d) of the MM towards the AB is no more than 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 times or greater, or between 1-5, 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times or greater than the dissociation constant of the AB towards the target.

[000126] In some embodiments, the MM does not interfere or compete with the AB for binding to CD166 when the activatable antibody is in a cleaved state.

[000127] In some embodiments, the MM is a polypeptide of about 2 to 40 amino acids in length. In some embodiments, the MM is a polypeptide of up to about 40 amino acids in length.

[000128] In some embodiments, the MM polypeptide sequence is different from that of CD166. In some embodiments, the MM polypeptide sequence is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM polypeptide sequence is different from that of CD166 and is no more than 40%, 30%, 25%, 20%, 15%, or 10% identical to any natural binding partner of the AB.

[000129] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind CD166 such that the dissociation constant (K_d) of the AB when coupled

to the MM towards CD166 is at least two times greater than the K_d of the AB when not coupled to the MM towards CD166.

[000130] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind CD166 such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD166 is at least five times greater than the K_d of the AB when not coupled to the MM towards CD166.

[000131] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind CD166 such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD166 is at least 10 times greater than the K_d of the AB when not coupled to the MM towards CD166.

[000132] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind CD166 such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD166 is at least 20 times greater than the K_d of the AB when not coupled to the MM towards CD166.

[000133] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind CD166 such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD166 is at least 40 times greater than the K_d of the AB when not coupled to the MM towards CD166.

[000134] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind CD166 such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD166 is at least 100 times greater than the K_d of the AB when not coupled to the MM towards CD166.

[000135] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind CD166 such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD166 is at least 1000 times greater than the K_d of the AB when not coupled to the MM towards CD166.

[000136] In some embodiments, the coupling of the MM to the AB reduces the ability of the AB to bind CD166 such that the dissociation constant (K_d) of the AB when coupled to the MM towards CD166 is at least 10,000 times greater than the K_d of the AB when not coupled to the MM towards CD166.

[000137] In some embodiments, in the presence of CD166, the MM reduces the ability of the AB to bind CD166 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 No. WO 2010/081173, the contents of which are hereby incorporated by reference in their entirety.

[000138] In some embodiments, MM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 135-238.

[000139] In some embodiments, the protease that cleaves the CM is active, e.g., up-regulated or otherwise unregulated, in diseased tissue, and the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease.

[000140] In some embodiments, the protease is co-localized with CD166 in a tissue, and the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease.

[000141] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD166 is reduced to occur with a dissociation constant that is at least twofold greater than the dissociation constant of an unmodified AB binding to CD166, whereas in the cleaved state (i.e., when the activatable antibody is in the cleaved state), the AB binds CD166.

[000142] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD166 is reduced to occur with a dissociation constant that is at least fivefold greater than the dissociation constant of an unmodified AB binding to CD166, whereas in the cleaved state (i.e., when the activatable antibody is in the cleaved state), the AB binds CD166.

[000143] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD166 is reduced to occur with a dissociation constant that is at least 10-fold greater than the dissociation constant of an unmodified AB binding to CD166, whereas in the cleaved state (i.e., when the activatable antibody is in the cleaved state), the AB binds CD166.

[000144] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD166 is reduced to occur with a dissociation constant that is at least 20-fold greater than the dissociation constant of an unmodified AB binding to CD166, whereas in

the cleaved state (i.e., when the activatable antibody is in the cleaved state), the AB binds CD166.

[000145] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD166 is reduced to occur with a dissociation constant that is at least 40-fold greater than the dissociation constant of an unmodified AB binding to CD166, whereas in the cleaved state, the AB binds CD166.

[000146] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD166 is reduced to occur with a dissociation constant that is at least 50-fold greater than the dissociation constant of an unmodified AB binding to CD166, whereas in the cleaved state, the AB binds CD166.

[000147] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD166 is reduced to occur with a dissociation constant that is at least 100-fold greater than the dissociation constant of an unmodified AB binding to CD166, whereas in the cleaved state, the AB binds CD166.

[000148] In some embodiments, the CM is positioned in the activatable antibody such that when the activatable antibody is in the uncleaved state, binding of the activatable antibody to CD166 is reduced to occur with a dissociation constant that is at least 200-fold greater than the dissociation constant of an unmodified AB binding to CD166, whereas in the cleaved state, the AB binds CD166.

[000149] In some embodiments, the CM is a polypeptide of up to 15 amino acids in length.

[000150] In some embodiments, the CM is a polypeptide that includes a first cleavable moiety (CM1) that is a substrate for at least one matrix metalloprotease (MMP) and a second cleavable moiety (CM2) that is a substrate for at least one serine protease (SP). In some embodiments, each of the CM1 substrate sequence and the CM2 substrate sequence of the CM1-CM2 substrate is independently a polypeptide of up to 15 amino acids in length.

[000151] In some embodiments, the CM is a substrate for at least one protease that is or is believed to be up-regulated or otherwise unregulated in cancer. In some embodiments, the CM is a substrate for at least one protease that is or is believed to be up-regulated in

inflammation. In some embodiments, the CM is a substrate for at least one protease that is or is believed to be up-regulated or otherwise unregulated in autoimmunity.

[000152] In some embodiments, the CM is a substrate for at least one protease selected from the group consisting of a matrix metalloprotease (MMP), thrombin, a neutrophil elastase, a cysteine protease, legumain, and a serine protease, such as matriptase (MT-SP1), and urokinase (uPA). Without being bound by theory, it is believed that these proteases are up-regulated or otherwise unregulated in at least one of cancer, inflammation, and/or autoimmunity.

[000153] Exemplary substrates include but are not limited to substrates cleavable by one or more of the following enzymes or proteases listed in Table 4.

[000154] In some embodiments, the CM is selected for use with a specific protease, for example a protease that is known to be co-localized with the target of the activatable antibody.

[000155] In some embodiments, the CM is a substrate for at least one MMP. Examples of MMPs include the MMPs listed in the Table 4. In some embodiments, the CM is a substrate for a protease selected from the group consisting of MMP 9, MMP14, MMP1, MMP3, MMP13, MMP17, MMP11, and MMP19. In some embodiments the CM is a substrate for MMP9. In some embodiments, the CM is a substrate for MMP14.

[000156] In some embodiments, the CM is a substrate that includes the sequence TGRGPSWV (SEQ ID NO: 18); SARGPSRW (SEQ ID NO: 19); TARGPSFK (SEQ ID NO: 20); LSGRSDNH (SEQ ID NO: 21); GGWHTGRN (SEQ ID NO: 22); HTGRSGAL (SEQ ID NO: 23); PLTGRSGG (SEQ ID NO: 24); AARGPAIH (SEQ ID NO: 25); RGPAFNPM (SEQ ID NO: 26); SSRGPAYL (SEQ ID NO: 27); RGPATPIM (SEQ ID NO: 28); RGPA (SEQ ID NO: 29); GGQPSGMWGW (SEQ ID NO: 30); FPRPLGITGL (SEQ ID NO: 31); VHMPLGFLGP (SEQ ID NO: 32); SPLTGRSG (SEQ ID NO: 33); SAGFSLPA (SEQ ID NO: 34); LAPLGLQRR (SEQ ID NO: 35); SGGPLGVR (SEQ ID NO: 36); PLGL (SEQ ID NO: 37); LSGRSGNH (SEQ ID NO: 318); SGRSANPRG (SEQ ID NO: 319); LSGRSDDH (SEQ ID NO: 320); LSGRSDIH (SEQ ID NO: 321); LSGRSDQH (SEQ ID NO: 322); LSGRSDTH (SEQ ID NO: 323); LSGRSDYH (SEQ ID NO: 324); LSGRSDNP (SEQ ID NO: 325); LSGRSANP (SEQ ID NO: 326); LSGRSANI (SEQ ID NO: 327); LSGRSDNI (SEQ ID NO: 328); MIAPVAYR (SEQ ID NO: 329); RPSPMWAY (SEQ ID NO: 330); WATPRPMR (SEQ ID NO: 331); FRLLDWQW (SEQ

ID NO: 332); ISSGL (SEQ ID NO: 333); ISSGLLS (SEQ ID NO: 334); and/or ISSGLL (SEQ ID NO: 335).

[000157] In some embodiments, the CM comprises the amino acid sequence LSGRSDNH (SEQ ID NO: 21). In some embodiments, the CM comprises the amino acid sequence TGRGPSWV (SEQ ID NO: 18). In some embodiments, the CM comprises the amino acid sequence PLTGRSGG (SEQ ID NO: 24). In some embodiments, the CM comprises the amino acid sequence GGQPSGMWGW (SEQ ID NO: 30). In some embodiments, the CM comprises the amino acid sequence FPRPLGITGL (SEQ ID NO: 31). In some embodiments, the CM comprises the amino acid sequence VHMPLGFLGP (SEQ ID NO: 32). In some embodiments, the CM comprises the amino acid sequence PLGL (SEQ ID NO: 37). In some embodiments, the CM comprises the amino acid sequence SARGPSRW (SEQ ID NO: 19). In some embodiments, the CM comprises the amino acid sequence TARGPSFK (SEQ ID NO: 20). In some embodiments, the CM comprises the amino acid sequence GGWHTGRN (SEQ ID NO: 22). In some embodiments, the CM comprises the amino acid sequence HTGRSGAL (SEQ ID NO: 23). In some embodiments, the CM comprises the amino acid sequence AARGPAIH (SEQ ID NO: 25). In some embodiments, the CM comprises the amino acid sequence RGPAFNPM (SEQ ID NO: 26). In some embodiments, the CM comprises the amino acid sequence SSRGPAYL (SEQ ID NO: 27). In some embodiments, the CM comprises the amino acid sequence RGPATPIM (SEQ ID NO: 28). In some embodiments, the CM comprises the amino acid sequence RGPA (SEQ ID NO: 29). In some embodiments, the CM comprises the amino acid sequence LSGRSGNH (SEQ ID NO: 315). In some embodiments, the CM comprises the amino acid sequence SGRSANPRG (SEQ ID NO: 319). In some embodiments, the CM comprises the amino acid sequence LSGRSDDH (SEQ ID NO: 320). In some embodiments, the CM comprises the amino acid sequence LSGRSDIH (SEQ ID NO: 321). In some embodiments, the CM comprises the amino acid sequence LSGRSDQH (SEQ ID NO: 322). In some embodiments, the CM comprises the amino acid sequence LSGRSDTH (SEQ ID NO: 323). In some embodiments, the CM comprises the amino acid sequence LSGRSDYH (SEQ ID NO: 324). In some embodiments, the CM comprises the amino acid sequence LSGRSDNP (SEQ ID NO: 325). In some embodiments, the CM comprises the amino acid sequence LSGRSANP (SEQ ID NO: 326). In some embodiments, the CM comprises the amino acid sequence LSGRSANI (SEQ ID NO: 327). In some embodiments, the CM comprises the amino acid sequence LSGRSDNI (SEQ ID NO: 328).

In some embodiments, the CM comprises the amino acid sequence MIAPVAYR (SEQ ID NO: 329). In some embodiments, the CM comprises the amino acid sequence RPSPMWAY (SEQ ID NO: 330). In some embodiments, the CM comprises the amino acid sequence WATPRPMR (SEQ ID NO: 331). In some embodiments, the CM comprises the amino acid sequence FRLLDWQW (SEQ ID NO: 332). In some embodiments, the CM comprises the amino acid sequence ISSGL (SEQ ID NO: 333). In some embodiments, the CM comprises the amino acid sequence ISSGLLS (SEQ ID NO: 334). In some embodiments, the CM comprises the amino acid sequence and/or ISSGLL (SEQ ID NO: 335).

[000158] In some embodiments, the CM is a substrate for an MMP and includes the sequence ISSGLSS (SEQ ID NO: 38); QNQALRMA (SEQ ID NO: 39); AQNLLGMV (SEQ ID NO: 40); STFPFGMF (SEQ ID NO: 41); PVGYTSSL (SEQ ID NO: 42); DWLYWPGI (SEQ ID NO: 43), ISSGLLSS (SEQ ID NO: 44), LKAAPRWA (SEQ ID NO: 45); GPSHLVLT (SEQ ID NO: 46); LPGGLSPW (SEQ ID NO: 47); MGLFSEAG (SEQ ID NO: 48); SPLPLRVP (SEQ ID NO: 49); RMHLRSLG (SEQ ID NO: 50); LAAPLGLL (SEQ ID NO: 51); AVGLLAPP (SEQ ID NO: 52); LLAPSHRA (SEQ ID NO: 53); and/or PAGLWLDP (SEQ ID NO: 54).

[000159] In some embodiments, the CM comprises the amino acid sequence ISSGLSS (SEQ ID NO: 38). In some embodiments, the CM comprises the amino acid sequence QNQALRMA (SEQ ID NO: 39). In some embodiments, the CM comprises the amino acid sequence AQNLLGMV (SEQ ID NO: 40). In some embodiments, the CM comprises the amino acid sequence STFPFGMF (SEQ ID NO: 41). In some embodiments, the CM comprises the amino acid sequence PVGYTSSL (SEQ ID NO: 42). In some embodiments, the CM comprises the amino acid sequence DWLYWPGI (SEQ ID NO: 43). In some embodiments, the CM comprises the amino acid sequence ISSGLLSS (SEQ ID NO: 44). In some embodiments, the CM comprises the amino acid sequence LKAAPRWA (SEQ ID NO: 45). In some embodiments, the CM comprises the amino acid sequence GPSHLVLT (SEQ ID NO: 46). In some embodiments, the CM comprises the amino acid sequence LPGGLSPW (SEQ ID NO: 47). In some embodiments, the CM comprises the amino acid sequence MGLFSEAG (SEQ ID NO: 48). In some embodiments, the CM comprises the amino acid sequence SPLPLRVP (SEQ ID NO: 49). In some embodiments, the CM comprises the amino acid sequence RMHLRSLG (SEQ ID NO: 50). In some embodiments, the CM comprises the amino acid sequence LAAPLGLL (SEQ ID NO: 51). In some embodiments, the CM comprises the amino acid sequence AVGLLAPP (SEQ ID NO: 52).

In some embodiments, the CM comprises the amino acid sequence LLAPSHRA (SEQ ID NO: 53). In some embodiments, the CM comprises the amino acid sequence PAGLWLDP (SEQ ID NO: 54).

[000160] In some embodiments, the CM is a substrate for thrombin. In some embodiments, the CM is a substrate for thrombin and includes the sequence GPRSFGL (SEQ ID NO: 55) or GPRSFG (SEQ ID NO: 56). In some embodiments, the CM comprises the amino acid sequence GPRSFGL (SEQ ID NO: 57). In some embodiments, the CM comprises the amino acid sequence GPRSFG (SEQ ID NO: 58).

[000161] In some embodiments, the CM comprises an amino acid sequence selected from the group consisting of NTLSGRSENHSG (SEQ ID NO: 59); NTLSGRSGNHGS (SEQ ID NO: 60); TSTSGRSANPRG (SEQ ID NO: 61); TSGRSANP (SEQ ID NO: 62); VAGRSMRP (SEQ ID NO: 63); VVPEGRRS (SEQ ID NO: 64); ILPRSPAF (SEQ ID NO: 65); MVLGRSLL (SEQ ID NO: 66); QGRAITFI (SEQ ID NO: 67); SPRSIMLA (SEQ ID NO: 68); and SMLRSMPL (SEQ ID NO: 69).

[000162] In some embodiments, the CM comprises the amino acid sequence NTLSGRSENHSG (SEQ ID NO: 59). In some embodiments, the CM comprises the amino acid sequence NTLSGRSGNHGS (SEQ ID NO: 60). In some embodiments, the CM comprises the amino acid sequence TSTSGRSANPRG (SEQ ID NO: 61). In some embodiments, the CM comprises the amino acid sequence TSGRSANP (SEQ ID NO: 62). In some embodiments, the CM comprises the amino acid sequence VAGRSMRP (SEQ ID NO: 63). In some embodiments, the CM comprises the amino acid sequence VVPEGRRS (SEQ ID NO: 64). In some embodiments, the CM comprises the amino acid sequence ILPRSPAF (SEQ ID NO: 65). In some embodiments, the CM comprises the amino acid sequence MVLGRSLL (SEQ ID NO: 66). In some embodiments, the CM comprises the amino acid sequence QGRAITFI (SEQ ID NO: 67). In some embodiments, the CM comprises the amino acid sequence SPRSIMLA (SEQ ID NO: 68). In some embodiments, the CM comprises the amino acid sequence SMLRSMPL (SEQ ID NO: 69).

[000163] In some embodiments, the CM is a substrate for a neutrophil elastase. In some embodiments, the CM is a substrate for a serine protease. In some embodiments, the CM is a substrate for uPA. In some embodiments, the CM is a substrate for legumain. In some embodiments, the CM is a substrate for matriptase. In some embodiments, the CM is a substrate for a cysteine protease. In some embodiments, the CM is a substrate for a cysteine protease, such as a cathepsin.

[000164] In some embodiments, the CM is a CM1-CM2 substrate and includes the sequence ISSGLLSGRSDNH (SEQ ID NO: 70), which is also referred to herein as substrate 2001; ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 71); AVGLLAPPGGTSTSGRSANPRG (SEQ ID NO: 72); TSTSGRSANPRGGGAVGLLAPP (SEQ ID NO: 73); VHMPLGFLGPGGTSTSGRSANPRG (SEQ ID NO: 74); TSTSGRSANPRGGGVHMPLGFLGP (SEQ ID NO: 75); AVGLLAPPGGLSGRSDNH (SEQ ID NO: 76), which is also referred to herein as substrate 3001; LSGRSDNHGGAVGLLAPP (SEQ ID NO: 77); VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 78); LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 79); LSGRSDNHGGSGGSISSGLLSS (SEQ ID NO: 80); LSGRSGNHGGSGGSISSGLLSS (SEQ ID NO: 81); ISSGLLSSGGSGGSLSGRSGNH (SEQ ID NO: 82); LSGRSDNHGGSGGSQNQALRMA (SEQ ID NO: 83); QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 84); LSGRSGNHGGSGGSQNQALRMA (SEQ ID NO: 85); QNQALRMAGGSGGSLSGRSGNH (SEQ ID NO: 86); ISSGLLSGRSGNH (SEQ ID NO: 87); GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 336); GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 337); ISSGLLSGRSANPRG (SEQ ID NO: 338), which is also referred to herein as substrate 2003; AVGLLAPPTSGRSANPRG (SEQ ID NO: 339), which is also referred to herein as substrate 2004; AVGLLAPPSGRSANPRG (SEQ ID NO: 340), which is also referred to herein as substrate 2005; ISSGLLSGRSDDH (SEQ ID NO: 341), which is also referred to herein as substrate 2006; ISSGLLSGRSDIH (SEQ ID NO: 342), which is also referred to herein as substrate 2007; ISSGLLSGRSDQH (SEQ ID NO: 343), which is also referred to herein as substrate 2008; ISSGLLSGRSDTH (SEQ ID NO: 344), which is also referred to herein as substrate 2009; ISSGLLSGRSDYH (SEQ ID NO: 345), which is also referred to herein as substrate 2010; ISSGLLSGRSDNP (SEQ ID NO: 346), which is also referred to herein as substrate 2011; ISSGLLSGRSANP (SEQ ID NO: 347), which is also referred to herein as substrate 2012; ISSGLLSGRSANI (SEQ ID NO: 348), which is also referred to herein as substrate 2013; AVGLLAPPGGLSGRSDDH (SEQ ID NO: 349), which is also referred to herein as substrate 3006; AVGLLAPPGGLSGRSDIH (SEQ ID NO: 350), which is also referred to herein as substrate 3007; AVGLLAPPGGLSGRSDQH (SEQ ID NO: 351), which is also referred to herein as substrate 3008; AVGLLAPPGGLSGRSDTH (SEQ ID NO: 352), which is also referred to herein as substrate 3009; AVGLLAPPGGLSGRSDYH (SEQ ID

NO: 353), which is also referred to herein as substrate 3010; AVGLLAPPGGLSGRSDNP (SEQ ID NO: 354), which is also referred to herein as substrate 3011; AVGLLAPPGGLSGRSANP (SEQ ID NO: 355), which is also referred to herein as substrate 3012; AVGLLAPPGGLSGRSANI (SEQ ID NO: 356), which is also referred to herein as substrate 3013; ISSGLLSGRSDNI (SEQ ID NO: 347), which is also referred to herein as substrate 2014; and/or AVGLLAPPGGLSGRSDNI (SEQ ID NO: 358), which is also referred to herein as substrate 3014.

[000165] In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDNH (SEQ ID NO: 70). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 71). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGTSTSGRSANPRG (SEQ ID NO: 72). In some embodiments, the CM1-CM2 substrate includes the sequence TSTSGRSANPRGGGAVGLLAPP (SEQ ID NO: 73). In some embodiments, the CM1-CM2 substrate includes the sequence VHMPLGFLGPGGTSTSGRSANPRG (SEQ ID NO: 74). In some embodiments, the CM1-CM2 substrate includes the sequence TSTSGRSANPRGGGVHMPLGFLGP (SEQ ID NO: 75). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDNH (SEQ ID NO: 76). In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSDNHGGAVGLLAPP (SEQ ID NO: 77). In some embodiments, the CM1-CM2 substrate includes the sequence VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 78). In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 79). In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSDNHGGSGGSISSGLLSS (SEQ ID NO: 80). In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSGNHGGSGGSISSGLLSS (SEQ ID NO: 81). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSSGGSGGSLSGRSGNH (SEQ ID NO: 82). In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSDNHGGSGGSQNQALRMA (SEQ ID NO: 83). In some embodiments, the CM1-CM2 substrate includes the sequence QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 84). In some embodiments, the CM1-CM2 substrate includes the sequence LSGRSGNHGGSGGSQNQALRMA (SEQ ID NO: 85). In some embodiments, the CM1-CM2 substrate includes the sequence QNQALRMAGGSGGSLSGRSGNH (SEQ ID NO: 86). In some embodiments, the CM1-

CM2 substrate includes the sequence ISSGLLSGRSGNH (SEQ ID NO: 87). In some embodiments, the CM1-CM2 substrate includes the sequence GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 336). In some embodiments, the CM1-CM2 substrate includes the sequence and/or GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 337). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSANPRG (SEQ ID NO: 338). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPTSGRSANPRG (SEQ ID NO: 339). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPSGRSANPRG (SEQ ID NO: 340). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDDH (SEQ ID NO: 341). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDIH (SEQ ID NO: 342). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDQH (SEQ ID NO: 343). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDTH (SEQ ID NO: 344). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDYH (SEQ ID NO: 345). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSDNP (SEQ ID NO: 346). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSANP (SEQ ID NO: 347). In some embodiments, the CM1-CM2 substrate includes the sequence ISSGLLSGRSANI (SEQ ID NO: 348). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDDH (SEQ ID NO: 349). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDIH (SEQ ID NO: 350). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDQH (SEQ ID NO: 351). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDTH (SEQ ID NO: 352). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDYH (SEQ ID NO: 353). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDNP (SEQ ID NO: 354). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSANP (SEQ ID NO: 355). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSANI (SEQ ID NO: 356), ISSGLLSGRSDNI (SEQ ID NO: 357). In some embodiments, the CM1-CM2 substrate includes the sequence AVGLLAPPGGLSGRSDNI (SEQ ID NO: 358).

[000166] In some embodiments, the CM is a substrate for at least two proteases. In some embodiments, each protease is selected from the group consisting of those shown in

Table 4. In some embodiments, the CM is a substrate for at least two proteases, wherein one of the proteases is selected from the group consisting of a MMP, thrombin, a neutrophil elastase, a cysteine protease, uPA, legumain and matriptase and the other protease is selected from the group consisting of those shown in Table 4. In some embodiments, the CM is a substrate for at least two proteases selected from the group consisting of a MMP, thrombin, a neutrophil elastase, a cysteine protease, uPA, legumain and matriptase.

[000167] In some embodiments, the activatable antibody includes at least a first CM and a second CM. In some embodiments, the first CM and the second CM are each polypeptides of no more than 15 amino acids long. In some embodiments, the first CM and the second CM in the activatable antibody in the uncleaved state have the structural arrangement from N-terminus to C-terminus as follows: MM-CM1-CM2-AB or AB-CM2-CM1-MM. In some embodiments, at least one of the first CM and the second CM is a polypeptide that functions as a substrate for a protease selected from the group consisting of a MMP, thrombin, a neutrophil elastase, a cysteine protease, uPA, legumain, and matriptase. In some embodiments, the first CM is cleaved by a first cleaving agent selected from the group consisting of a MMP, thrombin, a neutrophil elastase, a cysteine protease, uPA, legumain, and matriptase in a target tissue and the second CM is cleaved by a second cleaving agent in a target tissue. In some embodiments, the other protease is selected from the group consisting of those shown in Table 4. In some embodiments, the first cleaving agent and the second cleaving agent are the same protease selected from the group consisting of a MMP, thrombin, a neutrophil elastase, a cysteine protease, uPA, legumain, and matriptase, and the first CM and the second CM are different substrates for the enzyme. In some embodiments, the first cleaving agent and the second cleaving agent are the same protease selected from the group consisting of those shown in Table 4. In some embodiments, the first cleaving agent and the second cleaving agent are different proteases. In some embodiments, the first cleaving agent and the second cleaving agent are co-localized in the target tissue. In some embodiments, the first CM and the second CM are cleaved by at least one cleaving agent in the target tissue.

[000168] In some embodiments, the activatable antibody is exposed to and cleaved by a protease such that, in the activated or cleaved state, the activated antibody includes a light chain amino acid sequence that includes at least a portion of LP2 and/or CM sequence after the protease has cleaved the CM.

[000169] Suitable activatable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that binds to the same epitope on human CD166 and/or cynomolgus monkey CD166 as an anti-CD166 antibody comprising a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[000170] Suitable activatable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that binds to the same epitope on human CD166 and/or cynomolgus monkey CD166 as an anti-CD166 antibody comprising the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[000171] Suitable activatable anti-CD166 antibodies of the disclosure also include an antibody or antigen-binding fragment thereof that binds to the same epitope on human CD166 and/or cynomolgus monkey CD166 as an anti-CD166 antibody comprising a heavy chain variable region amino acid sequence selected from the group consisting of the heavy chain variable region sequences shown in Table 12 and a light chain variable region amino acid sequence selected from the group consisting of the light chain variable region sequences shown in Table 12.

[000172] Suitable activatable anti-CD166 antibodies of the disclosure also include an antibody or antigen-binding fragment thereof that binds to the same epitope on human CD166 and/or cynomolgus monkey CD166 as an anti-CD166 antibody comprising a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 13.

[000173] Suitable activatable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that cross-competes for binding to human

CD166 and/or cynomolgus monkey CD166 to an anti-CD166 antibody comprising a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[000174] Suitable activatable anti-CD166 antibodies of the disclosure also include an antibody or antigen binding fragment thereof that cross-competes for binding to human CD166 and/or cynomolgus monkey CD166 to an anti-CD166 antibody comprising the VH CDR1 sequence comprises the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 sequence comprises the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 sequence comprises the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 sequence comprises the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 sequence comprises the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 sequence comprises the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[000175] Suitable activatable anti-CD166 antibodies of the disclosure also include an antibody or antigen-binding fragment thereof that cross-competes for binding to human CD166 and/or cynomolgus monkey CD166 as an anti-CD166 antibody comprising a heavy chain variable region amino acid sequence selected from the group consisting of the heavy chain variable region sequences shown in Table 12 and a light chain variable region amino acid sequence selected from the group consisting of the light chain variable region sequences shown in Table 12.

[000176] Suitable activatable anti-CD166 antibodies of the disclosure also include an antibody or antigen-binding fragment thereof that cross-competes for binding to human CD166 and/or cynomolgus monkey CD166 as an anti-CD166 antibody comprising a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 13.

[000177] In some embodiments, the activatable antibody also includes an agent conjugated to the AB. In some embodiments, the agent conjugated to the AB or the AB of an activatable antibody 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 CM1-CM2 substrate sequence. In some embodiments, the agent is conjugated to the AB via a noncleavable linker. In some embodiments, the agent is conjugated to the AB via a linker that is cleavable in an intracellular or lysosomal environment. 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, a DNA cleaving agent, a DNA cross-linker, a DNA intercalator, or other DNA damaging agent. In some embodiments, the agent is an agent selected from the group listed in Table 5. 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. In some embodiments, the agent is a pyrrolobenzodiazepine dimer.

[000178] In some embodiments, the activatable antibody is conjugated to one or more equivalents of an agent. In some embodiments, the activatable antibody is conjugated to one equivalent of the agent. In some embodiments, the activatable antibody is conjugated to two, three, four, five, six, seven, eight, nine, ten, or greater than ten equivalents of the agent. In some embodiments, the activatable antibody is part of a mixture of activatable antibodies having a homogeneous number of equivalents of conjugated agents. In some embodiments, the activatable antibody is part of a mixture of activatable antibodies having a heterogeneous number of equivalents of conjugated agents. In some embodiments, the mixture of activatable antibodies is such that the average number of agents conjugated to each activatable antibody is between zero to one, between one to two, between two and three, between three and four, between four and five, between five and six, between six and seven, between seven and eight, between eight and nine, between nine and ten, and ten and greater. In some embodiments, the mixture of activatable antibodies is such that the average number of agents conjugated to each activatable antibody is one, two, three, four, five, six, seven, eight, nine, ten, or greater. In some embodiments, the activatable antibody comprises

one or more site-specific amino acid sequence modifications such that the number of lysine and/or cysteine residues is increased or decreased with respect to the original amino acid sequence of the activatable antibody, thus in some embodiments correspondingly increasing or decreasing the number of agents that can be conjugated to the activatable antibody, or in some embodiments limiting the conjugation of the agents to the activatable antibody in a site-specific manner. In some embodiments, the modified activatable antibody is modified with one or more non-natural amino acids in a site-specific manner, thus in some embodiments limiting the conjugation of the agents to only the sites of the non-natural amino acids.

[000179] In some embodiments, the agent is an anti-inflammatory agent.

[000180] In some embodiments, the activatable antibody also includes a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent.

[000181] In some embodiments, the activatable antibody also includes a signal peptide. In some embodiments, the signal peptide is conjugated to the activatable antibody via a spacer. In some embodiments, the spacer is conjugated to the activatable antibody in the absence of a signal peptide. In some embodiments, the spacer is joined directly to the MM of the activatable antibody. In some embodiments, the spacer is joined directly to the MM of the activatable antibody in the structural arrangement from N-terminus to C-terminus of spacer-MM-CM-AB. An example of a spacer joined directly to the N-terminus of MM of the activatable antibody is QGQSGQ (SEQ ID NO: 88). Other examples of a spacer joined directly to the N-terminus of MM of the activatable antibody include QGQSGQG (SEQ ID NO: 305), QGQSG (SEQ ID NO: 306), QGQS (SEQ ID NO: 307), QGQ (SEQ ID NO: 308), QG (SEQ ID NO: 309), and Q. Other examples of a spacer joined directly to the N-terminus of MM of the activatable antibody include GQSGQG (SEQ ID NO: 359), QSGQG (SEQ ID NO: 360), SGQG (SEQ ID NO: 361), GQG (SEQ ID NO: 362), and G. In some embodiments, no spacer is joined to the N-terminus of the MM. In some embodiments, the spacer includes at least the amino acid sequence QGQSGQ (SEQ ID NO: 88). In some embodiments, the spacer includes at least the amino acid sequence QGQSGQG (SEQ ID NO: 305). In some embodiments, the spacer includes at least the amino acid sequence QGQSG (SEQ ID NO: 306). In some embodiments, the spacer includes at least the amino acid sequence QGQS (SEQ ID NO: 307). In some embodiments, the spacer includes at least the amino acid sequence QGQ (SEQ ID NO: 308). In some embodiments, the spacer includes at least the amino acid sequence QG (SEQ ID NO: 309).

In some embodiments, the spacer includes at least the amino acid residue Q. In some embodiments, the spacer includes at least the amino acid sequence GQSGQG (SEQ ID NO: 359). In some embodiments, the spacer includes at least the amino acid sequence QSGQG (SEQ ID NO: 360). In some embodiments, the spacer includes at least the amino acid sequence SGQG (SEQ ID NO: 361). In some embodiments, the spacer includes at least the amino acid sequence GQG (SEQ ID NO: 362). In some embodiments, the spacer includes at least the amino acid sequence G. In some embodiments, the spacer is absent.

[000182] In some embodiments, the AB of the activatable antibody naturally contains one or more disulfide bonds. In some embodiments, the AB can be engineered to include one or more disulfide bonds.

[000183] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122.

[000184] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123-126.

[000185] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[000186] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 121, and 122, and a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123-126.

[000187] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain variable region amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain variable region amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122.

[000188] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123-126.

[000189] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 119, 121, and 122, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 120 and 123-126.

[000190] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a heavy chain variable region amino acid sequence comprising SEQ ID NO: 121 or SEQ ID NO: 122, and a nucleic acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of SEQ ID NO: 123-126.

[000191] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region amino acid sequence selected from the group consisting of the heavy chain variable region sequences shown in Table 12. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of the light chain variable region sequences shown in Table 12. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region amino acid sequence selected from the group consisting of the heavy chain variable region sequences shown in Table 12 and a nucleic acid sequence encoding a light chain variable region amino acid sequence selected from the group consisting of the light chain variable region sequences shown in Table 12.

[000192] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region 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 the heavy chain variable region sequences shown in Table 12. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain variable region 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 the light chain variable region sequences shown in Table 12. In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region 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 the heavy chain variable region sequences shown in Table 12 and a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain variable region 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 the light chain variable region sequences shown in Table 12.

[000193] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as

CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence shown in Table 13; a VH CDR2 sequence shown in Table 13; a VH CDR3 sequence shown in Table 13; a VL CDR1 sequence shown in Table 13; a VL CDR2 sequence shown in Table 13; and a VL CDR3 sequence shown in Table 13.

[000194] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR1 sequence shown in Table 13; a VH CD2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR2 sequence shown in Table 13; a VH CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VH CDR3 sequence shown in Table 13; a VL CDR1 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR1 sequence shown in Table 13; a VL CDR2 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR2 sequence shown in Table 13; and a VL CDR3 sequence that includes a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to a VL CDR3 sequence shown in Table 13.

[000195] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the six

CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 13.

[000196] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain variable region that comprise a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein the combination is a combination of the three light chain CDR sequences (VL CDR1, VL CDR2, VL CDR3) shown in a single row in Table 13.

[000197] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein the combination is a combination of the three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 13.

[000198] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a combination of a VH CDR1 sequence, a VH CDR2 sequence, a VH CDR3 sequence, a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of the six CDR sequences (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) shown in a single row in Table 13.

[000199] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a heavy chain variable region that comprise a combination of a VH CDR1 sequence, a VH CDR2 sequence, and a VH CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the corresponding CDR sequence in a combination of three heavy chain CDR sequences (VH CDR1, VH CDR2, VH CDR3) shown in a single row in Table 13.

[000200] In some embodiments, the activatable antibody is encoded by a nucleic acid sequence that comprises a nucleic acid sequence encoding a light chain variable region that comprise a combination of a VL CDR1 sequence, a VL CDR2 sequence, and a VL CDR3 sequence, wherein each CDR sequence in the combination comprises a sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the

corresponding CDR sequence in a combination of three light chain CDR sequences (VL CDR1, VL CDR2, VL CDR3) shown in a single row in Table 13.

[000201] In some embodiments, the activatable antibody includes one or more polypeptides that include the combination of sequences in a given row of Table A or any combination of a mask sequence (MM), a substrate sequence (CM), a light chain variable domain sequence or light chain variable domain CDR sequences, and a heavy chain variable domain sequence or heavy chain variable domain CDR sequences of Table B.

Table A: Anti-CD166 Activatable Antibody Combinations

Comb. No.	Mask Sequence (MM)	Substrate Sequence (CM)	VL CDRs SEQ ID NOs	VH CDRs SEQ ID NOs
1	LCHPLVLSAWESCSS (SEQ ID NO: 219)	LSGRSDNH (SEQ ID NO: 21)	130, 132, 134	127, 128, 129
2	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSS (SEQ ID NO: 44)	130, 132, 134	127, 128, 129
3	LCHPLVLSAWESCSS (SEQ ID NO: 219)	LSGRSGNH (SEQ ID NO: 318)	130, 132, 134	127, 128, 129
4	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPP (SEQ ID NO: 52)	130, 132, 134	127, 128, 129
5	LCHPLVLSAWESCSS (SEQ ID NO: 219)	VHMPLGFLGP (SEQ ID NO: 32)	130, 132, 134	127, 128, 129
6	LCHPLVLSAWESCSS (SEQ ID NO: 219)	TSTSGRSANPRG (SEQ ID NO: 61)	130, 132, 134	127, 128, 129
7	LCHPLVLSAWESCSS (SEQ ID NO: 219)	QNQALRMA (SEQ ID NO: 39)	130, 132, 134	127, 128, 129
8	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSDNH (SEQ ID NO: 70)	130, 132, 134	127, 128, 129
9	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSGNH (SEQ ID NO: 87)	130, 132, 134	127, 128, 129
10	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSANPRG (SEQ ID NO: 338)	130, 132, 134	127, 128, 129
11	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPTSGRSANPRG (SEQ ID NO: 339)	130, 132, 134	127, 128, 129
12	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPSGRSANPRG (SEQ ID NO: 340)	130, 132, 134	127, 128, 129
13	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSDDH (SEQ ID NO: 341)	130, 132, 134	127, 128, 129
14	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSDIH (SEQ ID NO: 342)	130, 132, 134	127, 128, 129
15	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSDQH (SEQ ID NO: 343)	130, 132, 134	127, 128, 129
16	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSDTH (SEQ ID NO: 344)	130, 132, 134	127, 128, 129
17	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSDYH (SEQ ID NO: 345)	130, 132, 134	127, 128, 129

18	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSDNP (SEQ ID NO: 346)	130, 132, 134	127, 128, 129
19	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSANP (SEQ ID NO: 347)	130, 132, 134	127, 128, 129
20	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSANI (SEQ ID NO: 348)	130, 132, 134	127, 128, 129
21	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSGRSDNI (SEQ ID NO: 357)	130, 132, 134	127, 128, 129
22	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSDNH (SEQ ID NO: 76)	130, 132, 134	127, 128, 129
23	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSDDH (SEQ ID NO: 349)	130, 132, 134	127, 128, 129
24	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSDIH (SEQ ID NO: 350)	130, 132, 134	127, 128, 129
25	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSDQH (SEQ ID NO: 351)	130, 132, 134	127, 128, 129
26	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSDTH (SEQ ID NO: 352)	130, 132, 134	127, 128, 129
27	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSDYH (SEQ ID NO: 353)	130, 132, 134	127, 128, 129
28	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSDNP (SEQ ID NO: 354)	130, 132, 134	127, 128, 129
29	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSANP (SEQ ID NO: 355)	130, 132, 134	127, 128, 129
30	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSANI (SEQ ID NO: 356)	130, 132, 134	127, 128, 129
31	LCHPLVLSAWESCSS (SEQ ID NO: 219)	AVGLLAPPGGLSGRSDNI (SEQ ID NO: 358)	130, 132, 134	127, 128, 129
32	LCHPLVLSAWESCSS (SEQ ID NO: 219)	ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 71)	130, 132, 134	127, 128, 129
33	LCHPAVLSAWESCSS (SEQ ID NO: 222)	LSGRSDNH (SEQ ID NO: 21)	130, 132, 134	127, 128, 129
34	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSS (SEQ ID NO: 44)	130, 132, 134	127, 128, 129
35	LCHPAVLSAWESCSS (SEQ ID NO: 222)	LSGRSGNH (SEQ ID NO: 318)	130, 132, 134	127, 128, 129
36	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPP (SEQ ID NO: 52)	130, 132, 134	127, 128, 129
37	LCHPAVLSAWESCSS (SEQ ID NO: 222)	VHMPLGFLGP (SEQ ID NO: 32)	130, 132, 134	127, 128, 129
38	LCHPAVLSAWESCSS (SEQ ID NO: 222)	TSTSGRSANPRG (SEQ ID NO: 61)	130, 132, 134	127, 128, 129
39	LCHPAVLSAWESCSS (SEQ ID NO: 222)	QNQALRMA (SEQ ID NO: 39)	130, 132, 134	127, 128, 129
40	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSDNH (SEQ ID NO: 70)	130, 132, 134	127, 128, 129
41	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSGNH (SEQ ID NO: 87)	130, 132, 134	127, 128, 129
42	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSANPRG (SEQ ID NO: 338)	130, 132, 134	127, 128, 129
43	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPTSGRSANPRG (SEQ ID NO: 339)	130, 132, 134	127, 128, 129

44	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPSGRSANPRG (SEQ ID NO: 340)	130, 132, 134	127, 128, 129
45	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSDDH (SEQ ID NO: 341)	130, 132, 134	127, 128, 129
46	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSDIH (SEQ ID NO: 342)	130, 132, 134	127, 128, 129
47	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSDQH (SEQ ID NO: 343)	130, 132, 134	127, 128, 129
48	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSDTH (SEQ ID NO: 344)	130, 132, 134	127, 128, 129
49	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSDYH (SEQ ID NO: 345)	130, 132, 134	127, 128, 129
50	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSDNP (SEQ ID NO: 346)	130, 132, 134	127, 128, 129
51	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSANP (SEQ ID NO: 347)	130, 132, 134	127, 128, 129
52	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSANI (SEQ ID NO: 348)	130, 132, 134	127, 128, 129
53	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSGRSDNI (SEQ ID NO: 357)	130, 132, 134	127, 128, 129
54	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSDNH (SEQ ID NO: 76)	130, 132, 134	127, 128, 129
55	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSDDH (SEQ ID NO: 349)	130, 132, 134	127, 128, 129
56	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSDIH (SEQ ID NO: 350)	130, 132, 134	127, 128, 129
57	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSDQH (SEQ ID NO: 351)	130, 132, 134	127, 128, 129
58	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSDTH (SEQ ID NO: 352)	130, 132, 134	127, 128, 129
59	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSDYH (SEQ ID NO: 353)	130, 132, 134	127, 128, 129
60	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSDNP (SEQ ID NO: 354)	130, 132, 134	127, 128, 129
61	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSANP (SEQ ID NO: 355)	130, 132, 134	127, 128, 129
62	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSANI (SEQ ID NO: 356)	130, 132, 134	127, 128, 129
63	LCHPAVLSAWESCSS (SEQ ID NO: 222)	AVGLLAPPGGLSGRSDNI (SEQ ID NO: 358)	130, 132, 134	127, 128, 129
64	LCHPAVLSAWESCSS (SEQ ID NO: 222)	ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 71)	130, 132, 134	127, 128, 129
65	LCHPLVASAWESCSS (SEQ ID NO: 224)	LSGRSDNH (SEQ ID NO: 21)	130, 132, 134	127, 128, 129
66	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSS (SEQ ID NO: 44)	130, 132, 134	127, 128, 129
67	LCHPLVASAWESCSS (SEQ ID NO: 224)	LSGRSGNH (SEQ ID NO: 318)	130, 132, 134	127, 128, 129
68	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPP (SEQ ID NO: 52)	130, 132, 134	127, 128, 129
69	LCHPLVASAWESCSS (SEQ ID NO: 224)	VHMPLGFLGP (SEQ ID NO: 32)	130, 132, 134	127, 128, 129

70	LCHPLVASAWESCSS (SEQ ID NO: 224)	TSTSGRSANPRG (SEQ ID NO: 61)	130, 132, 134	127, 128, 129
71	LCHPLVASAWESCSS (SEQ ID NO: 224)	QNQALRMA (SEQ ID NO: 39)	130, 132, 134	127, 128, 129
72	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSDNH (SEQ ID NO: 70)	130, 132, 134	127, 128, 129
73	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSGNH (SEQ ID NO: 87)	130, 132, 134	127, 128, 129
74	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSANPRG (SEQ ID NO: 338)	130, 132, 134	127, 128, 129
75	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPTSGRSANPRG (SEQ ID NO: 339)	130, 132, 134	127, 128, 129
76	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPSGRSANPRG (SEQ ID NO: 340)	130, 132, 134	127, 128, 129
77	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSDDH (SEQ ID NO: 341)	130, 132, 134	127, 128, 129
78	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSDIH (SEQ ID NO: 342)	130, 132, 134	127, 128, 129
79	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSDQH (SEQ ID NO: 343)	130, 132, 134	127, 128, 129
80	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSDTH (SEQ ID NO: 344)	130, 132, 134	127, 128, 129
81	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSDYH (SEQ ID NO: 345)	130, 132, 134	127, 128, 129
82	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSDNP (SEQ ID NO: 346)	130, 132, 134	127, 128, 129
83	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSANP (SEQ ID NO: 347)	130, 132, 134	127, 128, 129
84	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSANI (SEQ ID NO: 348)	130, 132, 134	127, 128, 129
85	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSGRSDNI (SEQ ID NO: 357)	130, 132, 134	127, 128, 129
86	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSDNH (SEQ ID NO: 76)	130, 132, 134	127, 128, 129
87	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSDDH (SEQ ID NO: 349)	130, 132, 134	127, 128, 129
88	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSDIH (SEQ ID NO: 350)	130, 132, 134	127, 128, 129
89	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSDQH (SEQ ID NO: 351)	130, 132, 134	127, 128, 129
90	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSDTH (SEQ ID NO: 352)	130, 132, 134	127, 128, 129
91	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSDYH (SEQ ID NO: 353)	130, 132, 134	127, 128, 129
92	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSDNP (SEQ ID NO: 354)	130, 132, 134	127, 128, 129
93	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSANP (SEQ ID NO: 355)	130, 132, 134	127, 128, 129
94	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSANI (SEQ ID NO: 356)	130, 132, 134	127, 128, 129
95	LCHPLVASAWESCSS (SEQ ID NO: 224)	AVGLLAPPGGLSGRSDNI (SEQ ID NO: 358)	130, 132, 134	127, 128, 129

96	LCHPLVASAWESCSS (SEQ ID NO: 224)	ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 71)	130, 132, 134	127, 128, 129
97	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	LSGRSDNH (SEQ ID NO: 21)	130, 132, 134	127, 128, 129
98	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSS (SEQ ID NO: 44)	130, 132, 134	127, 128, 129
99	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	LSGRSGNH (SEQ ID NO: 318)	130, 132, 134	127, 128, 129
100	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPP (SEQ ID NO: 52)	130, 132, 134	127, 128, 129
101	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	VHMPLGFLGP (SEQ ID NO: 32)	130, 132, 134	127, 128, 129
102	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	TSTSGRSANPRG (SEQ ID NO: 61)	130, 132, 134	127, 128, 129
103	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	QNQALRMA (SEQ ID NO: 39)	130, 132, 134	127, 128, 129
104	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSDNH (SEQ ID NO: 70)	130, 132, 134	127, 128, 129
105	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSGNH (SEQ ID NO: 87)	130, 132, 134	127, 128, 129
106	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSANPRG (SEQ ID NO: 338)	130, 132, 134	127, 128, 129
107	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPTSGRSANPRG (SEQ ID NO: 339)	130, 132, 134	127, 128, 129
108	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPSGRSANPRG (SEQ ID NO: 340)	130, 132, 134	127, 128, 129
109	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSDDH (SEQ ID NO: 341)	130, 132, 134	127, 128, 129
110	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSDIH (SEQ ID NO: 342)	130, 132, 134	127, 128, 129
111	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSDQH (SEQ ID NO: 343)	130, 132, 134	127, 128, 129
112	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSDTH (SEQ ID NO: 344)	130, 132, 134	127, 128, 129
113	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSDYH (SEQ ID NO: 345)	130, 132, 134	127, 128, 129
114	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSDNP (SEQ ID NO: 346)	130, 132, 134	127, 128, 129
115	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSANP (SEQ ID NO: 347)	130, 132, 134	127, 128, 129
116	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSANI (SEQ ID NO: 348)	130, 132, 134	127, 128, 129
117	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSGRSDNI (SEQ ID NO: 357)	130, 132, 134	127, 128, 129
118	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSDNH (SEQ ID NO: 76)	130, 132, 134	127, 128, 129
119	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSDDH (SEQ ID NO: 349)	130, 132, 134	127, 128, 129
120	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSDIH (SEQ ID NO: 350)	130, 132, 134	127, 128, 129
121	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSDQH (SEQ ID NO: 351)	130, 132, 134	127, 128, 129

122	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSDTH (SEQ ID NO: 352)	130, 132, 134	127, 128, 129
123	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSDYH (SEQ ID NO: 353)	130, 132, 134	127, 128, 129
124	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSDNP (SEQ ID NO: 354)	130, 132, 134	127, 128, 129
125	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSANP (SEQ ID NO: 355)	130, 132, 134	127, 128, 129
126	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSANI (SEQ ID NO: 356)	130, 132, 134	127, 128, 129
127	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AVGLLAPPGGLSGRSDNI (SEQ ID NO: 358)	130, 132, 134	127, 128, 129
128	LEGWCLHPLCLWGAG (SEQ ID NO: 230)	ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 71)	130, 132, 134	127, 128, 129

Table B: Anti-CD166 Activatable Antibody Components

Mask Sequence (MM)	Substrate Sequence (CM)	VL or VL CDRs	VH or VH CDRs
LCHPLVLSAWESCSS (SEQ ID NO: 219)	LSGRSDNH (SEQ ID NO: 21)	SEQ ID NOS: 130, 132, 134	SEQ ID NOS: 127, 128, 129
LCAPLVLSAWESCSS (SEQ ID NO: 220)	TGRGPSWV (SEQ ID NO: 18)	SEQ ID NO: 123	SEQ ID NO: 121
LCHALVLSAWESCSS (SEQ ID NO: 221)	PLTGRSGG (SEQ ID NO: 24)	SEQ ID NO: 124	SEQ ID NO: 122
LCHPAVLSAWESCSS (SEQ ID NO: 222)	TARGPSFK (SEQ ID NO: 20)	SEQ ID NO: 125	
LCHPLALSAWESCSS (SEQ ID NO: 223)	NTLSGRSENHSG (SEQ ID NO: 59)	SEQ ID NO: 126	
LCHPLVASAWESCSS (SEQ ID NO: 224)	NTLSGRSGNHGS (SEQ ID NO: 60)	SEQ ID NOS: 131, 133, 134	
LCHPLVLSAAESCSS (SEQ ID NO: 225)	TSTSGRSANPRG (SEQ ID NO: 61)		
LCHPLVLSAWASCSS (SEQ ID NO: 227)	TSGRSANP (SEQ ID NO: 62)		
HPLVL (SEQ ID NO: 228)	VHMPLGFLGP (SEQ ID NO: 32)		
HPL (SEQ ID NO: 229)	AVGLLAPP (SEQ ID NO: 52)		
LEGWCLHPLCLWGAG (SEQ ID NO: 230)	AQNLLGMV (SEQ ID NO: 40)		
LEGACLHPLCLWGAG (SEQ ID NO: 231)	QNQALRMA (SEQ ID NO: 39)		
LEGWCAHPLCLWGAG (SEQ ID NO: 232)	LAAPLGLL (SEQ ID NO: 51)		
LEGWCLAPLCLWGAG (SEQ ID NO: 233)	STFPFGMF (SEQ ID NO: 41)		
LEGWCLHACLWGAG (SEQ ID NO: 234)	ISSGLLSS (SEQ ID NO: 44)		

LEGWCLHPACLWGAG (SEQ ID NO: 235)	PAGLWLDP (SEQ ID NO: 54)		
LEGWCLHPLCAWGAG (SEQ ID NO: 236)	VAGRSMRP (SEQ ID NO: 63)		
LEGWCLHPLCLAGAG (SEQ ID NO: 237)	VVPEGRRS (SEQ ID NO: 64)		
CLHPLC (SEQ ID NO: 238)	ILPRSPAF (SEQ ID NO: 65)		
	MVLGRSLL (SEQ ID NO: 66)		
	QGRAITFI (SEQ ID NO: 67)		
	SPRSIMLA (SEQ ID NO: 68)		
	SMLRSMPL (SEQ ID NO: 69)		
	ISSGLLSGRSDNH (SEQ ID NO: 70)		
	AVGLLAPPGGLSGRSDNH (SEQ ID NO: 76)		
	ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 71)		
	LSGRSGNH (SEQ ID NO: 318)		
	SGRSANPRG (SEQ ID NO: 319)		
	LSGRSDDH (SEQ ID NO: 320)		
	LSGRSDIH (SEQ ID NO: 321)		
	LSGRSDQH (SEQ ID NO: 322)		
	LSGRSDTH (SEQ ID NO: 323)		
	LSGRSDYH (SEQ ID NO: 324)		
	LSGRSDNP (SEQ ID NO: 325)		
	LSGRSANP (SEQ ID NO: 326)		
	LSGRSANI (SEQ ID NO: 327)		
	LSGRSDNI (SEQ ID NO: 328)		
	MIAPVAYR (SEQ ID NO: 329)		
	RPSPMWAY (SEQ ID NO: 330)		
	WATPRPMR (SEQ ID NO: 331)		
	FRLLDWQW (SEQ ID NO: 332)		

	ISSGL (SEQ ID NO: 333)		
	ISSGLLS (SEQ ID NO: 334)		
	ISSGLL (SEQ ID NO: 335)		
	ISSGLLSGRSANPRG (SEQ ID NO: 338)		
	AVGLLAPPTSGRSANPRG (SEQ ID NO: 339)		
	AVGLLAPPSGRSANPRG (SEQ ID NO: 340)		
	ISSGLLSGRSDDH (SEQ ID NO: 341)		
	ISSGLLSGRSDIH (SEQ ID NO: 342)		
	ISSGLLSGRSDQH (SEQ ID NO: 343)		
	ISSGLLSGRSDTH (SEQ ID NO: 344)		
	ISSGLLSGRSDYH (SEQ ID NO: 345)		
	ISSGLLSGRSDNP (SEQ ID NO: 346)		
	ISSGLLSGRSANP (SEQ ID NO: 347)		
	ISSGLLSGRSANI (SEQ ID NO: 348)		
	AVGLLAPPGGLSGRSDDH (SEQ ID NO: 349)		
	AVGLLAPPGGLSGRSDIH (SEQ ID NO: 350)		
	AVGLLAPPGGLSGRSDQH (SEQ ID NO: 351)		
	AVGLLAPPGGLSGRSDTH (SEQ ID NO: 352)		
	AVGLLAPPGGLSGRSDYH (SEQ ID NO: 353)		
	AVGLLAPPGGLSGRSDNP (SEQ ID NO: 354)		
	AVGLLAPPGGLSGRSANP (SEQ ID NO: 355)		
	AVGLLAPPGGLSGRSANI (SEQ ID NO: 356)		
	ISSGLLSGRSDNI (SEQ ID NO: 357)		
	AVGLLAPPGGLSGRSDNI (SEQ ID NO: 358)		
	GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 336)		
	GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 337)		

[000202] In some embodiments, an activatable antibody of the present disclosure includes one or more polypeptides that include the combination of sequences selected from Table A or Table B, where the polypeptide includes a combination of a masking sequence selected from the column titled "Mask Sequence (MM)" of Table A or Table B, a substrate sequence from the column titled "Substrate Sequence (CM)" of Table A or Table B, a light chain variable domain or light chain CDRs from the column titled "VL or VL CDRs" or "VL CDRs SEQ ID NOs" of Table A or Table B, and a heavy chain variable domain or heavy chain CDRs from the column titled "VH or VH CDRs" or "VH CDRs SEQ ID Nos" of Table A or Table B. For example, an activatable antibody of the present disclosure includes the amino acid sequences of combination no. 54, which includes the masking sequence of SEQ ID NO: 222, the substrate sequence of SEQ ID NO: 76, a light chain variable domain that includes the VL CDR sequences of SEQ ID NOS: 130, 132, and 134, and a heavy chain variable domain that includes the VH CDR sequences of 127, 128, and 129. Therefore, an activatable antibody that includes at least the combination of sequences in any given row of Table A is described herein. Similarly, any combination of a mask sequence (MM), a substrate sequence (CM), a light chain variable domain sequence or light chain variable domain CDR sequences, and a heavy chain variable domain sequence or heavy chain variable domain CDR sequences of Table B is described herein. An activatable antibody that includes at least any combination of a masking sequence, a substrate sequence, a variable heavy chain or variable heavy chain CDRs, and a variable light chain or variable light chain CDRs selected from the corresponding columns Table A or Table B is also described herein. In some exemplary embodiments, an activatable antibody that includes at least the combination of sequences in any given row of Table A or any combination of a mask sequence (MM), a substrate sequence (CM), a light chain variable domain sequence or light chain variable domain CDR sequences, and a heavy chain variable domain sequence or heavy chain variable domain CDR sequences of Table B can be combined with one or more toxins, including a dolastatin or a derivative thereof, an auristatin or a derivative thereof, a maytansinoid or a derivative thereof, a duocarmycin or a derivative thereof, a calicheamicin or a derivative thereof, or a pyrrolobenzodiazepine or a derivative thereof. In some exemplary embodiments, an activatable antibody that includes at least the combination of sequences in any given row of Table A or any combination of a mask sequence (MM), a substrate sequence (CM), a light chain variable domain sequence or light chain variable domain CDR sequences, and a heavy chain variable domain sequence or

heavy chain variable domain CDR sequences of Table B can be combined with one or more toxins, including auristatin E, monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE), monomethyl auristatin D (MMAD), maytansinoid DM4, maytansinoid DM1, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, and/or a duocarmycin.

[000203] Any of the combinations in Table A or Table B as described above can be combined with human immunoglobulin constant regions to result in fully human IgGs including IgG1, IgG2, IgG4 or mutated constant regions to result in human IgGs with altered functions such as IgG1 N297A, IgG1 N297Q, or IgG4 S228P. The combinations described in Table A or Table B are not limited by the particular combinations shown in any given row, and thus may include any mask sequence from column 2 of Table A (or column 1 of Table B) combined with any substrate sequence from column 3 of Table A (or column 2 of Table B) combined with any VL sequence or set of VL CDR sequences from column 4 of Table A (or column 3 or Table B) combined with any VH sequence or set of VH CDR sequences from column 5 of Table A (or column 4 of Table B). In addition to the mask sequences disclosed in column 2 of Table A or column 1 of Table B, any mask sequence disclosed herein can be used in a combination. In addition to the substrate sequences disclosed in column 3 of Table A or column 2 of Table B, any CM disclosed herein can be used in a combination. In addition to the light chain variable region sequence or light chain CDR sequences disclosed in column 4 of Table A or column 3 of Table B, any light chain variable region sequence or light chain CDR sequences disclosed herein can be used in a combination. In addition to the heavy chain variable region sequence or heavy chain CDR sequences disclosed in column 5 of Table A or column 4 of Table B, any heavy chain variable region sequence or heavy chain CDR sequences disclosed herein can be used in a combination.

[000204] In some embodiments, the antibody drug conjugates (ADCs) and activatable antibody drug conjugates (AADCs) can include one or more polypeptides that include the combination of a light chain sequence or a light chain variable domain sequence, and a heavy chain sequence or a heavy chain variable domain sequences, a linker, and a toxin in a given row of Table C or any combination of a light chain sequence or a light chain variable domain sequence, and a heavy chain sequence or a heavy chain variable domain sequence, a linker, and a toxin of Table C.

Table C: Anti-CD166 ADC and Anti-CD166 Activatable ADC Combinations

Comb. No.	Heavy Chain (HC) or HC Variable Region SEQ ID NO.	Light Chain (LC) or LC Variable Region SEQ ID NO.	Linker	Toxin
1	122	123	vc	MMAD
2	122	123	PEG2-vc	MMAD
3	122	123	vc	MMAE
4	122	123	vc	duocarmycin
5	122	123	spdb	DM4
6	239	240	vc	MMAD
7	239	240	PEG2-vc	MMAD
8	239	240	vc	MMAE
9	239	240	vc	duocarmycin
10	239	240	spdb	DM4
11	239	242	vc	MMAD
12	239	242	PEG2-vc	MMAD
13	239	242	vc	MMAE
14	239	242	vc	duocarmycin
15	239	242	spdb	DM4
16	239	310	vc	MMAD
17	239	310	PEG2-vc	MMAD
18	239	310	vc	MMAE
19	239	310	vc	duocarmycin
20	239	310	spdb	DM4
21	122	363	vc	MMAD
22	122	363	PEG2-vc	MMAD
23	122	363	vc	MMAE
24	122	363	vc	duocarmycin
25	122	363	spdb	DM4
26	122	364	vc	MMAD
27	122	364	PEG2-vc	MMAD
28	122	364	vc	MMAE
29	122	364	vc	duocarmycin
30	122	364	spdb	DM4
31	239	244	vc	MMAD
32	239	244	PEG2-vc	MMAD
33	239	244	vc	MMAE
34	239	244	vc	duocarmycin
35	239	244	spdb	DM4
36	239	312	vc	MMAD
37	239	312	PEG2-vc	MMAD
38	239	312	vc	MMAE
39	239	312	vc	duocarmycin
40	239	312	spdb	DM4
41	122	365	vc	MMAD
42	122	365	PEG2-vc	MMAD

43	122	365	vc	MMAE
44	122	365	vc	duocarmycin
45	122	365	spdb	DM4
46	122	366	vc	MMAD
47	122	366	PEG2-vc	MMAD
48	122	366	vc	MMAE
49	122	366	vc	duocarmycin
50	122	366	spdb	DM4
51	239	246	vc	MMAD
52	239	246	PEG2-vc	MMAD
53	239	246	vc	MMAE
54	239	246	vc	duocarmycin
55	239	246	spdb	DM4
56	239	314	vc	MMAD
57	239	314	PEG2-vc	MMAD
58	239	314	vc	MMAE
59	239	314	vc	duocarmycin
60	239	314	spdb	DM4
61	122	367	vc	MMAD
62	122	367	PEG2-vc	MMAD
63	122	367	vc	MMAE
64	122	367	vc	duocarmycin
65	122	367	spdb	DM4
66	122	368	vc	MMAD
67	122	368	PEG2-vc	MMAD
68	122	368	vc	MMAE
69	122	368	vc	duocarmycin
70	122	368	spdb	DM4
71	239	303	vc	MMAD
72	239	303	PEG2-vc	MMAD
73	239	303	vc	MMAE
74	239	303	vc	duocarmycin
75	239	303	spdb	DM4
76	239	316	vc	MMAD
77	239	316	PEG2-vc	MMAD
78	239	316	vc	MMAE
79	239	316	vc	duocarmycin
80	239	316	spdb	DM4
81	122	369	vc	MMAD
82	122	369	PEG2-vc	MMAD
83	122	369	vc	MMAE
84	122	369	vc	duocarmycin
85	122	369	spdb	DM4
86	122	370	vc	MMAD
87	122	370	PEG2-vc	MMAD
88	122	370	vc	MMAE
89	122	370	vc	duocarmycin
90	122	370	spdb	DM4

91	239	387	vc	MMAD
92	239	387	PEG2-vc	MMAD
93	239	387	vc	MMAE
94	239	387	vc	duocarmycin
95	239	387	spdb	DM4
96	239	388	vc	MMAD
97	239	388	PEG2-vc	MMAD
98	239	388	vc	MMAE
99	239	388	vc	duocarmycin
100	239	388	spdb	DM4
101	122	389	vc	MMAD
102	122	389	PEG2-vc	MMAD
103	122	389	vc	MMAE
104	122	389	vc	duocarmycin
105	122	389	spdb	DM4
106	122	390	vc	MMAD
107	122	390	PEG2-vc	MMAD
108	122	390	vc	MMAE
109	122	390	vc	duocarmycin
110	122	390	spdb	DM4
111	239	391	vc	MMAD
112	239	391	PEG2-vc	MMAD
113	239	391	vc	MMAE
114	239	391	vc	duocarmycin
115	239	391	spdb	DM4
116	239	392	vc	MMAD
117	239	392	PEG2-vc	MMAD
118	239	392	vc	MMAE
119	239	392	vc	duocarmycin
120	239	392	spdb	DM4
121	122	393	vc	MMAD
122	122	393	PEG2-vc	MMAD
123	122	393	vc	MMAE
124	122	393	vc	duocarmycin
125	122	393	spdb	DM4
126	122	394	vc	MMAD
127	122	394	PEG2-vc	MMAD
128	122	394	vc	MMAE
129	122	394	vc	duocarmycin
130	122	394	spdb	DM4
131	239	395	vc	MMAD
132	239	395	PEG2-vc	MMAD
133	239	395	vc	MMAE
134	239	395	vc	duocarmycin
135	239	395	spdb	DM4
136	239	396	vc	MMAD
137	239	396	PEG2-vc	MMAD
138	239	396	vc	MMAE

139	239	396	vc	duocarmycin
140	239	396	spdb	DM4
141	122	397	vc	MMAD
142	122	397	PEG2-vc	MMAD
143	122	397	vc	MMAE
144	122	397	vc	duocarmycin
145	122	397	spdb	DM4
146	122	398	vc	MMAD
147	122	398	PEG2-vc	MMAD
148	122	398	vc	MMAE
149	122	398	vc	duocarmycin
150	122	398	spdb	DM4
151	239	399	vc	MMAD
152	239	399	PEG2-vc	MMAD
153	239	399	vc	MMAE
154	239	399	vc	duocarmycin
155	239	399	spdb	DM4
156	239	400	vc	MMAD
157	239	400	PEG2-vc	MMAD
158	239	400	vc	MMAE
159	239	400	vc	duocarmycin
160	239	400	spdb	DM4
161	122	401	vc	MMAD
162	122	401	PEG2-vc	MMAD
163	122	401	vc	MMAE
164	122	401	vc	duocarmycin
165	122	401	spdb	DM4
166	122	402	vc	MMAD
167	122	402	PEG2-vc	MMAD
168	122	402	vc	MMAE
169	122	402	vc	duocarmycin
170	122	402	spdb	DM4
171	239	427	vc	MMAD
172	239	427	PEG2-vc	MMAD
173	239	427	vc	MMAE
174	239	427	vc	duocarmycin
175	239	427	spdb	DM4
176	239	428	vc	MMAD
177	239	428	PEG2-vc	MMAD
178	239	428	vc	MMAE
179	239	428	vc	duocarmycin
180	239	428	spdb	DM4
181	122	429	vc	MMAD
182	122	429	PEG2-vc	MMAD
183	122	429	vc	MMAE
184	122	429	vc	duocarmycin
185	122	429	spdb	DM4
186	122	430	vc	MMAD

187	122	430	PEG2-vc	MMAD
188	122	430	vc	MMAE
189	122	430	vc	duocarmycin
190	122	430	spdb	DM4
191	239	431	vc	MMAD
192	239	431	PEG2-vc	MMAD
193	239	431	vc	MMAE
194	239	431	vc	duocarmycin
195	239	431	spdb	DM4
196	239	432	vc	MMAD
197	239	432	PEG2-vc	MMAD
198	239	432	vc	MMAE
199	239	432	vc	duocarmycin
200	239	432	spdb	DM4
201	122	433	vc	MMAD
202	122	433	PEG2-vc	MMAD
203	122	433	vc	MMAE
204	122	433	vc	duocarmycin
205	122	433	spdb	DM4
206	122	434	vc	MMAD
207	122	434	PEG2-vc	MMAD
208	122	434	vc	MMAE
209	122	434	vc	duocarmycin
210	122	434	spdb	DM4
211	239	435	vc	MMAD
212	239	435	PEG2-vc	MMAD
213	239	435	vc	MMAE
214	239	435	vc	duocarmycin
215	239	435	spdb	DM4
216	239	436	vc	MMAD
217	239	436	PEG2-vc	MMAD
218	239	436	vc	MMAE
219	239	436	vc	duocarmycin
220	239	436	spdb	DM4
221	122	437	vc	MMAD
222	122	437	PEG2-vc	MMAD
223	122	437	vc	MMAE
224	122	437	vc	duocarmycin
225	122	437	spdb	DM4
226	122	438	vc	MMAD
227	122	438	PEG2-vc	MMAD
228	122	438	vc	MMAE
229	122	438	vc	duocarmycin
230	122	438	spdb	DM4
231	239	439	vc	MMAD
232	239	439	PEG2-vc	MMAD
233	239	439	vc	MMAE
234	239	439	vc	duocarmycin

235	239	439	spdb	DM4
236	239	440	vc	MMAD
237	239	440	PEG2-vc	MMAD
238	239	440	vc	MMAE
239	239	440	vc	duocarmycin
240	239	440	spdb	DM4
241	122	441	vc	MMAD
242	122	441	PEG2-vc	MMAD
243	122	441	vc	MMAE
244	122	441	vc	duocarmycin
245	122	441	spdb	DM4
246	122	442	vc	MMAD
247	122	442	PEG2-vc	MMAD
248	122	442	vc	MMAE
249	122	442	vc	duocarmycin
250	122	442	spdb	DM4
251	239	451	vc	MMAD
252	239	451	PEG2-vc	MMAD
253	239	451	vc	MMAE
254	239	451	vc	duocarmycin
255	239	451	spdb	DM4
256	239	452	vc	MMAD
257	239	452	PEG2-vc	MMAD
258	239	452	vc	MMAE
259	239	452	vc	duocarmycin
260	239	452	spdb	DM4
261	122	453	vc	MMAD
262	122	453	PEG2-vc	MMAD
263	122	453	vc	MMAE
264	122	453	vc	duocarmycin
265	122	453	spdb	DM4
266	122	454	vc	MMAD
267	122	454	PEG2-vc	MMAD
268	122	454	vc	MMAE
269	122	454	vc	duocarmycin
270	122	454	spdb	DM4
271	239	455	vc	MMAD
272	239	455	PEG2-vc	MMAD
273	239	455	vc	MMAE
274	239	455	vc	duocarmycin
275	239	455	spdb	DM4
276	239	456	vc	MMAD
277	239	456	PEG2-vc	MMAD
278	239	456	vc	MMAE
279	239	456	vc	duocarmycin
280	239	456	spdb	DM4
281	122	457	vc	MMAD
282	122	457	PEG2-vc	MMAD

283	122	457	vc	MMAE
284	122	457	vc	duocarmycin
285	122	457	spdb	DM4
286	122	458	vc	MMAD
287	122	458	PEG2-vc	MMAD
288	122	458	vc	MMAE
289	122	458	vc	duocarmycin
290	122	458	spdb	DM4
291	239	459	vc	MMAD
292	239	459	PEG2-vc	MMAD
293	239	459	vc	MMAE
294	239	459	vc	duocarmycin
295	239	459	spdb	DM4
296	239	460	vc	MMAD
297	239	460	PEG2-vc	MMAD
298	239	460	vc	MMAE
299	239	460	vc	duocarmycin
300	239	460	spdb	DM4
301	122	461	vc	MMAD
302	122	461	PEG2-vc	MMAD
303	122	461	vc	MMAE
304	122	461	vc	duocarmycin
305	122	461	spdb	DM4
306	122	462	vc	MMAD
307	122	462	PEG2-vc	MMAD
308	122	462	vc	MMAE
309	122	462	vc	duocarmycin
310	122	462	spdb	DM4
311	239	463	vc	MMAD
312	239	463	PEG2-vc	MMAD
313	239	463	vc	MMAE
314	239	463	vc	duocarmycin
315	239	463	spdb	DM4
316	239	464	vc	MMAD
317	239	464	PEG2-vc	MMAD
478	239	464	vc	MMAE
319	239	464	vc	duocarmycin
320	239	464	spdb	DM4
321	122	465	vc	MMAD
322	122	465	PEG2-vc	MMAD
323	122	465	vc	MMAE
324	122	465	vc	duocarmycin
325	122	465	spdb	DM4
326	122	466	vc	MMAD
327	122	466	PEG2-vc	MMAD
328	122	466	vc	MMAE
329	122	466	vc	duocarmycin
330	122	466	spdb	DM4

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331	239	467	vc	MMAD
332	239	467	PEG2-vc	MMAD
333	239	467	vc	MMAE
334	239	467	vc	duocarmycin
335	239	467	spdb	DM4
336	239	468	vc	MMAD
337	239	468	PEG2-vc	MMAD
338	239	468	vc	MMAE
339	239	468	vc	duocarmycin
340	239	468	spdb	DM4
341	122	469	vc	MMAD
342	122	469	PEG2-vc	MMAD
343	122	469	vc	MMAE
344	122	469	vc	duocarmycin
345	122	469	spdb	DM4
346	122	470	vc	MMAD
347	122	470	PEG2-vc	MMAD
348	122	470	vc	MMAE
349	122	470	vc	duocarmycin
350	122	470	spdb	DM4
351	239	471	vc	MMAD
352	239	471	PEG2-vc	MMAD
353	239	471	vc	MMAE
354	239	471	vc	duocarmycin
355	239	471	spdb	DM4
356	239	472	vc	MMAD
357	239	472	PEG2-vc	MMAD
358	239	472	vc	MMAE
359	239	472	vc	duocarmycin
360	239	472	spdb	DM4
361	122	473	vc	MMAD
362	122	473	PEG2-vc	MMAD
363	122	473	vc	MMAE
364	122	473	vc	duocarmycin
365	122	473	spdb	DM4
366	122	474	vc	MMAD
367	122	474	PEG2-vc	MMAD
368	122	474	vc	MMAE
369	122	474	vc	duocarmycin
370	122	474	spdb	DM4

[000205] An antibody drug conjugate (ADC) of the present disclosure or activatable antibody drug conjugate (AADC) of the present disclosure may include one or more polypeptides that include the combination of amino acid sequences, a linker, and a toxin listed in a given row of Table C. Therefore, an activatable antibody drug conjugate (ADC) of the present disclosure or activatable antibody drug conjugate (AADC) of the present

disclosure that includes the combination of amino acid sequences, a linker, and a toxin listed in a given row or provided as a specific combination is described herein. For example, an activatable antibody drug conjugate of the present disclosure may include the amino acid sequences of combination no. 55, which includes a heavy chain comprising the amino acid sequence of SEQ ID NO: 239, a light chain comprising the amino acid sequence of SEQ ID NO: 246, and a spdb-DM4 linker-toxin. In another example of the AADCs disclosed and described herein, an activatable antibody drug conjugate of the present disclosure may include the amino acid sequences of combination no. 33, which includes a heavy chain comprising the amino acid sequence of SEQ ID NO: 239, a light chain comprising the amino acid sequence of SEQ ID NO: 244, and a vc-MMAE linker-toxin.

[000206] Any of the combinations in Table C that list a heavy chain and light chain variable region can be combined with human immunoglobulin constant regions to result in fully human IgGs including IgG1, IgG2, IgG4 or mutated constant regions to result in human IgGs with altered functions such as IgG1 N297A, IgG1 N297Q, or IgG4 S228P. The combinations described in Table C are not limited by the particular combinations shown in any given row, and thus can include any heavy chain sequence or heavy chain variable region sequence from column 2 of Table C combined with any light chain sequence or light chain variable region sequence from column 3 of Table C combined with any linker from column 4 combined with any toxin from column 5. In addition to the heavy chain sequences or heavy chain variable region sequences listed in column 2, any heavy chain sequence or heavy chain variable region sequence disclosed herein can be used in a combination. In addition to the light chain sequences or light chain variable region sequences listed in column 3, any light chain sequence or light chain variable region sequence disclosed herein can be used in a combination. In addition to the linkers listed in column 4, any linker disclosed herein can be used in a combination. In addition to the toxins listed in column 5, any toxin disclosed herein can be used in a combination.

[000207] In some embodiments, the serum half-life of the activatable antibody is longer than that of the corresponding antibody; e.g., the pK of the activatable antibody is longer than that of the corresponding antibody. In some embodiments, the serum half-life of the activatable antibody is similar to that of the corresponding antibody. In some embodiments, the serum half-life of the activatable antibody is at least 15 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 12 days when administered to an organism. In some embodiments, the

serum half-life of the activatable antibody is at least 11 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 10 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 9 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 8 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 7 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 6 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 5 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 4 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 3 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 2 days when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 24 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 20 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 18 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 16 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 14 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 12 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 10 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 8 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 6 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 4 hours when administered to an organism. In some embodiments, the serum half-life of the activatable antibody is at least 3 hours when administered to an organism.

[000208] In some embodiments, the activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody is monospecific. In some embodiments, the activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody is

multispecific, *e.g.*, by way of non-limiting example, bispecific or trifunctional. In some embodiments, the activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody is formulated as part of a pro-Bispecific T Cell Engager (BITE) molecule. In some embodiments, the activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody is formulated as part of a pro-Chimeric Antigen Receptor (CAR) modified T cell or other engineered receptor.

[000209] In some embodiments, the activatable antibody or antigen-binding fragment thereof is incorporated in a multispecific activatable antibody or antigen-binding fragment thereof, where at least one arm of the multispecific activatable antibody specifically binds CD166. In some embodiments, the activatable antibody or antigen-binding fragment thereof is incorporated in a bispecific antibody or antigen-binding fragment thereof, where at least one arm of the bispecific activatable antibody specifically binds CD166.

[000210] In some embodiments, the anti-CD166 antibodies, conjugated anti-CD166 antibodies, activatable anti-CD166 antibodies and/or conjugated activatable anti-CD166 antibodies described herein are used in conjunction with one or more additional agents or a combination of additional agents. Suitable additional agents include current pharmaceutical and/or surgical therapies for an intended application, such as, for example, cancer. For example, the anti-CD166 antibodies, conjugated anti-CD166 antibodies, activatable anti-CD166 antibodies and/or conjugated activatable anti-CD166 antibodies can be used in conjunction with an additional chemotherapeutic or anti-neoplastic agent.

[000211] In some embodiments, the additional agent(s) is a chemotherapeutic agent, such as a chemotherapeutic agent selected from the group consisting of docetaxel, paclitaxel, abraxane (i.e., albumin-conjugated paclitaxel), doxorubicin, oxaliplatin, carboplatin, cisplatin, irinotecan, and gemcitabine.

[000212] In some embodiments, the additional agent(s) is a checkpoint inhibitor, a kinase inhibitor, an agent targeting inhibitors in the tumor microenvironment, and/or a T cell or NK agonist. In some embodiments, the additional agent(s) is radiation therapy, alone or in combination with another additional agent(s) such as a chemotherapeutic or anti-neoplastic agent. In some embodiments, the additional agent(s) is a vaccine, an oncovirus, and/or a DC-activating agent such as, by way of non-limiting example, a toll-like receptor (TLR) agonist and/or α-CD40. In some embodiments, the additional agent(s) is a tumor-targeted antibody designed to kill the tumor via ADCC or via direct conjugation to a toxin (e.g., an antibody drug conjugate (ADC).

[000213] In some embodiments, the checkpoint inhibitor is an inhibitor of a target selected from the group consisting of CTLA-4, LAG-3, PD-1, CD166, TIGIT, TIM-3, B7H4, and Vista. In some embodiments, the kinase inhibitor is selected from the group consisting of B-RAFi, MEKi, and Btk inhibitors, such as ibrutinib. In some embodiments, the kinase inhibitor is crizotinib. In some embodiments, the tumor microenvironment inhibitor is selected from the group consisting of an IDO inhibitor, an α-CSF1R inhibitor, an α-CCR4 inhibitor, a TGF-beta, a myeloid-derived suppressor cell, or a T-regulatory cell. In some embodiments, the agonist is selected from the group consisting of Ox40, GITR, CD137, ICOS, CD27, and HVEM.

[000214] In some embodiments, the inhibitor is a CTLA-4 inhibitor. In some embodiments, the inhibitor is a LAG-3 inhibitor. In some embodiments, the inhibitor is a PD-1 inhibitor. In some embodiments, the inhibitor is a CD166 inhibitor. In some embodiments, the inhibitor is a TIGIT inhibitor. In some embodiments, the inhibitor is a TIM-3 inhibitor. In some embodiments, the inhibitor is a B7H4 inhibitor. In some embodiments, the inhibitor is a Vista inhibitor. In some embodiments, the inhibitor is a B-RAFi inhibitor. In some embodiments, the inhibitor is a MEKi inhibitor. In some embodiments, the inhibitor is a Btk inhibitor. In some embodiments, the inhibitor is ibrutinib. In some embodiments, the inhibitor is crizotinib. In some embodiments, the inhibitor is an IDO inhibitor. In some embodiments, the inhibitor is an α-CSF1R inhibitor. In some embodiments, the inhibitor is an α-CCR4 inhibitor. In some embodiments, the inhibitor is a TGF-beta. In some embodiments, the inhibitor is a myeloid-derived suppressor cell. In some embodiments, the inhibitor is a T-regulatory cell.

[000215] In some embodiments, the agonist is Ox40. In some embodiments, the agonist is GITR. In some embodiments, the agonist is CD137. In some embodiments, the agonist is ICOS. In some embodiments, the agonist is CD27. In some embodiments, the agonist is HVEM.

[000216] In some embodiments, the anti-CD166 antibody, conjugated antibody, activatable antibody and/or conjugated activatable antibody is administered during and/or after treatment in combination with one or more additional agents such as, for example, a chemotherapeutic agent, an anti-inflammatory agent, and/or a an immunosuppressive agent. In some embodiments, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and the additional agent are formulated into a single therapeutic composition, and the anti-CD166

antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and additional agent are administered simultaneously. Alternatively, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and additional agent are separate from each other, *e.g.*, each is formulated into a separate therapeutic composition, and the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and the additional agent are administered simultaneously, or the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and the additional agent are administered at different times during a treatment regimen. For example, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody is administered prior to the administration of the additional agent, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody is administered subsequent to the administration of the additional agent, or the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and the additional agent are administered in an alternating fashion. As described herein, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and additional agent are administered in single doses or in multiple doses.

[000217] In some embodiments, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and the additional agent(s) are administered simultaneously. For example, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 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 anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and the additional agent(s) are administered sequentially, or the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and the additional agent are administered at different times during a treatment regimen.

[000218] In some embodiments, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody is 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, an anti-inflammatory agent, and/or an immunosuppressive agent, such as an alkylating agent, an anti-metabolite, an anti-microtubule agent, a topoisomerase inhibitor, a cytotoxic antibiotic, and/or any other nucleic acid damaging agent. In some embodiments, the additional agent is a taxane, such as paclitaxel (e.g., Abraxane®). In some embodiments, the additional agent is an anti-metabolite, such as gemcitabine. In some embodiments, the additional agent is an alkylating agent, such as platinum-based chemotherapy, such as carboplatin or cisplatin. In some embodiments, the additional agent is a targeted agent, such as a kinase inhibitor, e.g., sorafenib or erlotinib. In some embodiments, the additional agent is a targeted agent, such as another antibody, e.g., a monoclonal antibody (e.g., bevacizumab), a bispecific antibody, or a multispecific antibody. In some embodiments, the additional agent is a proteosome inhibitor, such as bortezomib or carfilzomib. In some embodiments, the additional agent is an immune modulating agent, such as lenolidominde or IL-2. In some embodiments, the additional agent is radiation. In some embodiments, the additional agent is an agent considered standard of care by those skilled in the art. In some embodiments, the additional agent is a chemotherapeutic agent well known to those skilled in the art.

[000219] In some embodiments, the additional agent is another antibody or antigen-binding fragment thereof, another conjugated antibody or antigen-binding fragment thereof, another activatable antibody or antigen-binding fragment thereof and/or another conjugated activatable antibody or antigen-binding fragment thereof. In some embodiments the additional agent is another antibody or antigen-binding fragment thereof, another conjugated antibody or antigen-binding fragment thereof, another activatable antibody or antigen-binding fragment thereof and/or another conjugated activatable antibody or antigen-binding fragment thereof against the same target as the first antibody or antigen-binding fragment thereof, the first conjugated antibody or antigen-binding fragment thereof, activatable antibody or antigen-binding fragment thereof and/or a conjugated activatable antibody or antigen-binding fragment thereof, e.g., against CD166. In some embodiments the additional agent is another antibody or antigen-binding fragment thereof, another conjugated antibody or antigen-binding fragment thereof, another activatable antibody or

antigen-binding fragment thereof and/or another conjugated activatable antibody or antigen-binding fragment thereof against a target different than the target of the first antibody or antigen-binding fragment thereof, the first conjugated antibody or antigen-binding fragment thereof, activatable antibody or antigen-binding fragment thereof and/or a conjugated activatable antibody or antigen-binding fragment thereof.

[000220] As a non-limiting example, the antibody or antigen-binding fragment and/or the AB of an activatable antibody is a binding partner for any target listed in Table 1.

Table 1: Exemplary Targets

1-92-LFA-3	CD52	DL44	HVEM	LIF-R	STEAP1
Alpha-4 integrin	CD56	DLK1	Hyaluronidase	Lewis X	STEAP2
Alpha-V integrin	CD64	DLL4	ICOS	LIGHT	TAG-72
alpha4beta1 integrin	CD70	DPP-4	IFNalpha	LRP4	TAPA1
alpha4beta7 integrin	CD71	DSG1	IFNbeta	LRRC26	TGFbeta
AGR2	CD74	EGFR	IFNgamma	MCSP	TIGIT
Anti-Lewis-Y		EGFRviii	IgE	Mesothelin	TIM-3
Apelin J receptor	CD80	Endothelin B receptor (ETBR)	IgE Receptor (FceRI)	MRP4	TLR2
APRIL	CD81	ENPP3	IGF	MUC1	TLR4
B7-H4	CD86	EpCAM	IGF1R	Mucin-16 (MUC16, CA-125)	TLR6
BAFF	CD95	EPHA2	IL1B	Na/K ATPase	TLR7
BTLA	CD117	EPHB2	IL1R	Neutrophil elastase	TLR8
C5 complement	CD125	ERBB3	IL2	NGF	TLR9
C-242	CD132 (IL-2RG)	F protein of RSV	IL11	Nicastrin	TMEM31
CA9	CD133	FAP	IL12	Notch Receptors	TNFalpha
CA19-9 (Lewis a)	CD137	FGF-2	IL12p40	Notch 1	TNFR
Carbonic anhydrase 9	CD138	FGF8	IL-12R, IL-12Rbeta1	Notch 2	TNFRS12A
CD2	CD166	FGFR1	IL13	Notch 3	TRAIL-R1
CD3	CD172A	FGFR2	IL13R	Notch 4	TRAIL-R2
CD6	CD248	FGFR3	IL15	NOV	Transferrin

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

[000221] As a non-limiting example, the antibody or antigen-binding fragment and/or the AB of an activatable antibody is or is derived from an antibody listed in Table 2.

Table 2: Exemplary sources for Abs

Antibody Trade Name (antibody name)	**Target**
Avastin™ (bevacizumab)	VEGF
Lucentis™ (ranibizumab)	VEGF
Erbitux™ (cetuximab)	EGFR
Vectibix™ (panitumumab)	EGFR
Remicade™ (infliximab)	TNFα
Humira™ (adalimumab)	TNFα
Tysabri™ (natalizumab)	Integrinα4
Simulect™ (basiliximab)	IL2R
Soliris™ (eculizumab)	Complement C5
Raptiva™ (efalizumab)	CD11a
Bexxar™ (tositumomab)	CD20
Zevalin™ (ibritumomab tiuxetan)	CD20
Rituxan™ (rituximab)	CD20

Ocrelizumab	CD20
Arzerra™ (ofatumumab)	CD20
Gazyva™ (obinutuzumab)	CD20
Zenapax™ (daclizumab)	CD25
Adcetris™ (brentuximab vedotin)	CD30
Myelotarg™ (gemtuzumab)	CD33
Mylotarg™ (gemtuzumab ozogamicin)	CD33
Campath™ (alemtuzumab)	CD52
ReoPro™ (abiciximab)	Glycoprotein receptor IIb/IIIa
Xolair™ (omalizumab)	IgE
Herceptin™ (trastuzumab)	Her2
Kadcyla™ (trastuzumab emtansine)	Her2
Synagis™ (palivizumab)	F protein of RSV
(ipilimumab)	CTLA-4
(tremelimumab)	CTLA-4
Hu5c8	CD40L
(pertuzumab)	Her2-neu
(ertumaxomab)	CD3/Her2-neu
Orencia™ (abatacept)	CTLA-4
(tanezumab)	NGF
(bavituximab)	Phosphatidylserine
(zalutumumab)	EGFR
(mapatumumab)	EGFR
(matuzumab)	EGFR
(nimotuzumab)	EGFR
ICR62	EGFR
mAb 528	EGFR
CH806	EGFR
MDX-447	EGFR/CD64
(edrecolomab)	EpCAM
RAV12	RAAG12
huJ591	PSMA
Enbrel™ (etanercept)	TNF-R
Amevive™ (alefacept)	1-92-LFA-3
Antril™, Kineret™ (ankinra)	IL-1Ra
GC1008	TGFbeta
	Notch, e.g., Notch 1
	Jagged 1 or Jagged 2
(adecatumumab)	EpCAM
(figitumumab)	IGF1R
(tocilizumab)	IL-6 receptor
Stelara™ (ustekinumab)	IL-12/IL-23
Prolia™ (denosumab)	RANKL

[000222] In some embodiments, the additional antibody or antigen binding fragment thereof, conjugated antibody or antigen binding fragment thereof, activatable antibody or

antigen binding fragment thereof, and/or conjugated activatable antibody or antigen binding fragment thereof 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, the additional antibody or antigen binding fragment thereof, conjugated antibody or antigen binding fragment thereof, activatable antibody or antigen binding fragment thereof, and/or conjugated activatable antibody or antigen binding fragment thereof is a mouse, other rodent, chimeric, humanized or fully human monoclonal antibody.

[000223] The disclosure also provides methods of producing an anti-CD166 antibody and/or activatable anti-CD166 antibody polypeptide by culturing a cell under conditions that lead to expression of the polypeptide, wherein the cell comprises an isolated nucleic acid molecule encoding an antibody and/or an activatable antibody described herein, and/or vectors that include these isolated nucleic acid sequences. The disclosure provides methods of producing an antibody and/or activatable antibody by culturing a cell under conditions that lead to expression of the antibody and/or activatable antibody, wherein the cell comprises an isolated nucleic acid molecule encoding an antibody and/or an activatable antibody described herein, and/or vectors that include these isolated nucleic acid sequences.

[000224] The invention also provides a method of manufacturing activatable antibodies that in an activated state binds CD166 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 CD166, (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, when the activatable antibody is in an uncleaved state, the MM interferes with specific binding of the AB to CD166 and in a cleaved state the MM does not interfere or compete with specific binding of the AB to CD166; and (b) recovering the activatable antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000225] 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, the activatable antibody comprises a linking peptide between the MM and the CM. In some embodiments, the activatable antibody comprises a linking

peptide between the CM and the AB. In some embodiments, the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM. In some embodiments, the two linking peptides need not be identical to each other. In some embodiments, the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: spacer-MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM-spacer.

[000226] In some embodiments, at least one of LP1 or LP2 comprises an amino acid sequence selected from the group consisting of $(GS)_n$, $(GGS)_n$, $(GSGGS)_n$ (SEQ ID NO: 1) and $(GGGS)_n$ (SEQ ID NO: 2), where n is an integer of at least one.

[000227] In some embodiments, at least one of LP1 or LP2 comprises an amino acid sequence selected from the group consisting of GGSG (SEQ ID NO: 3), GGSGG (SEQ ID NO: 4), GSGSG (SEQ ID NO: 5), GSGGG (SEQ ID NO: 6), GGGSG (SEQ ID NO: 7), and GSSSG (SEQ ID NO: 8).

[000228] In some embodiments, LP1 comprises the amino acid sequence GSSGGSGGSGGSG (SEQ ID NO: 9), GSSGGSGGSGG (SEQ ID NO: 10), GSSGGSGGSGGS (SEQ ID NO: 11), GSSGGSGGSGGSGGGS (SEQ ID NO: 12), GSSGGSGGSG (SEQ ID NO: 13), or GSSGGSGGSGS (SEQ ID NO: 14).

[000229] In some embodiments, LP2 comprises the amino acid sequence GSS, GGS, GGGS (SEQ ID NO: 15), GSSGT (SEQ ID NO: 16) or GSSG (SEQ ID NO: 17).

[000230] The invention provides methods of preventing, delaying the progression of, treating, alleviating a symptom of, or otherwise ameliorating an CD166 mediated disease in a subject by administering a therapeutically effective amount of an anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody described herein to a subject in need thereof.

[000231] The invention also provides methods of preventing, delaying the progression of, treating, alleviating a symptom of, or otherwise ameliorating cancer in a subject by administering a therapeutically effective amount of an anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody described herein to a subject in need thereof. CD166 is known to be expressed in a variety of cancers, such as, by way of non-limiting example, any epithelial or squamous cell cancer, any carcinoid, and/or a neuroendocrine cancer. Examples of cancers

include, but are not limited to, adenocarcinoma, bile duct (biliary) cancer, bladder cancer, breast cancer, e.g., triple-negative breast cancer, Her2-negative breast cancer, estrogen receptor-positive breast cancer; carcinoid cancer; cervical cancer; cholangiocarcinoma; colorectal; endometrial; glioma; head and neck cancer, e.g., head and neck squamous cell cancer; leukemia; liver cancer; lung cancer, e.g., NSCLC, SCLC; lymphoma; melanoma; osopharyngeal cancer; ovarian cancer; pancreatic cancer; prostate cancer, *e.g.*, metastatic castration-resistant prostate carcinoma; renal cancer; skin cancer; squamous cell cancer; stomach cancer; testis cancer; thyroid cancer; and urothelial cancer.

[000232] In some embodiments, the cancer is any epithelial or squamous cancer. In some embodiments, the cancer is prostate cancer, breast cancer, lung cancer, cervical cancer, oropharyngeal cancer, and/or head and neck cancer.

[000233] In some embodiments, the cancer is a bladder cancer, a bone cancer, a breast cancer, a carcinoid, a cervical cancer, a colorectal cancer, a colon cancer, an endometrial cancer, an epithelial cancer, a glioma, a head and neck cancer, a liver cancer, a lung cancer, a melanoma, an oropharyngeal cancer, an ovarian cancer, a pancreatic cancer, a prostate cancer, a renal cancer, a sarcoma, a skin cancer, a stomach cancer, a testis cancer, a thyroid cancer, a urogenital cancer, and/or a urothelial cancer.

[000234] In some embodiments, the cancer is selected from the group consisting of triple negative breast cancer (TNBC), non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), Ras mutant colorectal carcinoma, a rare epithelial cancer, oropharyngeal cancer, cervical cancer, head and neck squamous cell carcinoma (HNSCC), and/or prostate cancer. In some embodiments, the cancer is associated with a CD166-expressing tumor. In some embodiments, the cancer is due to a CD166-expressing tumor.

[000235] An anti-CD166 antibody, a conjugated anti-CD166 antibody, an activatable anti-CD166 antibody and/or a conjugated activatable anti-CD166 antibody used in any of the embodiments of these methods and uses can be administered at any stage of the disease. For example, such an anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody can be administered to a patient suffering cancer of any stage, from early to metastatic. The terms subject and patient are used interchangeably herein.

[000236] In some embodiments, the subject is a mammal, such as a human, non-human primate, companion animal (*e.g.*, cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a human. In some embodiments, the subject is

a companion animal. In some embodiments, the subject is an animal in the care of a veterinarian.

[000237] The anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and therapeutic formulations thereof are administered to a subject suffering from or susceptible to a disease or disorder associated with aberrant CD166 expression and/or activity. A subject suffering from or susceptible to a disease or disorder associated with aberrant CD166 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/or stool analysis to evaluate health status. For example, subjects suffering from inflammation and/or an inflammatory disorder are identified using any of a variety of clinical and/or laboratory tests such as physical examination and/or bodily fluid analysis, e.g., blood, urine and/or stool analysis, to evaluate health status.

[000238] Administration of an anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody to a patient suffering from a disease or disorder associated with aberrant CD166 expression and/or activity is considered successful if any of a variety of laboratory or clinical objectives is achieved. For example, administration of an anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody to a patient suffering from a disease or disorder associated with aberrant CD166 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 anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody to a patient suffering from a disease or disorder associated with aberrant CD166 expression and/or activity is considered successful if the disease or disorder enters remission or does not progress to a further, *i.e.*, worse, state.

[000239] In some embodiments, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and therapeutic formulations thereof are administered to a subject suffering from or susceptible to a disease or disorder, such as subjects suffering from cancer or other neoplastic condition, wherein the subject's diseased cells are expressing CD166. In some

embodiments, the diseased cells are associated with aberrant CD166 expression and/or activity. In some embodiments, the diseased cells are associated with normal CD166 expression and/or activity. A subject suffering from or susceptible to a disease or disorder wherein the subject's diseased cells express CD166 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/or stool analysis to evaluate health status. For example, subjects suffering from inflammation and/or an inflammatory disorder are identified using any of a variety of clinical and/or laboratory tests such as physical examination and/or bodily fluid analysis, e.g., blood, urine and/or stool analysis, to evaluate health status.

[000240] In some embodiments, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and therapeutic formulations thereof are administered to a subject suffering from or susceptible to a disease or disorder associated with cells expressing CD166 or the presence, growth, proliferation, metastasis, and/or activity of such cells, such as subjects suffering from cancer or other neoplastic conditions. In some embodiments, the cells are associated with aberrant CD166 expression and/or activity. In some embodiments, the cells are associated with normal CD166 expression and/or activity. A subject suffering from or susceptible to a disease or disorder associated with cells that express CD166 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/or stool analysis to evaluate health status. For example, subjects suffering from inflammation and/or an inflammatory disorder are identified using any of a variety of clinical and/or laboratory tests such as physical examination and/or bodily fluid analysis, e.g., blood, urine and/or stool analysis, to evaluate health status.

[000241] Administration of an anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody to a patient suffering from a disease or disorder associated with cells expressing CD166 is considered successful if any of a variety of laboratory or clinical objectives is achieved. For example, administration of an anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody to a

patient suffering from a disease or disorder associated with cells expressing CD166 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 anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody to a patient suffering from a disease or disorder associated with cells expressing CD166 is considered successful if the disease or disorder enters remission or does not progress to a further, *i.e.*, worse, state.

[000242] In some embodiments, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody is administered during and/or after treatment in combination with one or more additional agents such as, for example, a chemotherapeutic agent, an anti-inflammatory agent, and/or an immunosuppressive agent. In some embodiments, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and the additional agent(s) are administered simultaneously. For example, the anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 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 anti-CD166 antibody, conjugated anti-CD166 antibody, activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody and the additional agent(s) are administered sequentially.

[000243] The invention also provides methods and kits for using the activatable anti-CD166 antibodies and/or conjugated activatable anti-CD166 antibodies in a variety of diagnostic and/or prophylactic indications. For example, the invention provides methods and kits for detecting the presence or absence of a cleaving agent and a target of interest in a subject or a sample by (i) contacting a subject or sample with an anti-CD166 activatable antibody, wherein the anti-CD166 activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, and an antigen binding domain or fragment thereof (AB) that specifically binds the target of interest, wherein the anti-CD166 activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to CD166, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the

AB and is not a modified form of a natural binding partner of the AB; and (b) wherein, when the AB is in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to CD166, and when the AB is in a cleaved, activated state the MM does not interfere or compete with specific binding of the AB to CD166; and (ii) measuring a level of activated anti-CD166 activatable antibody in the subject or sample, wherein a detectable level of activated anti-CD166 activatable antibody in the subject or sample indicates that the cleaving agent and CD166 are present in the subject or sample and wherein no detectable level of activated anti-CD166 activatable antibody in the subject or sample indicates that the cleaving agent, CD166 or both the cleaving agent and CD166 are absent in the subject or sample.

[000244] In some embodiments, the activatable anti-CD166 antibody is an activatable anti-CD166 antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable anti-CD166 antibody is not conjugated to an agent. In some embodiments, the activatable anti-CD166 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-CD166 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.

[000245] In some embodiments of these methods and kits, the activatable anti-CD166 antibody includes a detectable label. In some embodiments of these methods and kits, the detectable label includes an imaging agent, 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 kits, the imaging agent comprises a radioisotope. In some embodiments of these methods and kits, the radioisotope is indium or technetium. In some embodiments of these methods and kits, the contrasting agent comprises iodine, gadolinium or iron oxide. In some embodiments of these methods and kits, the enzyme comprises horseradish peroxidase, alkaline phosphatase, or β-galactosidase. In some embodiments of these methods and 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 kits, 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 and kits, the ligand-based label comprises biotin, avidin, streptavidin or one or more haptens.

[000246] In some embodiments of these methods and kits, 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 non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a rodent.

[000247] In some embodiments of these methods and kits, 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.

[000248] In some embodiments of the methods and kits, the method is used to identify or otherwise refine a patient population suitable for treatment with an anti-CD166 activatable antibody of the disclosure, followed by treatment by administering that activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody to a subject in need thereof. For example, patients that test positive for both the target (e.g., CD166) and a protease that cleaves the substrate in the cleavable moiety (CM) of the anti-CD166 activatable antibody being tested in these methods are identified as suitable candidates for treatment with such an anti-CD166 activatable antibody comprising such a CM, and the patient is then administered a therapeutically effective amount of the activatable anti-CD166 antibody and/or conjugated activatable anti-CD166 antibody that was tested. Likewise, patients that test negative for either or both of the target (*e.g.*, CD166) 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. In some embodiments, such patients can be tested with other anti-CD166 activatable antibodies until a suitable anti-CD166 activatable antibody for treatment is identified (e.g., an anti-CD166 activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments, the patient is then administered a therapeutically effective amount of the activatable anti-CD166 antibody and/or conjugated for which the patient tested positive. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000249] Pharmaceutical compositions according to the invention can include an 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

[000250] Figure 1 is a graph depicting binding of various anti-CD166 antibodies of the present disclosure to human CD166 protein.

[000251] Figure 2 is a schematic representation of the selection scheme for anti-CD166 masking peptides of the present disclosure. The boxes indicate populations with the sort parameters indicated between the boxes. All FACS sorts were conducted in 0.5% BSA with excess mouse Isotype to block non-specific binding to the Alexa Fluor 488 labeled M9 Mab (VH of SEQ ID NO: 119, VL of SEQ ID NO: 120).

[000252] Figures 3A and 3B are a series of graphs depicting the ability of the anti-CD166 antibody CD166 M9 vK1/HcB (VH of SEQ ID NO: 121, VL of SEQ ID NO: 123) of the present disclosure and various anti-CD166 activatable antibodies of the present disclosure to bind human CD166.

[000253] Figure 4 is a graph depicting the ability of various anti-CD166 activatable antibodies of the disclosure to bind human CD166 when proteolytically activated.

[000254] Figure 5 is a graph depicting the ability of various conjugated anti-CD166 activatable antibodies of the disclosure to bind human CD166 when proteolytically activated.

[000255] Figures 6A-6D are a series of images demonstrating that an activatable anti-CD166 antibody of the disclosure is activated (i.e., cleaved) in colon cancer tissue samples, and the activatable anti-CD166 antibody is not activated in healthy tissue samples. Figures 6A and 6C depict the results of IHC analysis on the tumor and healthy tissue samples, and Figures 6B and 6D depict the results of the *in situ* imaging assay on the tumor and healthy tissue samples.

[000256] Figures 7A-7D are a series of images demonstrating that an activatable anti-CD166 antibody of the disclosure is activated (i.e., cleaved) in lung cancer tissue samples, and the activatable anti-CD166 antibody is not activated in healthy tissue samples. Figures 7A and 7C depict the results of IHC analysis on the tumor and healthy tissue samples, and Figures 7B and 7D depict the results of the *in situ* imaging assay on the tumor and healthy tissue samples.

[000257] Figures 8A-8D are a series of images demonstrating that an activatable anti-CD166 antibody of the disclosure is not activated in healthy tissue samples. Figures 8A and 8C depict the results of IHC analysis on the healthy tissue samples, and Figures 8B and 8D depict the results of the *in situ* imaging assay on the healthy tissue samples.

[000258] Figure 9 is a series of graphs depicting the potency of a conjugated anti-CD166 antibody of the disclosure against a breast cancer cell line, a prostate cancer cell line, a pancreatic cancer cell line, a head and neck squamous cell cancer cell line, and a CD166 cell line as a negative control.

[000259] Figure 10 is a graph depicting the efficacy of a conjugated activatable anti-CD166 antibody (AADC, Activatable Antibody Drug Complex) of the disclosure in a breast cancer model.

[000260] Figure 11 is a graph depicting the efficacy of a CD166 AADC DM4 conjugate of the present disclosure (i.e., an activatable anti-CD166 antibody of the disclosure conjugated to DM4) in the H292 non-small cell lung cancer (NSCLC) model.

[000261] Figure 12 is a graph depicting the efficacy of the CD166 AADC DM4 conjugate of the present disclosure in the H1975 non-small cell lung cancer (NSCLC) model.

[000262] Figure 13 is a graph depicting the ability of the anti-CD166 antibodies of the disclosure to bind human and cynomolgus monkey CD166 with equal affinity.

[000263] Figure 14 is graph depicting the results of a tolerability study in cynomolgus monkeys using an activatable anti-CD166 antibody of the disclosure.

[000264] Figure 15 is a graph that demonstrates that the conjugated anti-CD166 activatable antibody of the present disclosure is well tolerated at the projected therapeutic dosage.

[000265] Figures 16A, 16B, and 16C are a series of graphs depicting the ability of various conjugated anti-CD166 activatable antibodies of the disclosure to bind human CD166 when such conjugated activatable antibodies are proteolytically activated.

[000266] Figure 17 is a graph depicting the ability of the anti-CD166 antibodies of the present disclosure to bind human H292 cells and cynomolgus monkey primary kidney epithelial cells with comparable affinity.

[000267] Figure 18 is a graph depicting the ability of the anti-CD166 antibodies of the present disclosure to inhibit human HuT-78 cells from binding CD6 receptor.

[000268] Figures 19A-19C are graphs depicting the ability of the anti-CD166 activatable antibody drug conjugates of the present disclosure to bind CD166 and human cells in a protease-activated (cleaved) and unactivated (cleaved) forms.

[000269] Figures 20A and 20B are graphs depicting an exemplary cytotoxicity assay of the anti-CD166 activatable antibody drug conjugates of the present disclosure to human H292 and HCC1806 cells.

[000270] Figures 21A-21D are graphs depicting the ability of the anti-CD166 activatable antibody drug conjugates of the present disclosure to induce an immunological response in cells.

[000271] Figure 22 is a graph depicting the ability of the anti-CD166 activatable antibody drug conjugates of the present disclosure to induce an antibody-dependent cell cytotoxicity in cells.

[000272] Figure 23 are graphs depicting the efficacy of the anti-CD166 activatable antibody drug conjugates of the present disclosure against multiple cell-derived and patient-derived xenograft tumor models.

[000273] Figures 24A to 24D are graphs depicting the cytotoxicity of the anti-CD166 activatable antibody drug conjugates of the present disclosure against multiple endometrial cancer-derived cell lines, and an assay showing the expression levels of CD166 on the endometrial cancer-derived cells.

[000274] Figures 25A and 25B depicts exemplary studies of *in situ* binding of anti-CD166 antibodies of the present disclosure in a lung cancer xenograft model.

[000275] Figure 26 is a graph depicting the efficacy of the anti-CD166 activatable antibody drug conjugates of the present disclosure against a mouse lung cancer xenograft model.

Detailed Description of the Invention

[000276] The present invention provides monoclonal antibodies (mAbs) and activatable monoclonal antibodies that specifically bind CD166, also known as activated leukocyte cell adhesion molecule (ALCAM). In some embodiments, the monoclonal antibodies and activatable monoclonal antibodies are internalized by CD166-containing cells. CD166 is a cell adhesion molecule that binds CD6, a cell surface receptor that belongs to the scavenger receptor cysteine-rich (SRCR) protein superfamily (SRCRSF). CD166 is known to be associated with cell-cell and cell-matrix interactions, cell adhesion, cell

migration, and T-cell activation and proliferation. Aberrant expression and/or activity of CD166 and CD166-related signaling has been implicated in the pathogenesis of many diseases and disorders, such as cancer, inflammation, and autoimmunity. For example, CD166 is highly expressed in a variety of cancer types such as, for example, prostate cancer, breast cancer, lung cancer such as NSCLC and/or SCLC, oropharyngeal cancer, cervical cancer, and head and neck cancer such as HNSCC.

[000277] The disclosure provides anti-CD166 antibodies, conjugated anti-CD166 antibodies, activatable anti-CD166 antibodies, and/or conjugated activatable anti-CD166 antibodies that are useful in methods of treating, preventing, delaying the progression of, ameliorating and/or alleviating a symptom of a disease or disorder associated with aberrant CD166 expression and/or activity. For example, the activatable anti-CD166 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.

[000278] The disclosure provides anti-CD166 antibodies, conjugated anti-CD166 antibodies, activatable anti-CD166 antibodies, and/or conjugated activatable anti-CD166 antibodies that are useful in methods of treating, preventing, delaying the progression of, ameliorating and/or alleviating a symptom of a disease or disorder associated with cells expressing CD166. In some embodiments, the cells are associated with aberrant CD166 expression and/or activity. In some embodiments, the cells are associated with normal CD166 expression and/or activity. For example, the activatable anti-CD166 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.

[000279] The disclosure provides anti-CD166 antibodies, conjugated anti-CD166 antibodies, activatable anti-CD166 antibodies, and/or conjugated activatable anti-CD166 antibodies that are useful in methods of treating, preventing, delaying the progression of, ameliorating and/or alleviating a symptom of a disease or disorder in which diseased cells express CD166. In some embodiments, the diseased cells are associated with aberrant CD166 expression and/or activity. In some embodiments, the diseased cells are associated with normal CD166 expression and/or activity. For example, the activatable anti-CD166 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.

[000280] The activatable anti-CD166 antibodies and/or conjugated activatable anti-CD166 antibodies include an antibody or antigen-binding fragment thereof that specifically

binds CD166 coupled to a masking moiety (MM), such that coupling of the MM reduces the ability of the antibody or antigen-binding fragment thereof to bind CD166. In some embodiments, the MM is coupled via a sequence that includes a substrate for a protease, for example, a protease that is co-localized with CD166 at a treatment site in a subject.

[000281] Exemplary activatable anti-CD166 antibodies of the invention include, for example, activatable antibodies that include a heavy chain and a light chain that are, or are derived from, the heavy chain variable and light chain variable sequences shown below (CDR sequences, which were defined according to the AbM definition provided in the website of Dr. Andrew C. R. Martin, available at www_bioinf_org_uk/abs/) are shown in bold and underline):

muM9 VH:
QVTLKESGPGILQPSQTLSLTCSFS**GFSLSTYGMGVG**WIRQPSGKGLEWLA**NIWWSEDKH**YNSALKS
RLTISKDTSNNQVFLKISSVDTADTATYYCVQ**IDYGNDYAFTY**WGQGTLVTVSA (SEQ ID
NO: 119)

muM9 VL:
DIVMTQAAFSNPVTLGTSASISC**RSSKSLLHSNGITYLY**WYLQKPGQSPQLLIY**QMSNLAS**GVPDRF
SSSGSGTDFTLRISRVEAEDVGVYYC**AQNLELPYT**FGGGTKLEIKR (SEQ ID NO: 120)

huM9b VH:
QITLKESGPTLVKPTQTLTLTCTFS**GFSLSTYGMGVG**WIRQPPGKALEWLANIWWSEDKHYSP
SLKSRLTITKDTSKNQVVLTMTNMDPVDTATYYCVQIDYGNDYAFTYWGQGTLVTVSS (SEQ
ID NO: 121)

huM9c VH:
QITLKESGPTLVKPTQTLTLTCTFS**GFSLSTYGMGVG**WIRQPPGKALEWLANIWWSEDKHYSP
SLKSRLTITKDTSKNQVVLTITNVDPVDTATYYCVQIDYGNDYAFTYWGQGTLVTVSS (SEQ
ID NO: 122)

hM9vK-1 VL:
DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSN
LASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK
(SEQ ID NO: 123)

hM9vK-2 VL:
DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSN
LASGVPDRFSSSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK
(SEQ ID NO: 124)

hM9vK-3a VL:
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGITYLYWYLQKPGQSPQLLIYQMSN
RASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK
(SEQ ID NO: 125)

hM9vK-3b VL:
DIVMTQSPLSLPVTPGEPASISCRSSQSLLHSNGITYLYWYLQKPGQSPQLLIYQMSN
RASGVPDRFSSSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK
(SEQ ID NO: 126)

[000282] Exemplary activatable anti-CD166 antibodies of the invention include, for example, activatable antibodies that include a heavy chain and a light chain that are, or are derived from, the heavy chain and light chain variable shown below:

HuCD166_HcC
Amino Acid sequence
QITLKESGPTLVKPTQTLTLTCTFSGFSLSTYGMGVGWIRQPPGKALEWLANIWWSEDKHYSPSLKS
RLTITKDTSKNQVVLTITNVDPVDTATYYCVQIDYGNDYAFTYWGQGTLVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 239)

HuCD166_Lc1
DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC (SEQ ID NO: 240)

[000283] Exemplary activatable anti-CD166 antibodies of the invention include, for example, activatable antibodies that include a combination of a variable heavy chain complementarity determining region 1 (VH CDR1, also referred to herein as CDRH1) sequence, a variable heavy chain complementarity determining region 2 (VH CDR2, also referred to herein as CDRH2) sequence, a variable heavy chain complementarity determining region 3 (VH CDR3, also referred to herein as CDRH3) sequence, a variable light chain complementarity determining region 1 (VL CDR1, also referred to herein as CDRL1) sequence, a variable light chain complementarity determining region 2 (VL CDR2, also referred to herein as CDRL2) sequence, and a variable light chain complementarity determining region 3 (VL CDR3, also referred to herein as CDRL3) sequence, wherein at least one CDR sequence is selected from the group consisting of a VH CDR1 sequence comprising the amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); a VH CDR2 sequence comprising the amino acid sequence NIWWSEDKH (SEQ ID NO: 128); a VH CDR3 sequence comprising the amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); a VL CDR1 sequence comprising the amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); a VL CDR2 sequence comprising the amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and a VL CDR3 sequence comprising the amino acid sequence AQNLELPYT (SEQ ID NO: 134).

[000284] In some embodiments, the activatable anti-CD166 antibody includes a heavy chain that comprises or is derived from a heavy chain amino acid sequence shown in US Patent Application Publication Nos. 20150071937, 20090070890, and/or 20090203538, the contents of each of which are hereby incorporated by reference in their entirety.

[000285] In some embodiments, the activatable anti-CD166 antibody includes a heavy chain that comprises a VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 sequence that comprises or is derived from a CDR acid sequence shown in US Patent Application Publication Nos. 20150071937, 20090070890, and/or 20090203538, the contents of each of which are hereby incorporated by reference in their entirety.

[000286] In some embodiments, the activatable anti-CD166 antibody includes a heavy chain that comprises or is derived from a heavy chain amino acid sequence shown in Table 12. In some embodiments, the activatable anti-CD166 antibody includes a light chain that comprises or is derived from a heavy chain amino acid sequence shown in Table 12. In some embodiments, the activatable anti-CD166 antibody includes a heavy chain that

comprises or is derived from a heavy chain amino acid sequence shown in Table 12, and a light chain that comprises or is derived from a light chain amino acid sequence shown in Table 12. In some embodiments, the activatable anti-CD166 antibody includes a combination of heavy chain variable region and light chain variable region sequences from the combinations shown in Group A in Table 12. In some embodiments, the activatable anti-CD166 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group B in Table 12. In some embodiments, the activatable anti-CD166 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group C in Table 12. In some embodiments, the activatable anti-CD166 antibody includes the combination of heavy chain variable region and light chain variable region sequences shown in Group D in Table 12.

[000287] In some embodiments, the activatable anti-CD166 antibody includes a combination of the complementarity determining region (CDR) sequences of a heavy chain sequence from the heavy chain sequences shown in Group A Table 12. In some embodiments, the activatable anti-CD166 antibody includes a combination of the CDRs of a light chain sequence from the light chain sequences shown in Group A Table 12. In some embodiments, the activatable anti-CD166 antibody includes a combination of the CDRs of a heavy chain sequence from the heavy chain sequences shown in Group A Table 12 and the CDRs of a light chain sequence from the heavy chain sequences shown in Group A Table 12.

[000288] In some embodiments, the activatable anti-CD166 antibody includes a combination of CDRs of a heavy chain sequence from the heavy chain sequences shown in Group B Table 12. In some embodiments, the activatable anti-CD166 antibody includes a combination of the CDRs of a light chain sequence from the light chain sequences shown in Group B Table 12. In some embodiments, the activatable anti-CD166 antibody includes a combination of the CDRs of a heavy chain sequence from the heavy chain sequences shown in Group B Table 12 and the CDRs of a light chain sequence from the heavy chain sequences shown in Group B Table 12.

[000289] In some embodiments, the activatable anti-CD166 antibody includes a combination of the CDRs of a heavy chain sequence from the heavy chain sequences shown in Group C Table 12. In some embodiments, the activatable anti-CD166 antibody includes a combination of the CDRs of a light chain sequence from the light chain sequences shown in Group C Table 12. In some embodiments, the activatable anti-CD166 antibody includes a

combination of the CDRs of a heavy chain sequence from the heavy chain sequences shown in Group C Table 12 and the CDRs of a light chain sequence from the heavy chain sequences shown in Group C Table 12.

[000290] In some embodiments, the activatable anti-CD166 antibody includes a combination of the CDRs of a heavy chain sequence from the heavy chain sequences shown in Group D Table 12. In some embodiments, the activatable anti-CD166 antibody includes a combination of the CDRs of a light chain sequence from the light chain sequences shown in Group D Table 12. In some embodiments, the activatable anti-CD166 antibody includes a combination of the CDRs of a heavy chain sequence from the heavy chain sequences shown in Group D Table 12 and the CDRs of a light chain sequence from the heavy chain sequences shown in Group D Table 12.

Table 12. Variable Heavy Chain Region (VH) and Variable Light Chain Region (VL) Sequences for Activatable Antibodies that Bind CD166

Group A	
VH	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISR DNSKDTLYLQMNSLRAEDTAVYYCASRSLLDYWGQGTLVTVSS (SEQ ID NO: 249)
VH	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMGWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISR DNSKDTLYLQMNSLRAEDTAVYYCASRSLLDYWGQGTLVTVSS (SEQ ID NO: 250)
VL	NFMLTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPLLVIYGKNNRPSGIPDRFSGSSSGNTASL TITGAQAEDEADYYCNSRDSSGNPVFGGGTKVTVL (SEQ ID NO: 251)
Group B	
VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCARGGGVVEFWGQGTLVTVSS (SEQ ID NO: 252)
VL	DIRMTQSPSFLSASVGDRVTITCRASQDISSYFAWYQQKPGKAPKLLIYAASTLRSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQSYSTPRITFGQGTRLEIK (SEQ ID NO: 253)
Group C	
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSTISGSGGSTYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCARGIVATSWGQGTLVTVSR (SEQ ID NO: 254)
VH	EVQLVESGGGVVQPGGSLRLSCAASGFNFDVYGMNWVRQVPGKGLEWVSLINGDGGLRYYADSVKGRFTVSR DNSRNSLYLQMNSLRSEDTALYYCVKGNFQQWGQGTLVTVSR (SEQ ID NO: 255)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAMHWVRQAPGKGLEWVSLISGDGGSTYYADSVKDRFTISR DNSKNSLYLQMNSLRAEDTAVYYCARGNYFDYWGQGTLVTVSR (SEQ ID NO: 256)
VH	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSYISSSSSTIYYADSVKGRFTISR DNAKNSLYLQMNSLRDEDTAVYYCARVMPSYYYYYGMDVWGQGTTVTVSR (SEQ ID NO: 257)

VH	EVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKGRFTISR DNSKNTLYLQMNSLRAEDTAVYYCADYGMDVWGQGTTVTVSR (SEQ ID NO: 258)
VL	SYELTQPPSVSVAPGQTARITCGGNKIGSKSVHWYQQKQGQAPVLVIYLDRDRPSGIPERFSGSNSGNTATL TITRVEAEDEADYYCHLWDSGSDQVFGGGTKLTVLG (SEQ ID NO: 259)
VL	SYVLTQPPSVSVAPGKTARITCGGNNIGSKSVHWYQQKPGQAPVLVIYYDSDRPSGIPERFSGSNSGNTATL TISRVEAGDEADYYCQVWDSSSDHVVFGGGTKLTVLG (SEQ ID NO: 260)
VL	SYELTQPLSVSVALGQTARITCGGNNIGSKNVHWYQQKPGQAPVLVIYRDSNRPSGIPERFSGSNSGNTATL TISRAQAGDEADYYCQVWDSSVVFGGGTKLTVLG (SEQ ID NO: 261)
VL	NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSAPTTVIYEDSERPSGVPDRFSGSIDSSSN SASLTISGLKTQDEADYYCQSYDGVNWVFGGGTKLTVLG (SEQ ID NO: 262)
VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQSYSTPVTFGQGTKVEIK (SEQ ID NO: 263)
Group D	
VH / VL	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISR DNSKDTLYLQMNSLRAEDTAVYYCASRSLLDYWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQDPAVSVAL GQTVRITCQGDSLRSYYASWYQQKPGQAPLLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYY CNSRDSSGNPVFGGGTKVTVL (SEQ ID NO: 475)
VH / VL	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMGWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISR DNSKDTLYLQMNSLRAEDTAVYYCASRSLLDYWGQGTLVTVSSGGGGSGGGGSGGGGSNFMLTQDPAVSVAL GQTVRITCQGDSLRSYYASWYQQKPGQAPLLVIYGKNNRPSGIPDRFSGSSSGNTASLTITGAQAEDEADYY CNSRDSSGNPVFGGGTKVTVL (SEQ ID NO: 476)

[000291] In some embodiments, the activatable anti-CD166 antibody includes a CDR sequence shown in Table 13, a combination of VL CDR sequences (VL CDR1, VL CDR2, VL CDR3) selected from the group consisting of those combinations shown in a single row Table 13, a combination of VH CDR sequences (VH CDR1, VH CDR2, VH CDR3) selected from the group consisting of those combinations shown in Table 13, or a combination of VL CDR and VH CDR sequences (VL CDR1, VL CDR2, VL CDR3, VH CDR1, VH CDR2, VH CDR3) selected the group consisting of those combinations shown in Table 13.

Table 13. CDR Sequences for Antibodies and Activatable Antibodies that Bind CD166

VL		
CDR1 (SEQ ID NO)	CDR2 (SEQ ID NO)	CDR3 (SEQ ID NO)
QGDSLRSYYAS (264)	YGKNNRPS (265)	NSRDSSGNPV (266)

VH		
CDR1 (SEQ ID NO)	CDR2 (SEQ ID NO)	CDR3 (SEQ ID NO)
SYAMS (267)	AISGSGGSTYYADSVKG (268)	RSLLDY (269)

QGDSLRSYYAS (264)	YGKNNRPS (265)	NSRDSSGNPV (266)
RASQDISSYFA (270)	AASTLRS (271)	QQSYSTPRIT (272)
GGNKIGSKSVH (274)	LDRDRPS (275)	HLWDSGSD (276)
GGNNIGSKSVH (279)	YDSDRPS (280)	QVWDSSSDH (281)
GGNNIGSKNVH (285)	RDSNRPS (286)	QVWDSS (287)
TGSSGSIASNY VQ (291)	EDSERPS (292)	QSYDGVN (293)
RASQSISSYLN (297)	AASSLQS (298)	QQSYSTP (299)

SYAMG (477)	AISGSGGSTYYADSVKG (268)	RSLLDY (269)
SYAMS (267)	AISGSGGSTYYADSVKG (268)	GGGVVEF (273)
SYAMS (267)	TISGSGGSTYYADSVKG (277)	GIVATS (278)
VYGMN (282)	LINGDGGLRYYADSVKG (283)	GNFQQ (284)
DYAMH (288)	LISGDGGSTYYADSVKD (289)	GNYFDY (290)
SYSMN (294)	YISSSSSTIYYADSVKG (295)	VMPSYYYYYGMDV (296)
SYGMH (300)	VISYDGSNKYYADSVKG (301)	YGMDV (302)

[000292] In some embodiments, the activatable anti-CD166 antibody comprises or is derived from an antibody that is manufactured, secreted or otherwise produced by a hybridoma, such as, for example, the hybridoma(s) disclosed in US Patent Application Publication No. 20040048319 and deposited with American Type Culture Collection (ATCC) under deposit number PTA-4478. In some embodiments, the activatable anti-CD166 antibody comprises or is derived from an antibody that is manufactured, secreted or otherwise produced by a hybridoma, such as, for example, the hybridoma(s) disclosed in US Patent No. 6,022,540 and deposited with ATCC under deposit number HB 11789.

[000293] In some embodiments, the activatable anti-CD166 antibody comprises or is derived from an antibody that is manufactured, secreted or otherwise produced by a hybridoma, such as, for example, the hybridoma(s) disclosed in US Patent No. 5,998,172 and deposited with ATCC under deposit number HB 12136, HB 12137, HB 12138, HB12139, HB 12140, and/or HB 12141.

[000294] The anti-CD166 antibodies and the ABs in the activatable antibodies of the disclosure specifically bind a CD166 target, such as, for example, mammalian CD166, and/or human CD166. Also included in the disclosure are anti-CD166 antibodies and ABs that bind to the same CD166 epitope as an antibody of the disclosure and/or an activated activatable antibody described herein. Also included in the disclosure are anti-CD166 antibodies and ABs that compete with an anti-CD166 antibody and/or an activated anti-CD166 activatable antibody described herein for binding to a CD166 target, e.g., human CD166. Also included in the disclosure are anti-CD166 antibodies and ABs that cross-compete with an anti-CD166 antibody and/or an activated anti-CD166 activatable antibody described herein for binding to a CD166 target, e.g., human CD166.

[000295] The activatable anti-CD166 antibodies provided herein include a masking moiety. In some embodiments, the masking moiety is an amino acid sequence that is coupled or otherwise attached to the anti-CD166 antibody and is positioned within the activatable anti-CD166 antibody construct such that the masking moiety reduces the ability of the anti-CD166 antibody to specifically bind CD166. Suitable masking moieties are identified using any of a variety of known techniques. For example, peptide masking moieties are identified using the methods described in PCT Publication No. WO 2009/025846 by Daugherty et al., the contents of which are hereby incorporated by reference in their entirety.

[000296] The activatable anti-CD166 antibodies provided herein include a cleavable moiety. In some embodiments, the cleavable moiety includes an amino acid sequence that is a substrate for a protease, usually an extracellular protease. Suitable substrates are identified using any of a variety of known techniques. For example, peptide substrates are identified using the methods described in U.S. Patent No. 7,666,817 by Daugherty et al.; in U.S. Patent No. 8,563,269 by Stagliano et al.; and in PCT Publication No. WO 2014/026136 by La Porte et al., the contents of each of which are hereby incorporated by reference in their entirety. (*See also* Boulware et al. "Evolutionary optimization of peptide substrates for proteases that exhibit rapid hydrolysis kinetics." Biotechnol Bioeng. 106.3 (2010): 339-46).

[000297] Exemplary substrates include but are not limited to substrates cleavable by one or more of the following enzymes or proteases listed in Table 4.

Table 4: Exemplary Proteases and/or Enzymes

ADAMS, ADAMTS, *e.g.*	Cysteine proteinases, *e.g.*,	Serine proteases, *e.g.*,
ADAM8	Cruzipain	activated protein C
ADAM9	Legumain	Cathepsin A
ADAM10	Otubain-2	Cathepsin G
ADAM12		Chymase
ADAM15	KLKs, *e.g.*,	coagulation factor proteases
ADAM17/TACE	KLK4	(*e.g.*, FVIIa, FIXa, FXa, FXIa,
ADAMDEC1	KLK5	FXIIa)
ADAMTS1	KLK6	Elastase
ADAMTS4	KLK7	Granzyme B
ADAMTS5	KLK8	Guanidinobenzoatase
	KLK10	HtrA1
Aspartate proteases, *e.g.*,	KLK11	Human Neutrophil Elastase
BACE	KLK13	Lactoferrin
Renin	KLK14	Marapsin
		NS3/4A

Aspartic cathepsins, *e.g.*,	Metallo proteinases, *e.g.*,	PACE4
Cathepsin D	Meprin	Plasmin
Cathepsin E	Neprilysin	PSA
	PSMA	tPA
Caspases, *e.g.*,	BMP-1	Thrombin
Caspase 1		Tryptase
Caspase 2	MMPs, *e.g.*,	uPA
Caspase 3	MMP1	
Caspase 4	MMP2	Type II Transmembrane
Caspase 5	MMP3	Serine Proteases (TTSPs), *e.g.*,
Caspase 6	MMP7	DESC1
Caspase 7	MMP8	DPP-4
Caspase 8	MMP9	FAP
Caspase 9	MMP10	Hepsin
Caspase 10	MMP11	Matriptase-2
Caspase 14	MMP12	MT-SP1/Matriptase
	MMP13	TMPRSS2
Cysteine cathepsins, *e.g.*,	MMP14	TMPRSS3
Cathepsin B	MMP15	TMPRSS4
Cathepsin C	MMP16	
Cathepsin K	MMP17	
Cathepsin L	MMP19	
Cathepsin S	MMP20	
Cathepsin V/L2	MMP23	
Cathepsin X/Z/P	MMP24	
	MMP26	
	MMP27	

[000298] The activatable anti-CD166 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. The activatable anti-CD166 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-CD166 antibodies remain masked until proteolytically activated at the site of disease. Starting with an anti-CD166 antibody as a parental therapeutic antibody, the activatable anti-CD166 antibodies of the invention were engineered by coupling the antibody to an inhibitory mask through a linker that incorporates a protease substrate.

[000299] When the AB is modified with a MM and is in the presence of the target, specific binding of the AB to its target is reduced or inhibited, as compared to the specific

binding of the AB not modified with an MM or the specific binding of the parental AB to the target.

[000300] The K_d of the AB modified with a MM towards the target is at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times greater than the K_d of the AB not modified with an MM or of the parental AB towards the target. Conversely, the binding affinity of the AB modified with a MM towards the target is at least 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times lower than the binding affinity of the AB not modified with an MM or of the parental AB towards the target.

[000301] The dissociation constant (K_d) of the MM towards the AB is generally greater than the K_d of the AB towards the target. The K_d of the MM towards the AB can be at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 100,000, 1,000,000 or even 10,000,000 times greater than the K_d of the AB towards the target. Conversely, the binding affinity of the MM towards the AB is generally lower than the binding affinity of the AB towards the target. The binding affinity of MM towards the AB can be at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 100,000, 1,000,000 or even 10,000,000 times lower than the binding affinity of the AB towards the target.

[000302] In some embodiments, the dissociation constant (Kd) of the MM towards the AB is approximately equal to the Kd of the AB towards the target. In some embodiments, the dissociation constant (Kd) of the MM towards the AB is no more than the dissociation constant of the AB towards the target.

[000303] In some embodiments, the dissociation constant (Kd) of the MM towards the AB is less than the dissociation constant of the AB towards the target.

[000304] In some embodiments, the dissociation constant (Kd) of the MM towards the AB is greater than the dissociation constant of the AB towards the target.

[000305] In some embodiments, the MM has a Kd for binding to the AB that is no more than the Kd for binding of the AB to the target.

[000306] In some embodiments, the MM has a Kd for binding to the AB that is no less than the Kd for binding of the AB to the target.

[000307] In some embodiments, the MM has a Kd for binding to the AB that is approximately equal to the Kd for binding of the AB to the target.

[000308] In some embodiments, the MM has a Kd for binding to the AB that is less than the Kd for binding of the AB to the target.

[000309] In some embodiments, the MM has a Kd for binding to the AB that is greater than the Kd for binding of the AB to the target.

[000310] In some embodiments, the MM has a Kd for binding to the AB that is no more than 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 fold greater than the Kd for binding of the AB to the target. In some embodiments, the MM has a Kd for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold greater than the Kd for binding of the AB to the target.

[000311] In some embodiments, the MM has an affinity for binding to the AB that is less than the affinity of binding of the AB to the target.

[000312] In some embodiments, the MM has an affinity for binding to the AB that is no more than the affinity of binding of the AB to the target.

[000313] In some embodiments, the MM has an affinity for binding to the AB that is approximately equal of the affinity of binding of the AB to the target.

[000314] In some embodiments, the MM has an affinity for binding to the AB that is no less than the affinity of binding of the AB to the target.

[000315] In some embodiments, the MM has an affinity for binding to the AB that is greater than the affinity of binding of the AB to the target.

[000316] In some embodiments, the MM has an affinity for binding to the AB that is 2, 3, 4, 5, 10, 25, 50, 100, 250, 500, or 1,000 less than the affinity of binding of the AB to the target. I In some embodiments, the MM has an affinity for binding to the AB that is between 1-5, 2-5, 2-10, 5-10, 5-20, 5-50, 5-100, 10-100, 10-1,000, 20-100, 20-1000, or 100-1,000 fold less than the affinity of binding of the AB to the target. In some embodiments, the MM has an affinity for binding to the AB that is 2 to 20 fold less than the affinity of binding of the AB to the target. In some embodiments, a MM not covalently linked to the

AB and at equimolar concentration to the AB does not inhibit the binding of the AB to the target.

[000317] When the AB is modified with a MM and is in the presence of the target specific binding of the AB to its target is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM or the specific binding of the parental AB to the target. When compared to the binding of the AB not modified with an MM or the binding of the parental AB to the target the AB's ability to bind the target when modified with an MM can be reduced by at least 50%, 60%, 70%, 80%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and even 100% for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours, or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or more when measured *in vivo* or in an *in vitro* assay.

[000318] The MM inhibits the binding of the AB to the target. The MM binds the antigen binding domain of the AB and inhibits binding of the AB to the target. The MM can sterically inhibit the binding of the AB to the target. The MM can allosterically inhibit the binding of the AB to its target. In these embodiments when the AB is modified or coupled to a MM and in the presence of target there is no binding or substantially no binding of the AB to the target, or no more than 0.001%, 0.01%, 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, or 50% binding of the AB to the target, as compared to the binding of the AB not modified with an MM, the parental AB, or the AB not coupled to an MM to the target, for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours, or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or longer when measured *in vivo* or in an *in vitro* assay.

[000319] When an AB is coupled to or modified by a MM, the MM 'masks' or reduces or otherwise inhibits the specific binding of the AB to the target. When an AB is coupled to or modified by a MM, such coupling or modification can effect a structural change that reduces or inhibits the ability of the AB to specifically bind its target.

[000320] An AB coupled to or modified with an MM can be represented by the following formulae (in order from an amino (N) terminal region to carboxyl (C) terminal region:

(MM)-(AB)

(AB)-(MM)

(MM)-L-(AB)

(AB)-L-(MM)

where MM is a masking moiety, the AB is an antibody or antibody fragment thereof, and the L is a linker. In many embodiments, it may be desirable to insert one or more linkers, *e.g.*, flexible linkers, into the composition so as to provide for flexibility.

[000321] In certain embodiments, the MM is not a natural binding partner of the AB. In some embodiments, the MM contains no or substantially no homology to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% similar to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 25% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 20% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 10% identical to any natural binding partner of the AB.

[000322] In some embodiments, the activatable antibodies include an AB that is modified by an MM and also includes one or more cleavable moieties (CM). Such activatable antibodies exhibit activatable/switchable binding, to the AB's target. Activatable antibodies generally include an antibody or antibody fragment (AB), modified by or coupled to a masking moiety (MM) and a modifiable or cleavable moiety (CM). In some embodiments, the CM contains an amino acid sequence that serves as a substrate for at least one protease.

[000323] The elements of the activatable antibodies are arranged so that the MM and CM are positioned such that in a cleaved (or relatively active) state and in the presence of a target, the AB binds a target while the activatable antibody is in an uncleaved (or relatively inactive) state in the presence of the target, specific binding of the AB to its target is reduced or inhibited. The specific binding of the AB to its target can be reduced due to the inhibition or masking of the AB's ability to specifically bind its target by the MM.

[000324] The K_d of the AB modified with a MM and a CM towards the target is at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000,

1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times greater than the K_d of the AB not modified with an MM and a CM or of the parental AB towards the target. Conversely, the binding affinity of the AB modified with a MM and a CM towards the target is at least 5, 10, 25, 50, 100, 250, 500, 1,000, 2,500, 5,000, 10,000, 50,000, 100,000, 500,000, 1,000,000, 5,000,000, 10,000,000, 50,000,000 or greater, or between 5-10, 10-100, 10-1,000, 10-10,000, 10-100,000, 10-1,000,000, 10-10,000,000, 100-1,000, 100-10,000, 100-100,000, 100-1,000,000, 100-10,000,000, 1,000-10,000, 1,000-100,000, 1,000-1,000,000, 1000-10,000,000, 10,000-100,000, 10,000-1,000,000, 10,000-10,000,000, 100,000-1,000,000, or 100,000-10,000,000 times lower than the binding affinity of the AB not modified with an MM and a CM or of the parental AB towards the target.

[000325] When the AB is modified with a MM and a CM and is in the presence of the target but not in the presence of a modifying agent (for example at least one protease), specific binding of the AB to its target is reduced or inhibited, as compared to the specific binding of the AB not modified with an MM and a CM or of the parental AB to the target. When compared to the binding of the parental AB or the binding of an AB not modified with an MM and a CM to its target, the AB's ability to bind the target when modified with an MM and a CM can be reduced by at least 50%, 60%, 70%, 80%, 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and even 100% for at least 2, 4, 6, 8, 12, 28, 24, 30, 36, 48, 60, 72, 84, or 96 hours or 5, 10, 15, 30, 45, 60, 90, 120, 150, or 180 days, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months or longer when measured *in vivo* or in an *in vitro* assay.

[000326] As used herein, the term cleaved state refers to the condition of the activatable antibodies following modification of the CM by at least one protease. The term uncleaved state, as used herein, refers to the condition of the activatable antibodies in the absence of cleavage of the CM by a protease. As discussed above, the term "activatable antibodies" is used herein to refer to an activatable antibody in both its uncleaved (native) state, as well as in its cleaved state. It will be apparent to the ordinarily skilled artisan that in some embodiments a cleaved activatable antibody may lack an MM due to cleavage of the CM by protease, resulting in release of at least the MM (*e.g.*, where the MM is not joined to the activatable antibodies by a covalent bond (*e.g.*, a disulfide bond between cysteine residues).

[000327] By activatable or switchable is meant that the activatable antibody exhibits a first level of binding to a target when the activatable antibody is in a inhibited, masked or

uncleaved state (*i.e.*, a first conformation), and a second level of binding to the target in the uninhibited, unmasked and/or cleaved state (*i.e.*, a second conformation), where the second level of target binding is greater than the first level of binding. In general, the access of target to the AB of the activatable antibody is greater in the presence of a cleaving agent capable of cleaving the CM, i.e., a protease, than in the absence of such a cleaving agent. Thus, when the activatable antibody is in the uncleaved state, the AB is inhibited from target binding and can be masked from target binding (*i.e.*, the first conformation is such the AB cannot bind the target), and in the cleaved state the AB is not inhibited or is unmasked to target binding.

[000328] The CM and AB of the activatable antibodies are selected so that the AB represents a binding moiety for a given target, and the CM represents a substrate for a protease. In some embodiments, the protease is co-localized with the target at a treatment site or diagnostic site in a subject. As used herein, co-localized refers to being at the same site or relatively close nearby. In some embodiments, a protease cleaves a CM yielding an activated antibody that binds to a target located nearby the cleavage site. The activatable antibodies disclosed herein find particular use where, for example, a protease capable of cleaving a site in the CM, i.e., a protease, is present at relatively higher levels in target-containing tissue of a treatment site or diagnostic site than in tissue of non-treatment sites (for example in healthy tissue). In some embodiments, a CM of the disclosure is also cleaved by one or more other proteases. In some embodiments, it is the one or more other proteases that is co-localized with the target and that is responsible for cleavage of the CM *in vivo*.

[000329] In some embodiments activatable antibodies provide for reduced toxicity and/or adverse side effects that could otherwise result from binding of the AB at non-treatment sites if the AB were not masked or otherwise inhibited from binding to the target.

[000330] In general, an activatable antibody can be designed by selecting an AB of interest and constructing the remainder of the activatable antibody so that, when conformationally constrained, the MM provides for masking of the AB or reduction of binding of the AB to its target. Structural design criteria can be to be taken into account to provide for this functional feature.

[000331] Activatable antibodies exhibiting a switchable phenotype of a desired dynamic range for target binding in an inhibited versus an uninhibited conformation are provided. Dynamic range generally refers to a ratio of (a) a maximum detected level of a

parameter under a first set of conditions to (b) a minimum detected value of that parameter under a second set of conditions. For example, in the context of an activatable antibody, the dynamic range refers to the ratio of (a) a maximum detected level of target protein binding to an activatable antibody in the presence of at least one protease capable of cleaving the CM of the activatable antibodies to (b) a minimum detected level of target protein binding to an activatable antibody in the absence of the protease. The dynamic range of an activatable antibody can be calculated as the ratio of the dissociation constant of an activatable antibody cleaving agent (*e.g.*, enzyme) treatment to the dissociation constant of the activatable antibodies cleaving agent treatment. The greater the dynamic range of an activatable antibody, the better the switchable phenotype of the activatable antibody. Activatable antibodies having relatively higher dynamic range values (*e.g.*, greater than 1) exhibit more desirable switching phenotypes such that target protein binding by the activatable antibodies occurs to a greater extent (*e.g.*, predominantly occurs) in the presence of a cleaving agent (*e.g.*, enzyme) capable of cleaving the CM of the activatable antibodies than in the absence of a cleaving agent.

[000332] Activatable antibodies can be provided in a variety of structural configurations. Exemplary formulae for activatable antibodies are provided below. It is specifically contemplated that the N- to C-terminal order of the AB, MM and CM may be reversed within an activatable antibody. It is also specifically contemplated that the CM and MM may overlap in amino acid sequence, *e.g.*, such that the CM is contained within the MM.

[000333] For example, activatable antibodies can be represented by the following formula (in order from an amino (N) terminal region to carboxyl (C) terminal region:

(MM)-(CM)-(AB)

(AB)-(CM)-(MM)

where MM is a masking moiety, CM is a cleavable moiety, and AB is an antibody or fragment thereof. It should be noted that although MM and CM are indicated as distinct components in the formulae above, in all exemplary embodiments (including formulae) disclosed herein it is contemplated that the amino acid sequences of the MM and the CM could overlap, *e.g.*, such that the CM is completely or partially contained within the MM. In addition, the formulae above provide for additional amino acid sequences that may be positioned N-terminal or C-terminal to the activatable antibodies elements.

[000334] In certain embodiments, the MM is not a natural binding partner of the AB. In some embodiments, the MM contains no or substantially no homology to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% similar to any natural binding partner of the AB. In some embodiments, the MM is no more than 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or 80% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 50% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 25% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 20% identical to any natural binding partner of the AB. In some embodiments, the MM is no more than 10% identical to any natural binding partner of the AB.

[000335] In many embodiments it may be desirable to insert one or more linkers, *e.g.*, flexible linkers, into the activatable antibody construct so as to provide for flexibility at one or more of the MM-CM junction, the CM-AB junction, or both. For example, the AB, MM, and/or CM may not contain a sufficient number of residues (*e.g.*, Gly, Ser, Asp, Asn, especially Gly and Ser, particularly Gly) to provide the desired flexibility. As such, the switchable phenotype of such activatable antibody constructs may benefit from introduction of one or more amino acids to provide for a flexible linker. In addition, as described below, where the activatable antibody is provided as a conformationally constrained construct, a flexible linker can be operably inserted to facilitate formation and maintenance of a cyclic structure in the uncleaved activatable antibody.

[000336] For example, in certain embodiments an activatable antibody comprises one of the following formulae (where the formula below represent an amino acid sequence in either N- to C-terminal direction or C- to N-terminal direction):

(MM)-L1-(CM)-(AB)

(MM)-(CM)-L2-(AB)

(MM)-L1-(CM)-L2-(AB)

wherein MM, CM, and AB are as defined above; wherein L1 and L2 are each independently and optionally present or absent, are the same or different flexible linkers that include at least 1 flexible amino acid (*e.g.*, Gly). In addition, the formulae above provide for additional amino acid sequences that may be positioned N-terminal or C-terminal to the activatable antibodies elements. Examples include, but are not limited to, targeting moieties (*e.g.*, a

ligand for a receptor of a cell present in a target tissue) and serum half-life extending moieties (*e.g.*, polypeptides that bind serum proteins, such as immunoglobulin (*e.g.*, IgG) or serum albumin (*e.g.*, human serum albumin (HAS)).

[000337] The CM is specifically cleaved by at least one protease at a rate of about 0.001-1500 x 10^4 $M^{-1}S^{-1}$ or at least 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2.5, 5, 7.5, 10, 15, 20, 25, 50, 75, 100, 125, 150, 200, 250, 500, 750, 1000, 1250, or 1500 x 10^4 $M^{-1}S^{-1}$. In some embodiments, the CM is specifically cleaved at a rate of about 100,000 $M^{-1}S^{-1}$. In some embodiments, the CM is specifically cleaved at a rate from about 1x10E2 to about 1x10E6 $M^{-1}S^{-1}$ (i.e., from about 1x10^2 to about 1x10^6 $M^{-1}S^{-1}$).

[000338] For specific cleavage by an enzyme, contact between the enzyme and CM is made. When the activatable antibody comprising an AB coupled to a MM and a CM is in the presence of target and sufficient enzyme activity, the CM can be cleaved. Sufficient enzyme activity can refer to the ability of the enzyme to make contact with the CM and effect cleavage. It can readily be envisioned that an enzyme may be in the vicinity of the CM but unable to cleave because of other cellular factors or protein modification of the enzyme.

[000339] Linkers suitable for use in compositions described herein are generally ones that provide flexibility of the modified AB or the activatable antibodies to facilitate the inhibition of the binding of the AB to the target. Such linkers are generally referred to as flexible linkers. Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (*e.g.*, Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length.

[000340] Exemplary flexible linkers include glycine polymers (G)n, glycine-serine polymers (including, for example, (GS)n, (GSGGS)n (SEQ ID NO: 1) and (GGGS)n (SEQ ID NO: 2), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are relatively unstructured, and therefore may be able to serve as a neutral tether between components. Glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, Rev. Computational Chem. 11173-142 (1992)). Exemplary flexible linkers include, but are not

limited to Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Ser-Gly-Gly (SEQ ID NO: 4), Gly-Ser-Gly-Ser-Gly (SEQ ID NO: 5), Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 7), Gly-Ser-Ser-Ser-Gly (SEQ ID NO: 8), and the like. The ordinarily skilled artisan will recognize that design of an activatable antibodies can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure to provide for a desired activatable antibodies structure.

[000341] The disclosure also provides compositions and methods that include an activatable anti-CD166 antibody that includes an antibody or antibody fragment (AB) that specifically binds CD166, where the AB is coupled to a masking moiety (MM) that decreases the ability of the AB to bind its target. In some embodiments, the activatable anti-CD166 antibody further includes a cleavable moiety (CM) that is a substrate for a protease. The compositions and methods provided herein enable the attachment of one or more agents to one or more cysteine residues in the AB without compromising the activity (e.g., the masking, activating or binding activity) of the activatable anti-CD166 antibody. In some embodiments, the compositions and methods provided herein enable the attachment of one or more agents to one or more cysteine residues in the AB without reducing or otherwise disturbing one or more disulfide bonds within the MM. The compositions and methods provided herein produce an activatable anti-CD166 antibody that is conjugated to one or more agents, *e.g.*, any of a variety of therapeutic, diagnostic and/or prophylactic agents, for example, in some embodiments, without any of the agent(s) being conjugated to the MM of the activatable anti-CD166 antibody. The compositions and methods provided herein produce conjugated activatable anti-CD166 antibodies in which the MM retains the ability to effectively and efficiently mask the AB of the activatable antibody in an uncleaved state. The compositions and methods provided herein produce conjugated activatable anti-CD166 antibodies in which the activatable antibody is still activated, *i.e.*, cleaved, in the presence of a protease that can cleave the CM.

[000342] The activatable anti-CD166 antibodies have at least one point of conjugation for an agent, but in the methods and compositions provided herein less than all possible points of conjugation are available for conjugation to an agent. In some embodiments, the one or more points of conjugation are sulfur atoms involved in disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms involved in interchain disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur

atoms involved in interchain sulfide bonds, but not sulfur atoms involved in intrachain disulfide bonds. In some embodiments, the one or more points of conjugation are sulfur atoms of cysteine or other amino acid residues containing a sulfur atom. Such residues may occur naturally in the antibody structure or may be incorporated into the antibody by site-directed mutagenesis, chemical conversion, or mis-incorporation of non-natural amino acids.

[000343] Also provided are methods of preparing a conjugate of an activatable anti-CD166 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 is provided. The method generally includes 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-CD166 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.

[000344] In yet other embodiments, a method of reducing and conjugating an agent, e.g., a drug, to an activatable anti-CD166 antibody resulting in selectivity in the placement of the agent is provided. The method generally includes partially reducing the activatable anti-CD166 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-CD166 antibody will vary depending on the activatable antibody. In some embodiments, the ratio of reducing agent to activatable anti-CD166 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. In some embodiments, the ratio is in a range from about 8:1 to about 1:1. In some embodiments, the ratio is in a range of from about 2.5:1 to 1:1.

[000345] In some embodiments, a method of reducing interchain disulfide bonds in the AB of an activatable anti-CD166 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. In some embodiments, the ratio is in a range from about 8:1 to about 1:1. In some embodiments, the ratio is in a range of from about 2.5:1 to 1:1.

[000346] 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.

[000347] The invention also provides partially reduced activatable anti-CD166 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 CD166, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the CD166 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.

[000348] The disclosure also provides partially reduced activatable 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 the target, e.g., CD166, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the target, and a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for at least one 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.

[000349] In yet other embodiments, a method of reducing and conjugating an agent, e.g., a drug, to an activatable anti-CD166 antibody resulting in selectivity in the placement of the agent by providing an activatable anti-CD166 antibody with a defined number and positions of lysine and/or cysteine residues. In some embodiments, the defined number of lysine and/or cysteine residues is higher or lower than the number of corresponding residues in the amino acid sequence of the parent antibody or activatable antibody. In some embodiments, the defined number of lysine and/or cysteine residues may result in a defined number of agent equivalents that can be conjugated to the anti-CD166 antibody or activatable anti-CD166 antibody. In some embodiments, the defined number of lysine and/or cysteine residues may result in a defined number of agent equivalents that can be conjugated to the anti-CD166 antibody or activatable anti-CD166 antibody in a site-specific manner. In some embodiments, the modified activatable antibody is modified with one or more non-natural amino acids in a site-specific manner, thus in some embodiments limiting the conjugation of the agents to only the sites of the non-natural amino acids. In some embodiments, the anti-CD166 antibody or activatable anti-CD166 antibody with a defined number and positions of lysine and/or cysteine residues may be partially reduced with a reducing agent as discussed herein 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.

[000350] In some embodiments, the activatable 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 anti-inflammatory and/or an antineoplastic agent. In such embodiments, the agent is conjugated to a carbohydrate moiety of the activatable antibody, for example, 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.

[000351] 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).

[000352] 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

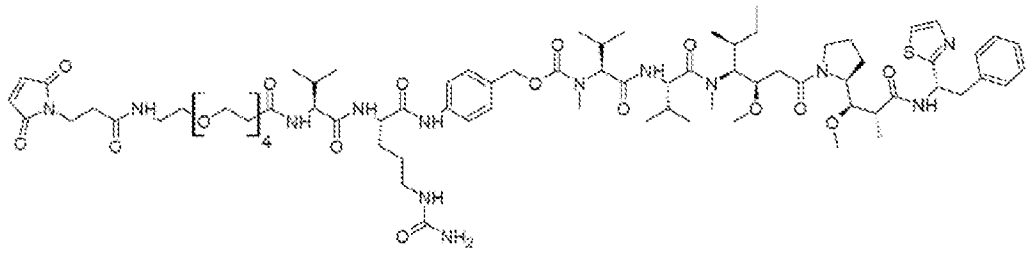
containing a fluorescent marker or enzymatic activity that can be detected by optical or calorimetric methods), one or more radioisotopes or radionuclides, one or more fluorescent labels, one or more enzymatic labels, and/or one or more chemiluminescent agents. In some embodiments, detectable moieties are attached by spacer molecules.

[000353] The disclosure also pertains to immunoconjugates comprising an antibody conjugated to 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, dolastatins and derivatives thereof (*e.g.* auristatin E, AFP, MMAF, MMAE, MMAD, DMAF, DMAE). For example, the agent is monomethyl auristatin E (MMAE) or monomethyl auristatin D (MMAD). In some embodiments, the agent is an agent selected from the group listed in Table 5. 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.

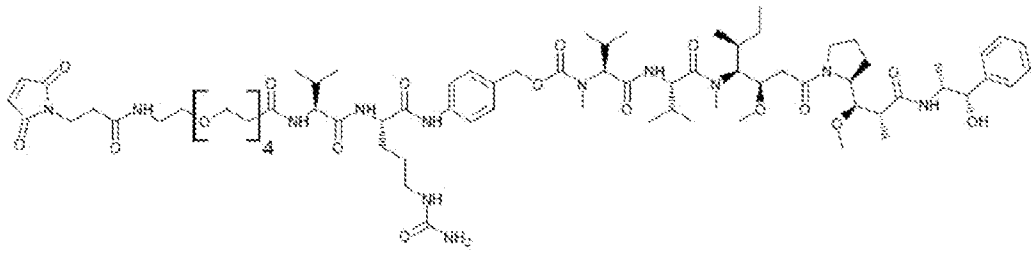
[000354] In some embodiments, the agent is linked to the AB using a maleimide caproyl-valine-citrulline linker or a maleimide PEG-valine-citrulline linker. In some embodiments, the agent is linked to the AB using a maleimide caproyl-valine-citrulline linker. In some embodiments, the agent is linked to the AB using a maleimide PEG-valine-citrulline linker In some embodiments, the agent is monomethyl auristatin D (MMAD) linked to the AB using a maleimide PEG-valine-citrulline-para-aminobenzyloxycarbonyl linker, and this linker payload construct is referred to herein as "vc-MMAD." In some embodiments, the agent is monomethyl auristatin E (MMAE) linked to the AB using a maleimide PEG-valine-citrulline-para-aminobenzyloxycarbonyl linker, and this linker payload construct is referred to herein as "vc-MMAE." In some embodiments, the agent is linked to the AB using a maleimide PEG-valine-citrulline linker In some embodiments, the agent is monomethyl auristatin D (MMAD) linked to the AB using a maleimide bis-PEG-valine-citrulline-para-aminobenzyloxycarbonyl linker, and this linker payload construct is

referred to herein as "PEG2-vc-MMAD." The structures of vc-MMAD, vc-MMAE, and PEG2-vc-MMAD are shown below:

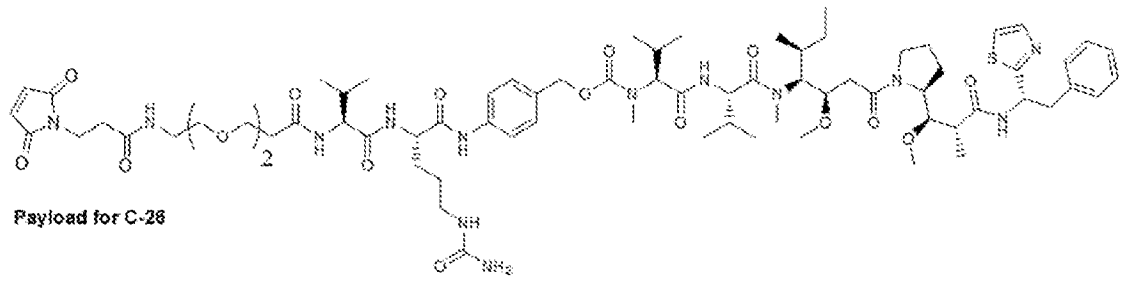
vc-MMAD:



vc-MMAE:



PEG2-vc-MMAD:



[000355] The disclosure also provides conjugated activatable antibodies that include an activatable antibody linked to monomethyl auristatin D (MMAD) payload, wherein the activatable antibody includes an antibody or an antigen binding fragment thereof (AB) that specifically binds to a target, a masking moiety (MM) that inhibits the binding of the AB of the activatable antibody in an uncleaved state to the target, and cleavable moiety (CM) coupled to the AB, and the CM is a polypeptide that functions as a substrate for at least one MMP protease.

[000356] In some embodiments, the MMAD-conjugated activatable antibody can be conjugated using any of 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.

[000357] In some embodiments, the MMAD payload is conjugated to the AB via a linker. In some embodiments, the MMAD payload is conjugated to a cysteine in the AB via a linker. In some embodiments, the MMAD payload is conjugated to a lysine in the AB via a linker. In some embodiments, the MMAD payload is conjugated to another residue of the AB via a linker, such as those residues disclosed herein. In some embodiments, the linker is a thiol-containing linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the linker is a non-cleavable linker. In some embodiments, the linker is selected from the group consisting of the linkers shown in Tables 6 and 7. In some embodiments, the activatable antibody and the MMAD payload are linked via a maleimide caproyl-valine-citrulline linker. In some embodiments, the activatable antibody and the MMAD payload are linked via a maleimide PEG-valine-citrulline linker. In some embodiments, the activatable antibody and the MMAD payload are linked via a maleimide caproyl-valine-citrulline-para-aminobenzyloxycarbonyl linker. In some embodiments, the activatable antibody and the MMAD payload are linked via a maleimide PEG-valine-citrulline-para-aminobenzyloxycarbonyl linker. In some embodiments, the MMAD payload is conjugated to the AB using the partial reduction and conjugation technology disclosed herein.

[000358] In some embodiments, the polyethylene glycol (PEG) component of a linker of the present disclosure is formed from 2 ethylene glycol monomers, 3 ethylene glycol monomers, 4 ethylene glycol monomers, 5 ethylene glycol monomers, 6 ethylene glycol monomers, 7 ethylene glycol monomers 8 ethylene glycol monomers, 9 ethylene glycol monomers, or at least 10 ethylene glycol monomers. In some embodiments of the present disclosure, the PEG component is a branched polymer. In some embodiments of the present disclosure, the PEG component is an unbranched polymer. In some embodiments, the PEG polymer component is functionalized with an amino group or derivative thereof, a carboxyl group or derivative thereof, or both an amino group or derivative thereof and a carboxyl group or derivative thereof.

[000359] In some embodiments, the PEG component of a linker of the present disclosure is an amino-tetra-ethylene glycol-carboxyl group or derivative thereof. In some

embodiments, the PEG component of a linker of the present disclosure is an amino-tri-ethylene glycol-carboxyl group or derivative thereof. In some embodiments, the PEG component of a linker of the present disclosure is an amino-di-ethylene glycol-carboxyl group or derivative thereof. In some embodiments, an amino derivative is the formation of an amide bond between the amino group and a carboxyl group to which it is conjugated. In some embodiments, a carboxyl derivative is the formation of an amide bond between the carboxyl group and an amino group to which it is conjugated. In some embodiments, a carboxyl derivative is the formation of an ester bond between the carboxyl group and an hydroxyl group to which it is conjugated.

[000360] 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, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, 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, ^{131}In, ^{90}Y, and ^{186}Re.

[000361] 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 glutareldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 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).

[000362] Table 5 lists some of the exemplary pharmaceutical agents that may be employed in the herein described disclosure but in no way is meant to be an exhaustive list.

Table 5: Exemplary Pharmaceutical Agents for Conjugation

CYTOTOXIC AGENTS

Auristatins
Auristatin E
Monomethyl auristatin D (MMAD)
Monomethyl auristatin E (MMAE)
Desmethyl auristatin E (DMAE)
Auristatin F
Monomethyl auristatin F (MMAF)
Desmethyl auristatin F (DMAF)
Auristatin derivatives, *e.g.*, amides thereof
Auristatin tyramine
Auristatin quinoline
Dolastatins
Dolastatin derivatives
Dolastatin 16 DmJ
Dolastatin 16 Dpv
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

Turbostatin
Phenstatins
Hydroxyphenstatin
Spongistatin 5
Spongistatin 7
Halistatin 1
Halistatin 2
Halistatin 3
Modified Bryostatins
Halocomstatins
Pyrrolobenzimidazoles (PBI)
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)

RADIOPHARMACEUTICALS
^{125}I
^{131}I
^{89}Zr
^{111}In
^{123}I
^{131}I
^{99}mTc
^{201}Tl

Alkeran
Velban
Oncovin
Fluorouracil
Methotrexate
Thiotepa
Bisantrene
Novantrone
Thioguanine
Procarabizine
Cytarabine

ANTI-BACTERIALS
Aminoglycosides
Streptomycin
Neomycin
Kanamycin
Amikacin
Gentamicin
Tobramycin
Streptomycin B
Spectinomycin
Ampicillin
Sulfanilamide
Polymyxin
Chloramphenicol

^{133}Xe
^{11}C
^{62}Cu
^{18}F
^{68}Ga
^{13}N
^{15}O
^{38}K
^{82}Rb
^{99}mTc (Technetium)

HEAVY METALS
Barium
Gold
Platinum

ANTI-MYCOPLASMALS
Tylosine
Spectinomycin

[000363] 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 disclosure. (*See, for example*, "Conjugate Vaccines", Contributions to Microbiology and Immunology, J. M. Cruse and R. E. Lewis, Jr (eds), Carger Press, New York, (1989), the entire contents of which are incorporated herein by reference).

[000364] 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 disclosure, 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).

[000365] In some embodiments, in addition to the compositions and methods provided herein, the conjugated activatable antibody can also 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 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.

[000366] 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. In some embodiments, suitable linkers include: (i) EDC (1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride; (ii) SMPT (4-succinimidyloxycarbonyl-alpha-methyl-alpha-(2-pridyl-dithio)-toluene (Pierce Chem. Co., Cat. (21558G); (iii) SPDP (succinimidyl-6 [3-(2-pyridyldithio) propionamido]hexanoate (Pierce Chem. Co., Cat #21651G); (iv) Sulfo-LC-SPDP (sulfosuccinimidyl 6 [3-(2-pyridyldithio)-propianamide] hexanoate (Pierce Chem. Co. Cat. #2165-G); and (v) sulfo-NHS (N-hydroxysulfo-succinimide: Pierce Chem. Co., Cat. #24510) conjugated to EDC. Additional linkers include, but are not limited to, SMCC ((succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate), sulfo-SMCC (sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate), SPDB (N-succinimidyl-4-(2-pyridyldithio) butanoate), or sulfo-SPDB (N-succinimidyl-4-(2-pyridyldithio)-2-sulfo butanoate).

[000367] The linkers described above contain components that have different attributes, thus leading to conjugates with differing physio-chemical properties. For example, sulfo-NHS esters of alkyl carboxylates are more stable than sulfo-NHS esters of aromatic carboxylates. NHS-ester containing linkers are less soluble than sulfo-NHS esters. Further, 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. Sulfo-NHS, in particular, can enhance the stability of carbodimide couplings. Carbodimide couplings (such as EDC) when used in conjunction with sulfo-NHS, forms esters that are more resistant to hydrolysis than the carbodimide coupling reaction alone.

[000368] 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, *i.e.*, cleavable or non-cleavable, or the two or more linkers are different, *i.e.*, at least one cleavable and at least one non-cleavable.

[000369] The present disclosure 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 disclosure, 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.

[000370] According to the present disclosure, 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).

[000371] According to the present disclosure, 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.

[000372] According to the present disclosure, suitable linkers for attachment to neither oxidized nor reduced Abs 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.

[000373] 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 some embodiments, the AB is first attached to the MM, CM and associated linkers and then attached to the linker for conjugation purposes.

[000374] *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.

[000375] In some embodiments, 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.

[000376] *Cleavable Linkers*: Peptide linkers that are susceptible to cleavage by enzymes of the complement system, such as but not limited to u-plasminogen activator, tissue plasminogen activator, trypsin, plasmin, or another enzyme having proteolytic activity may be used in one embodiment of the present disclosure. According to one method of the present disclosure, 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 disclosure, an agent is attached via a linker susceptible to cleavage by enzymes having a proteolytic activity such as a u-plasminogen activator, 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 5.

[000377] Non-limiting examples of cleavable linker sequences are provided in Table 6.

Table 6: Exemplary Linker Sequences for Conjugation

Types of Cleavable Sequences	Amino Acid Sequence
Plasmin cleavable sequences	
Pro-urokinase	PRFKIIGG (SEQ ID NO: 89) PRFRIIGG (SEQ ID NO: 90)
TGFβ	SSRHRRALD (SEQ ID NO: 91)
Plasminogen	RKSSIIIRMRDVVL (SEQ ID NO: 92)
Staphylokinase	SSSFDKGKYKKGDDA (SEQ ID NO: 93) SSSFDKGKYKRGDDA (SEQ ID NO: 94)
Factor Xa cleavable sequences	IEGR (SEQ ID NO: 95) IDGR (SEQ ID NO: 96) GGSIDGR (SEQ ID NO: 97)
MMP cleavable sequences	
Gelatinase A	PLGLWA (SEQ ID NO: 98)
Collagenase cleavable sequences	
Calf skin collagen (α1(I) chain)	GPQGIAGQ (SEQ ID NO: 99)
Calf skin collagen (α2(I) chain)	GPQGLLGA (SEQ ID NO: 100)

Bovine cartilage collagen (α1(II) chain)	GIAGQ (SEQ ID NO: 101)
Human liver collagen (α1(III) chain)	GPLGIAGI (SEQ ID NO: 102)
Human α_2M	GPEGLRVG (SEQ ID NO: 103)
Human PZP	YGAGLGVV (SEQ ID NO: 104)
	AGLGVVER (SEQ ID NO: 105)
	AGLGISST (SEQ ID NO: 106)
Rat α_1M	EPQALAMS (SEQ ID NO: 107)
	QALAMSAI (SEQ ID NO: 108)
Rat α_2M	AAYHLVSQ (SEQ ID NO: 109)
	MDAFLESS (SEQ ID NO: 110)
Rat α_1I_3(2J)	ESLPVVAV (SEQ ID NO: 111)
Rat α_1I_3(27J)	SAPAVESE (SEQ ID NO: 112)
Human fibroblast collagenase	DVAQFVLT (SEQ ID NO: 113)
(autolytic cleavages)	VAQFVLTE (SEQ ID NO: 114)
	AQFVLTEG (SEQ ID NO: 115)
	PVQPIGPQ (SEQ ID NO: 116)

[000378] 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 some embodiments, the reducing agent that would modify a CM would also modify the linker of the conjugated activatable antibody.

[000379] *Spacers and Cleavable Elements*: In some embodiments, 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:

$$W - (CH_2)n - Q$$

wherein

W is either --NH--CH_2-- or --CH_2--;

Q is an amino acid, peptide; and

n is an integer from 0 to 20.

[000380] In some 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.

[000381] 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.

[000382] *Serum Complement and Selection of Linkers*: According to one method of the present disclosure, 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 disclosure, 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 carbodimide 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.

[000383] 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.

[000384] 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 5.

[000385] *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.

[000386] *Biochemical Cross Linkers*: In some 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.

[000387] 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.

[000388] Exemplary hetero-bifunctional cross-linkers are referenced in Table 7.

Table 7: Exemplary Hetero-Bifunctional Cross Linkers

HETERO-BIFUNCTIONAL CROSS-LINKERS			
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 conjugation	11.6 Å
Sulfo-SMCC	Primary amines	Stable maleimide reactive group	11.6 Å

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

[000389] *Non-Cleavable Linkers or Direct Attachment*: In some embodiments of the disclosure, 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.

[000390] 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

$$W - (CH_2)n - Q$$

wherein

W is either --NH--CH_2-- or --CH_2--;

Q is an amino acid, peptide; and

n is an integer from 0 to 20.

[000391] *Non-Cleavable Conjugates*: In some embodiments, 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.

[000392] The antibodies disclosed herein can also be formulated as immunoliposomes. Liposomes containing the antibody are prepared by methods known in the art, such as

described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al., Proc. Natl Acad. Sci. USA, 77: 4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556.

[000393] Particularly useful liposomes can be generated by the reverse-phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present disclosure can be conjugated to the liposomes as described in Martin et al., J. Biol. Chem., 257: 286-288 (1982) via a disulfide-interchange reaction.

Definitions:

[000394] Unless otherwise defined, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art. The term "a" entity or "an" entity refers to one or more of that entity. For example, a compound refers to one or more compounds. As such, the terms "a", "an", "one or more" and "at least one" can be used interchangeably. 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.

[000395] As utilized in accordance with the present disclosure, the following terms, unless otherwise indicated, shall be understood to have the following meanings:

[000396] As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active, e.g., antigen-binding, 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 ($K_d > 10^{-6}$). Antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, domain antibody, single chain, Fab, and $F(ab')_2$ fragments, scFvs, and an Fab expression library.

[000397] 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 IgG_1, IgG_2, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

[000398] 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.

[000399] 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).

[000400] 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 ≤ 1 μM; in some embodiments, ≤ 100 nM and in some embodiments, ≤ 10 nM.

[000401] As used herein, the terms "specific binding," "immunological binding," and "immunological binding properties" refer to the non-covalent interactions of the type which 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 disclosure is said to specifically bind to the target, when the binding constant (K_d) is ≤1 μM, in some embodiments ≤ 100 nM, in some embodiments ≤ 10 nM, and in some embodiments ≤ 100 pM to about 1 pM, as measured by assays such as radioligand binding assays or similar assays known to those skilled in the art.

[000402] 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 which 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 disclosure 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.

[000403] 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.

[000404] 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 disclosure 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.

[000405] 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 that has not been intentionally modified by man in the laboratory or otherwise is naturally-occurring.

[000406] 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.

[000407] 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.

[000408] 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 disclosure are either sense or antisense oligonucleotides.

[000409] 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, phosphoroselerloate, phosphorodiselenoate, phosphoroanilothioate, phoshoraniladate, phosphoronmidate, 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.

[000410] 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. Green, Eds., Sinauer Associates, Sunderland, 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 disclosure. 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.

[000411] 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".

[000412] 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.

[000413] In some embodiments, residue positions that are not identical differ by conservative amino acid substitutions.

[000414] As discussed herein, minor variations in the amino acid sequences of antibodies or immunoglobulin molecules are contemplated as being encompassed by the present disclosure, 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. Suitable 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 disclosure.

[000415] Suitable 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 (5) confer or modify other physicochemical or functional properties of such analogs. Analogs can include various muteins of a sequence other than the naturally-occurring peptide sequence. For example, single or multiple amino acid substitutions (for example, conservative amino acid substitutions) may be made in the naturally-occurring sequence (for example, 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 at. Nature 354:105 (1991).

[000416] 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 the target, under suitable binding conditions. Typically, polypeptide analogs comprise a conservative amino acid substitution (or addition or deletion) with respect to the naturally-occurring sequence. Analogs 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.

[000417] 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.

[000418] 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.,* ^{3}H, ^{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.

[000419] 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)).

[000420] 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.

[000421] 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.

[000422] The term patient includes human and veterinary subjects.

[000423] Antibodies and/or activatable antibodies of the disclosure specifically bind a given target, e.g., a human target protein such as human CD166. Also included in the disclosure are antibodies and/or activatable antibodies that bind to the same epitope as the antibodies and/or activatable antibodies described herein. Also included in the disclosure are antibodies and/or antibodies activatable antibodies that compete with an anti-CD166 antibody and/or an anti-CD166 activatable antibody described herein for binding to CD166, e.g., human CD166. Also included in the disclosure are antibodies and/or antibodies activatable antibodies that cross-compete with an anti-CD166 antibody and/or an anti-CD166 activatable antibody described herein for binding to CD166, e.g., human CD166.

[000424] 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 the target. If the monoclonal antibody being tested competes with the monoclonal antibody of the disclosure, as shown by a decrease in binding by the monoclonal antibody of the disclosure, 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 disclosure is to pre-incubate the monoclonal antibody of the disclosure with the target and then add the monoclonal antibody being tested to determine if the monoclonal antibody being tested is inhibited in its ability to bind the target. 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 disclosure.

Multispecific Activatable Antibodies

[000425] The disclosure also provides multispecific anti-CD166 activatable antibodies. The multispecific activatable antibodies provided herein are multispecific antibodies that recognize CD166 and at least one or more different antigens or epitopes and that include at least one masking moiety (MM) linked to at least one antigen- or epitope-binding domain of the multispecific antibody such that coupling of the MM reduces the ability of the antigen- or epitope-binding domain to bind its target. In some embodiments, the MM is coupled to the antigen- or epitope-binding domain of the multispecific antibody via a cleavable moiety (CM) that functions as a substrate for at least one protease. The

activatable multispecific antibodies provided herein are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *i.e.*, healthy tissue, and, when activated, exhibit binding to a target that is at least comparable to the corresponding, unmodified multispecific antibody.

[000426] In some embodiments, the multispecific activatable antibodies are designed to engage immune effector cells, also referred to herein as immune-effector cell engaging multispecific activatable antibodies. In some embodiments, the multispecific activatable antibodies are designed to engage leukocytes, also referred to herein as leukocyte engaging multispecific activatable antibodies. In some embodiments, the multispecific activatable antibodies are designed to engage T cells, also referred to herein as T-cell engaging multispecific activatable antibodies. In some embodiments, the multispecific activatable antibodies engage a surface antigen on a leukocyte, such as on a T cell, on a natural killer (NK) cell, on a myeloid mononuclear cell, on a macrophage, and/or on another immune effector cell. In some embodiments, the immune effector cell is a leukocyte. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is a NK cell. In some embodiments, the immune effector cell is a mononuclear cell, such as a myeloid mononuclear cell. In some embodiments, the multispecific activatable antibodies are designed to bind or otherwise interact with more than one target and/or more than one epitope, also referred to herein as multi-antigen targeting activatable antibodies. As used herein, the terms "target" and "antigen" are used interchangeably.

[000427] In some embodiments, immune effector cell engaging multispecific activatable antibodies of the disclosure include a targeting antibody or antigen-binding fragment thereof that binds CD166 and an immune effector cell engaging antibody or antigen-binding portion thereof, where at least one of the targeting antibody or antigen-binding fragment thereof and/or the immune effector cell engaging antibody or antigen-binding portion thereof is masked. In some embodiments, the immune effector cell engaging antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, immune effector cell engaging target, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind the first target. In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds CD166, where the AB2 is attached to a masking moiety (MM2) such that coupling of

the MM2 reduces the ability of the AB2 to bind CD166. In some embodiments, the immune effector cell engaging antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, immune effector cell engaging target, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind the first target, and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds CD166, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind CD166. In some embodiments, the non-immune effector cell engaging antibody is a cancer targeting antibody. In some embodiments the non-immune cell effector antibody is an IgG. In some embodiments the immune effector cell engaging antibody is a scFv. In some embodiments the CD166-targeting antibody (e.g., non-immune cell effector antibody) is an IgG and the immune effector cell engaging antibody is a scFv. In some embodiments, the immune effector cell is a leukocyte. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is a NK cell. In some embodiments, the immune effector cell is a myeloid mononuclear cell.

[000428] In some embodiments, T-cell engaging multispecific activatable antibodies of the disclosure include a CD166-targeting antibody or antigen-binding fragment thereof and a T-cell engaging antibody or antigen-binding portion thereof, where at least one of the CD166-targeting antibody or antigen-binding fragment thereof and/or the T-cell engaging antibody or antigen-binding portion thereof is masked. In some embodiments, the T-cell engaging antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, T-cell engaging target, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind the first target. In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds CD166, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind CD166. In some embodiments, the T-cell engaging antibody or antigen binding fragment thereof includes a first antibody or antigen-binding fragment thereof (AB1) that binds a first, T-cell engaging target, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to

bind the first target, and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds CD166, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind CD166.

[000429] In some embodiments of an immune effector cell engaging multispecific activatable antibody, one antigen is CD166, and another antigen is typically a stimulatory or inhibitory receptor present on the surface of a T-cell, natural killer (NK) cell, myeloid mononuclear cell, macrophage, and/or other immune effector cell, such as, but not limited to, B7-H4, BTLA, CD3, CD4, CD8, CD16a, CD25, CD27, CD28, CD32, CD56, CD137, CTLA-4, GITR, HVEM, ICOS, LAG3, NKG2D, OX40, PD-1, TIGIT, TIM3, or VISTA. In some embodiments, the antigen is a stimulatory receptor present on the surface of a T cell or NK cell; examples of such stimulatory receptors include, but are not limited to, CD3, CD27, CD28, CD137 (also referred to as 4-1BB), GITR, HVEM, ICOS, NKG2D, and OX40. In some embodiments, the antigen is an inhibitory receptor present on the surface of a T-cell; examples of such inhibitory receptors include, but are not limited to, BTLA, CTLA-4, LAG3, PD-1, TIGIT, TIM3, and NK-expressed KIRs. The antibody domain conferring specificity to the T-cell surface antigen may also be substituted by a ligand or ligand domain that binds to a T-cell receptor, a NK-cell receptor, a macrophage receptor, and/or other immune effector cell receptor, such as, but not limited to, B7-1, B7-2, B7H3, PDL1, PDL2, or TNFSF9.

[000430] In some embodiments, the T-cell engaging multispecific activatable antibody includes an anti-CD3 epsilon (CD3ε, also referred to herein as CD3e and CD3) scFv and a targeting antibody or antigen-binding fragment thereof, where at least one of the anti-CD3ε scFv and/or the targeting antibody or antigen-binding portion thereof is masked. In some embodiments, the CD3ε scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3ε, where the AB1 is attached to a masking moiety (MM1) such that coupling of the MM1 reduces the ability of the AB1 to bind CD3ε. In some embodiments, the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds CD166, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind CD166. In some embodiments, the CD3ε scFv includes a first antibody or antigen-binding fragment thereof (AB1) that binds CD3ε, where the AB1 is attached to a masking moiety (MM1) such that coupling of

the MM1 reduces the ability of the AB1 to bind CD3ε, and the targeting antibody or antigen-binding fragment thereof includes a second antibody or fragment thereof that includes a second antibody or antigen-binding fragment thereof (AB2) that binds CD166, where the AB2 is attached to a masking moiety (MM2) such that coupling of the MM2 reduces the ability of the AB2 to bind CD166.

[000431] In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies include at least a first antibody or antigen-binding fragment thereof that binds a first target and/or first epitope and a second antibody or antigen-binding fragment thereof that binds a second target and/or a second epitope. In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies bind two or more different targets. In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies bind two or more different epitopes on the same target. In some embodiments, the multi-antigen targeting antibodies and/or multi-antigen targeting activatable antibodies bind a combination of two or more different targets and two or more different epitopes on the same target.

[000432] In some embodiments, a multispecific activatable antibody comprising an IgG has the IgG variable domains masked. In some embodiments, a multispecific activatable antibody comprising a scFv has the scFv domains masked. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where at least one of the IgG variable domains is coupled to a masking moiety. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where at least one of the scFv domains is coupled to a masking moiety. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where at least one of the IgG variable domains is coupled to a masking moiety and at least one of the scFv domains is coupled to a masking moiety. In some embodiments, a multispecific activatable antibody has both IgG variable domains and scFv domains, where each of the IgG variable domains and the scFv domains is coupled to its own masking moiety. In some embodiments, one antibody domain of a multispecific activatable antibody has specificity for a target antigen and another antibody domain has specificity for a T-cell surface antigen. In some embodiments, one antibody domain of a multispecific activatable antibody has specificity for a target antigen and another antibody domain has specificity for another target antigen. In some embodiments, one antibody

domain of a multispecific activatable antibody has specificity for an epitope of a target antigen and another antibody domain has specificity for another epitope of the target antigen.

[000433] In a multispecific activatable antibody, a scFv can be fused to the carboxyl terminus of the heavy chain of an IgG activatable antibody, to the carboxyl terminus of the light chain of an IgG activatable antibody, or to the carboxyl termini of both the heavy and light chains of an IgG activatable antibody. In a multispecific activatable antibody, a scFv can be fused to the amino terminus of the heavy chain of an IgG activatable antibody, to the amino terminus of the light chain of an IgG activatable antibody, or to the amino termini of both the heavy and light chains of an IgG activatable antibody. In a multispecific activatable antibody, a scFv can be fused to any combination of one or more carboxyl termini and one or more amino termini of an IgG activatable antibody. In some embodiments, a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of the IgG. In some embodiments, a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of at least one scFv. In some embodiments, a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of an IgG and a masking moiety (MM) linked to a cleavable moiety (CM) is attached to and masks an antigen binding domain of at least one scFv.

[000434] The disclosure provides examples of multispecific activatable antibody structures which include, but are not limited to, the following: $(VL\text{-}CL)_2$:$(VH\text{-}CH1\text{-}CH2\text{-}CH3\text{-}L4\text{-}VH^*\text{-}L3\text{-}VL^*\text{-}L2\text{-}CM\text{-}L1\text{-}MM)_2$; $(VL\text{-}CL)_2$:$(VH\text{-}CH1\text{-}CH2\text{-}CH3\text{-}L4\text{-}VL^*\text{-}L3\text{-}VH^*\text{-}L2\text{-}CM\text{-}L1\text{-}MM)_2$; $(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VL\text{-}CL)_2$:$(VH\text{-}CH1\text{-}CH2\text{-}CH3\text{-}L4\text{-}VH^*\text{-}L3\text{-}VL^*)_2$; $(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VL\text{-}CL)_2$:$(VH\text{-}CH1\text{-}CH2\text{-}CH3\text{-}L4\text{-}VL^*\text{-}L3\text{-}VH^*)_2$; $(VL\text{-}CL)_2$:$(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VL^*\text{-}L3\text{-}VH^*\text{-}L4\text{-}VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(VL\text{-}CL)_2$:$(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VH^*\text{-}L3\text{-}VL^*\text{-}L4\text{-}VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VL\text{-}CL)_2$:$(VL^*\text{-}L3\text{-}VH^*\text{-}L4\text{-}VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VL\text{-}CL)_2$:$(VH^*\text{-}L3\text{-}VL^*\text{-}L4\text{-}VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(VL\text{-}CL\text{-}L4\text{-}VH^*\text{-}L3\text{-}VL^*\text{-}L2\text{-}CM\text{-}L1\text{-}MM)_2$:$(VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(VL\text{-}CL\text{-}L4\text{-}VL^*\text{-}L3\text{-}VH^*\text{-}L2\text{-}CM\text{-}L1\text{-}MM)_2$:$(VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VL^*\text{-}L3\text{-}VH^*\text{-}L4\text{-}VL\text{-}CL)_2$:$(VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VH^*\text{-}L3\text{-}VL^*\text{-}L4\text{-}VL\text{-}CL)_2$:$(VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(VL\text{-}CL\text{-}L4\text{-}VH^*\text{-}L3\text{-}VL^*\text{-}L2\text{-}CM\text{-}L1\text{-}MM)_2$: $(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VL^*\text{-}L3\text{-}VH^*\text{-}L4\text{-}VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(VL\text{-}CL\text{-}L4\text{-}VH^*\text{-}L3\text{-}VL^*\text{-}L2\text{-}CM\text{-}L1\text{-}MM)_2$: $(MM\text{-}L1\text{-}CM\text{-}L2\text{-}VH^*\text{-}L3\text{-}VL^*\text{-}L4\text{-}VH\text{-}CH1\text{-}CH2\text{-}CH3)_2$; $(VL\text{-}CL\text{-}L4\text{-}VL^*\text{-}L3\text{-}VH^*\text{-}L2\text{-}CM\text{-}$

L1-MM)$_2$: (MM-L1-CM-L2-VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*-L2-CM-L1-MM)$_2$: (MM-L1-CM-L2-VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VH*-L3-VL*)$_2$: (MM-L1-CM-L2-VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VH*-L3-VL*)$_2$: (MM-L1-CM-L2-VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*)$_2$: (MM-L1-CM-L2-VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*)$_2$: (MM-L1-CM-L2-VH*-L3-VL*-L4-VH-CH1-CH2-CH3)2; (VL-CL-L4-VH*-L3-VL*-L2-CM-L1-MM)$_2$: (VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VH*-L3-VL*-L2-CM-L1-MM)$_2$: (VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$; (VL-CL-L4-VL*-L3-VH*-L2-CM-L1-MM)$_2$: (VL*-L3-VH*-L4-VH-CH1-CH2-CH3)$_2$; or (VL-CL-L4-VL*-L3-VH*-L2-CM-L1-MM)$_2$: (VH*-L3-VL*-L4-VH-CH1-CH2-CH3)$_2$, wherein: VL and VH represent the light and heavy variable domains of the first specificity, contained in the IgG; VL* and VH* represent the variable domains of the second specificity, contained in the scFv; L1 is a linker peptide connecting the masking moiety (MM) and the cleavable moiety (CM); L2 is a linker peptide connecting the cleavable moiety (CM), and the antibody; L3 is a linker peptide connecting the variable domains of the scFv; L4 is a linker peptide connecting the antibody of the first specificity to the antibody of the second specificity; CL is the light-chain constant domain; and CH1, CH2, CH3 are the heavy chain constant domains. The first and second specificities may be toward any antigen or epitope.

[000435] In some embodiments of a T-cell engaging multispecific activatable antibody, one antigen is CD166, and another antigen is typically a stimulatory (also referred to herein as activating) or inhibitory receptor present on the surface of a T-cell, natural killer (NK) cell, myeloid mononuclear cell, macrophage, and/or other immune effector cell, such as, but not limited to, B7-H4, BTLA, CD3, CD4, CD8, CD16a, CD25, CD27, CD28, CD32, CD56, CD137 (also referred to as TNFRSF9), CTLA-4, GITR, HVEM, ICOS, LAG3, NKG2D, OX40, PD-1, TIGIT, TIM3, or VISTA. The antibody domain conferring specificity to the T-cell surface antigen may also be substituted by a ligand or ligand domain that binds to a T-cell receptor, a NK-cell receptor, a macrophage receptor, and/or other immune effector cell receptor.

[000436] In some embodiments, the targeting antibody is an anti-CD166 antibody disclosed herein. In some embodiments, the targeting antibody can be in the form an activatable antibody. In some embodiments, the scFv(s) can be in the form of a Pro-scFv (see, e.g., WO 2009/025846, WO 2010/081173).

[000437] In some embodiments, the scFv is specific for binding CD3ε, and comprises or is derived from an antibody or fragment thereof that binds CD3ε, e.g., CH2527, FN18, H2C, OKT3, 2C11, UCHT1, or V9. In some embodiments, the scFv is specific for binding CTLA-4 (also referred to herein as CTLA and CTLA4).

[000438] In some embodiments, the anti-CTLA-4 scFv includes the amino acid sequence:

GGGSGGGGSGSGGGSGGGGSGGGEIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQ
QKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPLTF
GGGTKVEIKRSGGSTITSYNVYYTKLSSSGTQVQLVQTGGGVVQPGRSLRLSCAASGSTFS
SYAMSWVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDT
AVYYCATNSLYWYFDLWGRGTLVTVSSAS (SEQ ID NO: 117)

[000439] In some embodiments, the anti-CTLA-4 scFv includes the amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 117.

[000440] In some embodiments, the anti-CD3ε scFv includes the amino acid sequence:

GGGSGGGGSGSGGGSGGGGSGGGQVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVK
QRPGQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYYCARY
YDDHYCLDYWGQGTTLTVSSGGGGSGGGGSGGGGSQIVLTQSPAIMSASPGEKVTMTCSAS
SSVSYMNWYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAATYYC
QQWSSNPFTFGSGTKLEINR (SEQ ID NO: 118)

[000441] In some embodiments, the anti-CD3ε scFv includes the amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of SEQ ID NO: 118.

[000442] In some embodiments, the scFv is specific for binding one or more T-cells, one or more NK-cells and/or one or more macrophages. In some embodiments, the scFv is specific for binding a target selected from the group consisting of B7-H4, BTLA, CD3, CD4, CD8, CD16a, CD25, CD27, CD28, CD32, CD56, CD137, CTLA-4, GITR, HVEM, ICOS, LAG3, NKG2D, OX40, PD-1, TIGIT, TIM3, or VISTA.

[000443] In some embodiments, the multispecific 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. In some embodiments, the agent is conjugated to the multispecific activatable antibody via a linker. In some embodiments, the agent is conjugated to the AB via a cleavable linker. In some embodiments, the linker is a non-cleavable linker. In some embodiments, the agent is a microtubule inhibitor. In some embodiments, the agent is a nucleic acid damaging agent, such as a DNA alkylator or DNA intercalator, or other DNA damaging agent. In some embodiments, the linker is a cleavable linker. In some embodiments, the agent is an agent selected from the group listed in Table 5. 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. In some embodiments, the agent is a pyrrolobenzodiazepine dimer.

[000444] In some embodiments, the multispecific activatable antibody also includes a detectable moiety. In some embodiments, the detectable moiety is a diagnostic agent.

[000445] In some embodiments, the multispecific activatable antibody naturally contains one or more disulfide bonds. In some embodiments, the multispecific activatable antibody can be engineered to include one or more disulfide bonds.

[000446] The disclosure also provides an isolated nucleic acid molecule encoding a multispecific activatable antibody described herein, as well as vectors that include these isolated nucleic acid sequences. The disclosure provides methods of producing a multispecific activatable antibody by culturing a cell under conditions that lead to expression of the activatable antibody, wherein the cell comprises such a nucleic acid molecule. In some embodiments, the cell comprises such a vector.

[000447] The disclosure also provides a method of manufacturing multispecific activatable antibodies of the disclosure by (a) culturing a cell comprising a nucleic acid construct that encodes the multispecific activatable antibody under conditions that lead to expression of the multispecific activatable, and (b) recovering the multispecific activatable

antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000448] The disclosure also provides multispecific activatable antibodies and/or multispecific activatable antibody compositions that include at least a first antibody or antigen-binding fragment thereof (AB1) that specifically binds a first target or first epitope and a second antibody or antigen-biding fragment thereof (AB2) that binds a second target or a second epitope, where at least AB1 is coupled or otherwise attached to a masking moiety (MM1), such that coupling of the MM1 reduces the ability of AB1 to bind its target. In some embodiments, the MM1 is coupled to AB1 via a first cleavable moiety (CM1) sequence that includes a substrate for a protease, for example, a protease that is co-localized with the target of AB1 at a treatment site or a diagnostic site in a subject. The multispecific activatable antibodies provided herein are stable in circulation, activated at intended sites of therapy and/or diagnosis but not in normal, *i.e.*, healthy tissue, and, when activated, exhibit binding to the target of AB1 that is at least comparable to the corresponding, unmodified multispecific antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000449] The disclosure also provides compositions and methods that include a multispecific activatable antibody that includes at least a first antibody or antibody fragment (AB1) that specifically binds a target and a second antibody or antibody fragment (AB2), where at least the first AB in the multispecific activatable antibody is coupled to a masking moiety (MM1) that decreases the ability of AB1 to bind its target. In some embodiments, each AB is coupled to a MM that decreases the ability of its corresponding AB to each target. For example, in bispecific activatable antibody embodiments, AB1 is coupled to a first masking moiety (MM1) that decreases the ability of AB1 to bind its target, and AB2 is coupled to a second masking moiety (MM2) that decreases the ability of AB2 to bind its target. In some embodiments, the multispecific activatable antibody comprises more than two AB regions; in such embodiments, AB1 is coupled to a first masking moiety (MM1) that decreases the ability of AB1 to bind its target, AB2 is coupled to a second masking moiety (MM2) that decreases the ability of AB2 to bind its target, AB3 is coupled to a third masking moiety (MM3) that decreases the ability of AB3 to bind its target, and so on for each AB in the multispecific activatable antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

[000450] In some embodiments, the multispecific activatable antibody further includes at least one cleavable moiety (CM) that is a substrate for a protease, where the CM links a MM to an AB. For example, in some embodiments, the multispecific activatable antibody includes at least a first antibody or antibody fragment (AB1) that specifically binds a target and a second antibody or antibody fragment (AB2), where at least the first AB in the multispecific activatable antibody is coupled via a first cleavable moiety (CM1) to a masking moiety (MM1) that decreases the ability of AB1 to bind its target. In some bispecific activatable antibody embodiments, AB1 is coupled via CM1 to MM1, and AB2 is coupled via a second cleavable moiety (CM2) to a second masking moiety (MM2) that decreases the ability of AB2 to bind its target. In some embodiments, the multispecific activatable antibody comprises more than two AB regions; in some of these embodiments, AB1 is coupled via CM1 to MM1, AB2 is coupled via CM2 to MM2, and AB3 is coupled via a third cleavable moiety (CM3) to a third masking moiety (MM3) that decreases the ability of AB3 to bind its target, and so on for each AB in the multispecific activatable antibody. Suitable AB, MM, and/or CM include any of the AB, MM, and/or CM disclosed herein.

Activatable antibodies Having Non-Binding Steric Moieties or Binding Partners for Non-Binding Steric Moieties

[000451] The disclosure also provides activatable antibodies that include non-binding steric moieties (NB) or binding partners (BP) for non-binding steric moieties, where the BP recruits or otherwise attracts the NB to the activatable antibody. The activatable antibodies provided herein include, for example, an activatable antibody that includes a non-binding steric moiety (NB), a cleavable linker (CL) and antibody or antibody fragment (AB) that binds a target; an activatable antibody that includes a binding partner for a non-binding steric moiety (BP), a CL and an AB; and an activatable antibody that includes a BP to which an NB has been recruited, a CL and an AB that binds the target. Activatable antibodies in which the NB is covalently linked to the CL and AB of the activatable antibody or is associated by interaction with a BP that is covalently linked to the CL and AB of the activatable antibody are referred to herein as "NB-containing activatable antibodies." By activatable or switchable is meant that the activatable antibody exhibits a first level of binding to a target when the activatable antibody is in an inhibited, masked or uncleaved state (*i.e.*, a first conformation), and a second level of binding to the target when

the activatable antibody is in an uninhibited, unmasked and/or cleaved state (*i.e.*, a second conformation, *i.e.*, activated antibody), where the second level of target binding is greater than the first level of target binding. The activatable antibody compositions can exhibit increased bioavailability and more favorable biodistribution compared to conventional antibody therapeutics.

[000452] In some embodiments, activatable antibodies provide for reduced toxicity and/or adverse side effects that could otherwise result from binding of the at non-treatment sites and/or non-diagnostic sites if the AB were not masked or otherwise inhibited from binding to such a site.

[000453] Anti-CD166 activatable antibodies that include a non-binding steric moiety (NB) can be made using the methods set forth in PCT Publication No. WO 2013/192546, the contents of which are hereby incorporated by reference in their entirety.

Use Of Antibodies, Conjugated Antibodies, Activatable Antibodies, and Conjugated Activatable Antibodies

[000454] It will be appreciated that administration of therapeutic entities in accordance with the disclosure will be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences (15th ed, Mack Publishing Company, Easton, PA (1975)), particularly Chapter 87 by Blaug, Seymour, therein. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as Lipofectin™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Any of the foregoing mixtures may be appropriate in treatments and therapies in accordance with the present disclosure, provided that the active ingredient in the formulation is not inactivated by the formulation and the formulation is physiologically compatible and tolerable with the route of administration. *See* also Baldrick P. "Pharmaceutical excipient development: the need for preclinical guidance." Regul. Toxicol Pharmacol. 32(2):210-8 (2000), Wang W. "Lyophilization and development of solid protein pharmaceuticals." Int. J. Pharm. 203(1-2):1-60 (2000), Charman WN "Lipids, lipophilic drugs, and oral drug delivery-some emerging concepts." J Pharm Sci.89(8):967-

78 (2000), Powell *et al.* "Compendium of excipients for parenteral formulations" PDA J Pharm Sci Technol. 52:238-311 (1998) and the citations therein for additional information related to formulations, excipients and carriers well known to pharmaceutical chemists.

[000455] Therapeutic formulations of the disclosure, which include an anti-CD166 antibody and/or activatable anti-CD166 antibody, such as by way of non-limiting example, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody, are used to prevent, treat or otherwise ameliorate a disease or disorder associated with aberrant target expression and/or activity. For example, therapeutic formulations of the disclosure, which include an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody, are used to treat or otherwise ameliorate a cancer or other neoplastic condition, inflammation, an inflammatory disorder, and/or an autoimmune disease . In some embodiments, the cancer is a solid tumor or a hematologic malignancy where the target is expressed. In some embodiments, the cancer is a solid tumor where the target is expressed. In some embodiments, the cancer is a hematologic malignancy where the target is expressed. In some embodiments, the target is expressed on parenchyma (e.g., in cancer, the portion of an organ or tissue that often carries out function(s) of the organ or tissue). In some embodiments, the target is expressed on a cell, tissue, or organ. In some embodiments, the target is expressed on stroma (i.e., the connective supportive framework of a cell, tissue, or organ). In some embodiments, the target is expressed on an osteoblast. In some embodiments, the target is expressed on the endothelium (vasculature). In some embodiments, the target is expressed on a cancer stem cell. In some embodiments, the agent to which the antibody and/or the activatable antibody is conjugated is a microtubule inhibitor. In some embodiments, the agent to which the antibody and/or the activatable antibody is conjugated is a nucleic acid damaging agent.

[000456] Efficaciousness of prevention, amelioration or treatment is determined in association with any known method for diagnosing or treating the disease or disorder associated with target expression and/or activity, such as, for example, aberrant target expression and/or activity. Prolonging the survival of a subject or otherwise delaying the progression of the disease or disorder associated with target expression and/or activity, e.g., aberrant target expression and/or activity, in a subject indicates that the antibody, conjugated antibody, activatable antibody and/or conjugated activatable antibody confers a clinical benefit.

[000457] An antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody 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.

[000458] In some embodiments where antibody fragments are used, the smallest fragment that specifically binds to the binding domain of the target protein is selected. 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 compounds as necessary for the particular indication being treated, for example, in some embodiments, those with complementary activities that do not adversely affect each other. In some embodiments, 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.

[000459] 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-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles, and nanocapsules) or in macroemulsions.

[000460] The formulations to be used for *in vivo* administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

[000461] 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 DEPOT ™ (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.

[000462] In some embodiments, the antibody, the conjugated antibody, activatable antibody and/or conjugated 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 disclosure 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. Christopoulus, 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.

[000463] The antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable antibodies of the disclosure are also useful in a variety of diagnostic and prophylactic formulations. In one embodiment, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody is administered to patients that are at risk of developing one or more of the aforementioned disorders. A patient's or organ's predisposition to one or more of the aforementioned disorders can be determined using genotypic, serological or biochemical markers.

[000464] In some embodiments of the disclosure, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody is administered to human individuals diagnosed with a clinical indication associated with one or more of the aforementioned disorders. Upon diagnosis, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody is administered to mitigate or reverse the effects of the clinical indication.

[000465] An antibody, a conjugated antibody, an activatable antibody, and/or a conjugated activatable antibody of the disclosure is also useful in the detection of a target in patient samples and accordingly are useful as diagnostics. For example, the antibodies and/or activatable antibodies, and conjugated versions thereof, of the disclosure are used in *in vitro* assays, *e.g.*, ELISA, to detect target levels in a patient sample.

[000466] In one embodiment, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody of the disclosure is immobilized on a solid support (*e.g.*, the well(s) of a microtiter plate). The immobilized antibody, conjugated antibody, activatable antibody and/or conjugated activatable antibody serves as a capture antibody for any target that may be present in a test sample. Prior to contacting the immobilized antibody and/or activatable antibody, and/or conjugated versions thereof, 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.

[000467] Subsequently the wells are treated with a test sample suspected of containing the antigen, or with a solution containing a standard amount of the antigen. Such a sample is, *e.g.*, a serum sample from a subject suspected of having levels of circulating antigen considered to be diagnostic of a pathology. After rinsing away the test sample or standard, the solid support is treated with a second antibody that is detectably labeled. The labeled

second antibody serves as a detecting antibody. The level of detectable label is measured, and the concentration of target antigen in the test sample is determined by comparison with a standard curve developed from the standard samples.

[000468] It will be appreciated that based on the results obtained using the antibodies and activatable antibodies of the disclosure, and conjugated versions thereof, in an *in vitro* diagnostic assay, it is possible to stage a disease in a subject based on expression levels of the target 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.

[000469] An antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody 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 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 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 antibodies (i.e., antibodies resulting from cleavage of an activatable antibody) in a given cell or tissue of a given host organism. Such accumulation of activated 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.

[000470] For example, the CM can be selected to be substrate for at least one 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 as disclosed herein, or when appropriate, methods familiar to one skilled in the art, a detectable label (*e.g.*, a fluorescent label or radioactive label or radiotracer) can be conjugated to an AB or other region of an

antibody and/or activatable 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 at least one protease whose activity is elevated in the disease tissue of interest, activatable 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 antibodies in the disease tissue is enhanced relative to non-disease tissues.

[000471] In another example, activatable antibodies can be used to detect the presence or absence of a cleaving agent in a sample. For example, where the activatable antibodies contain a CM susceptible to cleavage by an enzyme, the activatable antibodies can be used to detect (either qualitatively or quantitatively) the presence of an enzyme in the sample. In another example, where the activatable antibodies contain a CM susceptible to cleavage by reducing agent, the activatable 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 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 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 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.

[000472] 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 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. 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 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.

[000473] Activatable 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).

[000474] 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.

[000475] In these techniques, an activatable antibody is labeled with a detectable label. The detectable label may be a fluorescent dye, (e.g. a fluorophore, Fluorescein Isothiocyanate (FITC), Rhodamine Isothiocyanate (TRITC), an Alexa Fluor® label), a near infrared (NIR) dye (e.g., Qdot® nanocrystals), a colloidal metal, a hapten, a radioactive marker, biotin and an amplification reagent such as streptavidin, or an enzyme (e.g. horseradish peroxidase or alkaline phosphatase).

[000476] Detection of the label in a sample that has been incubated with the labeled, activatable antibody indicates that the sample contains the target and contains a protease that is specific for the CM of the activatable 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 and inhibits the proteolytic activity of matriptase; 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 that is specific for the CM of the activatable antibody. In some embodiments, the presence of the target can be confirmed using an agent that is specific for the target, *e.g.*, another antibody, or the detectable label can be competed with unlabeled target. In some embodiments, unlabeled activatable

antibody could be used, with detection by a labeled secondary antibody or more complex detection system.

[000477] Similar techniques are also useful for *in vivo* imaging where detection of the fluorescent signal in a subject, *e.g.*, a mammal, including a human, indicates that the disease site contains the target and contains a protease that is specific for the CM of the activatable antibody.

[000478] 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 antibody.

[000479] The disclosure provides methods of using the antibodies and/or activatable antibodies in a variety of diagnostic and/or prophylactic indications. For example, the disclosure provides methods of detecting presence or absence of a cleaving agent and a target of interest in a subject or a sample by (i) contacting a subject or sample with an activatable antibody, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target of interest, wherein the activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB and is not a modified form of a natural binding partner of the AB; and (b) wherein, in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to the target, and in a cleaved, activated state the MM does not interfere or compete with specific binding of the AB to the target; and (ii) measuring a level of activated activatable antibody in the subject or sample, wherein a detectable level of activated activatable antibody in the subject or sample indicates that the cleaving agent and the target are present in the subject or sample and wherein no detectable level of activated activatable antibody in the subject or sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or sample. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the 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 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.

[000480] The disclosure 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 activatable antibody in the presence of a target of interest, *e.g.*, the target, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target of interest, wherein the activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB and is not a modified form of a natural binding partner of the AB; and (b) wherein, in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to the target, and in a cleaved, activated state the MM does not interfere or compete with specific binding of the AB to the target; and (ii) measuring a level of activated activatable antibody in the subject or sample, wherein a detectable level of activated 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 activatable antibody in the subject or sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or sample. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the 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 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.

[000481] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample, where the kits include at least an activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM)

that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target of interest, wherein the activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB and is not a modified form of a natural binding partner of the AB; and (b) wherein, in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to the target, and in a cleaved, activated state the MM does not interfere or compete with specific binding of the AB to the target; and (ii) measuring a level of activated activatable antibody in the subject or sample, wherein a detectable level of activated 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 activatable antibody in the subject or sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or sample. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the 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 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.

[000482] The disclosure 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 activatable antibody, wherein the activatable antibody comprises a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, an antigen binding domain (AB) that specifically binds the target, and a detectable label, wherein the activatable antibody in an uncleaved, non-activated state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB and is not a modified form of a natural binding partner of the AB; wherein, in an uncleaved, non-activated state, the MM interferes with specific binding of the AB to the target, and in a

cleaved, activated state the MM does not interfere or compete with specific binding of the AB to the target; and wherein the detectable label is positioned on a portion of the 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 and/or not sufficiently present 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. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the 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 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.

[000483] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample, where the kits include at least an activatable antibody and/or conjugated activatable antibody (e.g., an activatable antibody to which a therapeutic agent is conjugated) described herein for use in contacting a subject or biological sample and means for detecting the level of activated activatable antibody and/or conjugated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or biological sample, such that the target binding and/or protease cleavage of the activatable antibody cannot be detected in the subject or biological sample.

[000484] The disclosure also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or biological sample with an activatable antibody in the presence of the target, and (ii) measuring a level of activated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving

agent is present in the subject or biological sample and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or biological sample at a detectable level, such that protease cleavage of the activatable antibody cannot be detected in the subject or biological sample. Such an activatable antibody includes a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target, wherein the activatable antibody in an uncleaved (*i.e.*, non-activated) state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB; and (b) wherein the MM of the activatable antibody in an uncleaved state interferes with specific binding of the AB to the target, and wherein the MM of an activatable antibody in a cleaved (*i.e.*, activated) state does not interfere or compete with specific binding of the AB to the target. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the detectable label is attached to the masking moiety. In some embodiments, the detectable label is attached to the cleavable moiety N-terminal to the protease cleavage site. In some embodiments, a single antigen binding site of the AB is masked. In some embodiments wherein an antibody of the disclosure has at least two antigen binding sites, at least one antigen binding site is masked and at least one antigen binding site is not masked. In some embodiments all antigen binding sites are masked. In some embodiments, the measuring step includes use of a secondary reagent comprising a detectable label.

[000485] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample, where the kits include at least an activatable antibody and/or conjugated activatable antibody described herein for use in contacting a subject or biological sample with an activatable antibody in the presence of the target, and measuring a level of activated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is present in the subject or biological sample and wherein no detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is absent and/or not sufficiently present

in the subject or biological sample at a detectable level, such that protease cleavage of the activatable antibody cannot be detected in the subject or biological sample. Such an activatable antibody includes a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, e.g., a protease, and an antigen binding domain or fragment thereof (AB) that specifically binds the target, wherein the activatable antibody in an uncleaved (*i.e.*, non-activated) state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB; and (b) wherein the MM of the activatable antibody in an uncleaved state interferes with specific binding of the AB to the target, and wherein the MM of an activatable antibody in a cleaved (*i.e.*, activated) state does not interfere or compete with specific binding of the AB to the target. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the detectable label is attached to the masking moiety. In some embodiments, the detectable label is attached to the cleavable moiety N-terminal to the protease cleavage site. In some embodiments, a single antigen binding site of the AB is masked. In some embodiments wherein an antibody of the disclosure has at least two antigen binding sites, at least one antigen binding site is masked and at least one antigen binding site is not masked. In some embodiments all antigen binding sites are masked. In some embodiments, the measuring step includes use of a secondary reagent comprising a detectable label.

[000486] The disclosure 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 activatable antibody and/or conjugated activatable antibody described herein for use in contacting a subject or biological sample and means for detecting the level of activated activatable antibody and/or conjugated activatable antibody in the subject or biological sample, wherein the activatable antibody includes a detectable label that is positioned on a portion of the activatable antibody that is released following cleavage of the CM, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or biological sample such that the target binding and/or protease cleavage of the activatable antibody cannot be detected in the subject or biological sample, and wherein no detectable

level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent is present in the subject or biological sample at a detectable level.

[000487] The disclosure provides methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample by (i) contacting a subject or biological sample with an activatable antibody, wherein the activatable antibody includes a detectable label that is positioned on a portion of the activatable antibody that is released following cleavage of the CM and (ii) measuring a level of activated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or biological sample, such that the target binding and/or protease cleavage of the activatable antibody cannot be detected in the subject or biological sample, and wherein a reduced detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample. A reduced level of detectable label is, for example, a reduction of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% and/or about 100%. Such an activatable antibody includes a masking moiety (MM), a cleavable moiety (CM) that is cleaved by the cleaving agent, and an antigen binding domain or fragment thereof (AB) that specifically binds the target, wherein the activatable antibody in an uncleaved (*i.e.*, non-activated) state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB; and (b) wherein the MM of the activatable antibody in an uncleaved state interferes with specific binding of the AB to the target, and wherein the MM of an activatable antibody in a cleaved (*i.e.*, activated) state does not interfere or compete with specific binding of the AB to the target. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the 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 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.

[000488] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent and the target in a subject or a sample, where the kits include at least an activatable antibody and/or conjugated activatable antibody described herein for use in contacting a subject or biological sample and means for detecting the level of activated activatable antibody and/or conjugated activatable antibody in the subject or biological sample, wherein a detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent, the target or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or biological sample, such that the target binding and/or protease cleavage of the activatable antibody cannot be detected in the subject or biological sample, and wherein a reduced detectable level of activated activatable antibody in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample. A reduced level of detectable label is, for example, a reduction of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% and/or about 100%.

[000489] The disclosure also provides methods of detecting presence or absence of a cleaving agent in a subject or a sample by (i) contacting a subject or biological sample with an activatable antibody, wherein the activatable antibody includes a detectable label that is positioned on a portion of the activatable antibody that is released following cleavage of the CM; and (ii) measuring a level of detectable label in the subject or biological sample, wherein a detectable level of the detectable label in the subject or biological sample indicates that the cleaving agent is absent and/or not sufficiently present in the subject or biological sample at a detectable level, such that protease cleavage of the activatable antibody cannot be detected in the subject or biological sample, and wherein a reduced detectable level of the detectable label in the subject or biological sample indicates that the cleaving agent is present in the subject or biological sample. A reduced level of detectable label is, for example, a reduction of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% and/or about 100%. Such an activatable antibody includes a masking moiety (MM), a cleavable

moiety (CM) that is cleaved by the cleaving agent, and an antigen binding domain or fragment thereof (AB) that specifically binds the target, wherein the activatable antibody in an uncleaved (*i.e.*, non-activated) state comprises a structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM; (a) wherein the MM is a peptide that inhibits binding of the AB to the target, and wherein the MM does not have an amino acid sequence of a naturally occurring binding partner of the AB; and (b) wherein the MM of the activatable antibody in an uncleaved state interferes with specific binding of the AB to the target, and wherein the MM of an activatable antibody in a cleaved (*i.e.*, activated) state does not interfere or compete with specific binding of the AB to the target. In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the 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 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.

[000490] The disclosure also provides kits for use in methods of detecting presence or absence of a cleaving agent of interest in a subject or a sample, where the kits include at least an activatable antibody and/or conjugated activatable antibody described herein for use in contacting a subject or biological sample and means for detecting the level of activated activatable antibody and/or conjugated activatable antibody in the subject or biological sample, wherein the activatable antibody includes a detectable label that is positioned on a portion of the activatable antibody that is released following cleavage of the CM, wherein a detectable level of the detectable label in the subject or biological sample indicates that the cleaving agent, the target, or both the cleaving agent and the target are absent and/or not sufficiently present in the subject or biological sample, such that the target binding and/or protease cleavage of the activatable antibody cannot be detected in the subject or biological sample, and wherein a reduced detectable level of the detectable label in the subject or biological sample indicates that the cleaving agent and the target are present in the subject or biological sample. A reduced level of detectable label is, for example, a reduction of about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about

40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95% and/or about 100%.

[000491] In some embodiments of these methods and kits, the activatable antibody includes a detectable label. In some embodiments of these methods and kits, the detectable label includes an imaging agent, 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 kits, the imaging agent comprises a radioisotope. In some embodiments of these methods and kits, the radioisotope is indium or technetium. In some embodiments of these methods and kits, the contrasting agent comprises iodine, gadolinium or iron oxide. In some embodiments of these methods and kits, the enzyme comprises horseradish peroxidase, alkaline phosphatase, or β-galactosidase. In some embodiments of these methods and 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 kits, 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 and kits, the ligand-based label comprises biotin, avidin, streptavidin or one or more haptens.

[000492] In some embodiments of these methods and kits, the subject is a mammal. In some embodiments of these methods and kits, the subject is a human. In some embodiments, the subject is a non-human mammal, such as a non-human primate, companion animal (e.g., cat, dog, horse), farm animal, work animal, or zoo animal. In some embodiments, the subject is a rodent.

[000493] 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.

[000494] 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 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.

[000495] In some embodiments *in situ* imaging is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target (e.g., the target) and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested (e.g., accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative for either or both of the target (*e.g.*, the target) 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. In some embodiments, such patients that test negative with respect to a first activatable antibody can be tested with other activatable antibodies comprising different CMs until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments, the patient is then administered a therapeutically effective amount of the activatable antibody for which the patient tested positive.

[000496] In some embodiments *in vivo* imaging is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target (e.g., the target) and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested (e.g., accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative might be identified as suitable candidates for another form of therapy. In some embodiments, such patients that test negative with respect to a first activatable antibody can be tested with other activatable antibodies comprising different CMs until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments, the patient is then administered a therapeutically effective amount of the activatable antibody for which the patient tested positive.

[000497] In some embodiments of the methods and kits, the method or kit is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target (e.g., the target) and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested in these methods are identified as suitable candidates for

treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative for both of the targets (*e.g.*, the target) 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. In some embodiments, such patients can be tested with other activatable antibodies until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments, patients that test negative for either of the target (*e.g.*, the target) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. In some embodiments, patients that test negative for either of the target (*e.g.*, the target) are identified as not being suitable candidates for treatment with such an activatable antibody comprising such a CM. In some embodiments, such patients can be tested with other activatable antibodies until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments, the activatable antibody is an activatable antibody to which a therapeutic agent is conjugated. In some embodiments, the activatable antibody is not conjugated to an agent. In some embodiments, the 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 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.

[000498] In some embodiments, a method or kit is used to identify or otherwise refine a patient population suitable for treatment with an anti-the target activatable antibody and/or conjugated activatable antibody (e.g., activatable antibody to which a therapeutic agent is conjugated) of the disclosure, followed by treatment by administering that activatable antibody and/or conjugated activatable antibody to a subject in need thereof. For example, patients that test positive for both the targets (e.g., the target) and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody and/or conjugated activatable antibody being tested in these methods are identified as suitable candidates for treatment with such antibody and/or such a conjugated activatable antibody comprising such a CM, and the patient is then administered a therapeutically effective amount of the activatable antibody and/or conjugated activatable antibody that was tested. Likewise,

patients that test negative for either or both of the target (e.g., the target) 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. In some embodiments, such patients can be tested with other antibody and/or conjugated activatable antibody until a suitable antibody and/or conjugated activatable antibody for treatment is identified (*e.g.*, an activatable antibody and/or conjugated activatable antibody comprising a CM that is cleaved by the patient at the site of disease). In some embodiments, the patient is then administered a therapeutically effective amount of the activatable antibody and/or conjugated activatable antibody for which the patient tested positive.

[000499] In some embodiments of these methods and kits, the MM is a peptide having a length from about 4 to 40 amino acids. In some embodiments of these methods and kits, the activatable antibody comprises a linker peptide, wherein the linker peptide is positioned between the MM and the CM. In some embodiments of these methods and kits, the activatable antibody comprises a linker peptide, where the linker peptide is positioned between the AB and the CM. In some embodiments of these methods and kits, the activatable antibody comprises a first linker peptide (L1) and a second linker peptide (L2), wherein the first linker peptide is positioned between the MM and the CM and the second linker peptide is positioned between the AB and the CM. In some embodiments of these methods and kits, each of L1 and L2 is a peptide of about 1 to 20 amino acids in length, and wherein each of L1 and L2 need not be the same linker. In some embodiments of these methods and kits, one or both of L1 and L2 comprises a glycine-serine polymer. In some embodiments of these methods and kits, at least one of L1 and L2 comprises an amino acid sequence selected from the group consisting of (GS)n, (GSGGS)n (SEQ ID NO: 1) and (GGGS)n (SEQ ID NO: 2), where n is an integer of at least one. In some embodiments of these methods and kits, at least one of L1 and L2 comprises an amino acid sequence having the formula (GGS)n, where n is an integer of at least one. In some embodiments of these methods and kits, at least one of L1 and L2 comprises an amino acid sequence selected from the group consisting of Gly-Gly-Ser-Gly (SEQ ID NO: 3), Gly-Gly-Ser-Gly-Gly (SEQ ID NO: 4), Gly-Ser-Gly-Ser-Gly (SEQ ID NO: 5), Gly-Ser-Gly-Gly-Gly (SEQ ID NO: 6), Gly-Gly-Gly-Ser-Gly (SEQ ID NO: 7), and Gly-Ser-Ser-Ser-Gly (SEQ ID NO: 8).

[000500] In some embodiments of these methods and kits, the AB comprises an antibody or antibody fragment sequence selected from the cross-reactive antibody

sequences presented herein. In some embodiments of these methods and kits, the AB comprises a Fab fragment, a scFv or a single chain antibody (scAb).

[000501] In some embodiments of these methods and kits, the cleaving agent is a protease that is co-localized in the subject or sample with the target and the CM is a polypeptide that functions as a substrate for the protease, wherein the protease cleaves the CM in the activatable antibody when the activatable antibody is exposed to the protease. In some embodiments of these methods and kits, the CM is a polypeptide of up to 15 amino acids in length. In some embodiments of these methods and kits, the CM is coupled to the N-terminus of the AB. In some embodiments of these methods and kits, the CM is coupled to the C-terminus of the AB. In some embodiments of these methods and kits, the CM is coupled to the N-terminus of a VL chain of the AB.

[000502] The antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable antibodies of the disclosure are used in diagnostic and prophylactic formulations. In one embodiment, an activatable antibody is administered to patients that are at risk of developing one or more of the aforementioned inflammation, inflammatory disorders, cancer or other disorders.

[000503] A patient's or organ's predisposition to one or more of the aforementioned disorders can be determined using genotypic, serological or biochemical markers.

[000504] In some embodiments of the disclosure, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody is administered to human individuals diagnosed with a clinical indication associated with one or more of the aforementioned disorders. Upon diagnosis, an antibody, a conjugated antibody, an activatable antibody and/or a conjugated activatable antibody is administered to mitigate or reverse the effects of the clinical indication.

[000505] Antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable antibodies of the disclosure are also useful in the detection of the target in patient samples and accordingly are useful as diagnostics. For example, the antibodies, conjugated antibodies, the activatable antibodies and/or conjugated activatable antibodies of the disclosure are used in *in vitro* assays, *e.g.*, ELISA, to detect target levels in a patient sample.

[000506] In one embodiment, an antibody and/or activatable antibody of the disclosure 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 target that may be

present in a test sample. Prior to contacting the immobilized antibody and/or activatable 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.

[000507] Subsequently the wells are treated with a test sample suspected of containing the antigen, or with a solution containing a standard amount of the antigen. Such a sample is, *e.g.*, a serum sample from a subject suspected of having levels of circulating antigen considered to be diagnostic of a pathology. After rinsing away the test sample or standard, the solid support is treated with a second antibody that is detectably labeled. The labeled second antibody serves as a detecting antibody. The level of detectable label is measured, and the concentration of target antigen in the test sample is determined by comparison with a standard curve developed from the standard samples.

[000508] It will be appreciated that based on the results obtained using the antibodies and/or activatable antibodies of the disclosure in an *in vitro* diagnostic assay, it is possible to stage a disease in a subject based on expression levels of the Target 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.

[000509] Antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable 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 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 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 antibodies (i.e., antibodies resulting from cleavage of an activatable antibody) in a given cell or tissue of a given host organism. Such accumulation of activated 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.

[000510] 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 activatable 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 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 antibodies in the disease tissue is enhanced relative to non-disease tissues.

[000511] In another example, activatable antibodies can be used to detect the presence or absence of a cleaving agent in a sample. For example, where the activatable antibodies contain a CM susceptible to cleavage by an enzyme, the activatable antibodies can be used to detect (either qualitatively or quantitatively) the presence of an enzyme in the sample. In another example, where the activatable antibodies contain a CM susceptible to cleavage by reducing agent, the activatable 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 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 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 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.

[000512] 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 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. 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 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.

[000513] Activatable 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).

[000514] 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.

[000515] In these techniques, an activatable antibody is labeled with a detectable label. The detectable label may be a fluorescent dye, (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).

[000516] Detection of the label in a sample that has been incubated with the labeled, activatable antibody indicates that the sample contains the target and contains a protease that is specific for the CM of the activatable 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 and inhibits the

proteolytic activity of matriptase; 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 antibody. In some embodiments, the presence of the target can be confirmed using an agent that is specific for the target, *e.g.*, another antibody, or the detectable label can be competed with unlabeled target. In some embodiments, unlabeled activatable antibody could be used, with detection by a labeled secondary antibody or more complex detection system.

[000517] Similar techniques are also useful for *in vivo* imaging where detection of the fluorescent signal in a subject, *e.g.*, a mammal, including a human, indicates that the disease site contains the target and contains a protease that is specific for the CM of the activatable antibody.

[000518] 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 antibody.

[000519] 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 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.

[000520] In some embodiments *in situ* imaging is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested (e.g., accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative for either or both of the target and the protease that cleaves the substrate in the CM in the activatable antibody being tested using these methods are identified as suitable candidates for another form of therapy (*i.e.*, not suitable for treatment with the activatable antibody being tested). In some embodiments, such patients that test negative with respect to a first activatable antibody can be tested with other activatable antibodies comprising different CMs until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease).

[000521] In some embodiments *in vivo* imaging is used to identify or otherwise refine a patient population suitable for treatment with an activatable antibody of the disclosure. For example, patients that test positive for both the target and a protease that cleaves the substrate in the cleavable moiety (CM) of the activatable antibody being tested (e.g., accumulate activated antibodies at the disease site) are identified as suitable candidates for treatment with such an activatable antibody comprising such a CM. Likewise, patients that test negative are identified as suitable candidates for another form of therapy (*i.e.*, not suitable for treatment with the activatable antibody being tested). In some embodiments, such patients that test negative with respect to a first activatable antibody can be tested with other activatable antibodies comprising different CMs until a suitable activatable antibody for treatment is identified (e.g., an activatable antibody comprising a CM that is cleaved by the patient at the site of disease).

Pharmaceutical compositions

[000522] The antibodies, conjugated antibodies, activatable antibodies and/or conjugated activatable antibodies of the disclosure (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 antibody, the conjugated antibody, activatable antibody and/or conjugated 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. Suitable examples of such carriers or diluents include, but are not limited to, water, saline, ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[000523] A pharmaceutical composition of the disclosure is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral (*e.g.*, inhalation), transdermal (*i.e.,* topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[000524] 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 some embodiments, it will be desirable 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.

[000525] 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.

[000526] 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.

[000527] 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.

[000528] 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.

[000529] 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.

[000530] 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.

[000531] It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

[000532] The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

[000533] 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. Characterization of Anti-CD166 Antibodies

[000534] The studies provided herein were designed to evaluate binding of anti-CD166 antibodies of the disclosure.

[000535] Binding of various anti-CD166 antibodies of the disclosure was confirmed by ELISA (Figure 1). Anti-human CD166 antibodies comprising the following VH and VL sequences were tested:

Antibody Name	VH SEQ	VL SEQ
CD166-M9_vK1/HcB	SEQ ID NO: 121	SEQ ID NO: 123
CD166-M9_vK2/HcB	SEQ ID NO: 121	SEQ ID NO: 124
CD166-M9_vK3a/HcB	SEQ ID NO: 121	SEQ ID NO: 125
CD166-M9_vK3b/HcB	SEQ ID NO: 121	SEQ ID NO: 126
CD166-M9_vK1/HcC	SEQ ID NO: 122	SEQ ID NO: 123
CD166-M9_vK2/HcC	SEQ ID NO: 122	SEQ ID NO: 124
CD166-M9_vK3a/HcC	SEQ ID NO: 122	SEQ ID NO: 125
CD166-M9_vK3b/HcC	SEQ ID NO: 122	SEQ ID NO: 126

[000536] The M9 mAb was obtained using mouse hybridoma technology, and the remaining sequences were generated by humanizing the M9 mAb sequence. Those of ordinary skill in the art will appreciate that the ability to raise anti-CD166 antibodies was hampered for a long time, because researchers could not generate hybridomas and/or antibodies in mice that were administered human CD166. In contrast, the anti-CD166 antibodies presented herein were generated against human CD166 in mice.

[000537] The antibody CD166-M9 antibody comprising VH of SEQ ID NO: 119, VL of SEQ ID NO: 120 was used a positive control, and an isotype control antibody was used a negative control.

[000538] As shown in Figure 1, all of the humanized ant-CD166 antibodies showed comparable binding to CD166 M9. Using a standard ELISA protocol, human CD166 protein was absorbed to ELISA plates and subsequently incubated with the indicated concentration of antibody. Bound antibody was detected with an anti-human FAB-peroxidase secondary.

EXAMPLE 2. Mask Discovery

[000539] The studies provided herein were designed to identify and characterize masking moieties for use in activatable anti-CD166 antibodies of the disclosure.

[000540] The mouse anti-CD166 Mab M9 (VH of SEQ ID NO: 119, VL of SEQ ID NO: 120 was used to screen a cysteine constrained X_{15} peptide library with a total diversity of $3x10^{11}$, where X is any amino acid, using a method similar to that described in PCT International Publication Number WO 2010/081173, published 15 July 2010. The screening consisted of two rounds of MACS and four rounds of FACS sorting. The sort process is outlined in Figure 2.

[000541] Individual clones from the M2F1.1, M2F2.1, M2F3.1, and M2F4.1 populations were sequenced and the results are shown in Table 9.

Table 9. Masking Peptide Sequences:

M2F1.1

JF16490 YLCQRHPLALKYCTN (SEQ ID NO: 135)
JF16492 PLCVPTQLLRSCYNY (SEQ ID NO: 136)
JF16493 AVCHPLANVETQCLD (SEQ ID NO: 137)
JF16494 PHCHPLFNNTYCYRH (SEQ ID NO: 138)
JF16495 PLCRPIELLASCPMK (SEQ ID NO: 139)
JF16496 GAACVSAWGFFCECC (SEQ ID NO: 140)
JF16498 DCAKDILHLMPHCSM (SEQ ID NO: 141)
JF16501 NTCMHPLLLQGCKTY (SEQ ID NO: 142)
JF16503 YLGCLLYAGPGCEGG (SEQ ID NO: 143)
JF16506 ARCPHPLLLSICENN (SEQ ID NO: 144)
JF16507 ELCPHPLPFGFCNNY (SEQ ID NO: 145)
JF16508 ALYCHPPYIRCEEMT (SEQ ID NO: 146)

M2F2.1

JF16534 TSLCHPVMIMYCKTG (SEQ ID NO: 147)
JF16535 PLCHPLEQASWCNMD (SEQ ID NO: 148)
JF16536 PHPCPRTGSRMCHFS (SEQ ID NO: 149)
JF16537 SGCRHPLPLKACGTN (SEQ ID NO: 150)
JF16538 GLCHPIRLHNTQCTI (SEQ ID NO: 151)
JF16539 KCMHPLNLHNINCNH (SEQ ID NO: 152)
JF16540 PICHPLREFMNTCFK (SEQ ID NO: 153)
JF16541 NCHPLDVVGWLGCMK (SEQ ID NO: 154)

JF16542 YNNVCHPLFCSQHTY (SEQ ID NO: 155)
JF16543 TFCHPLFSLNYCGHK (SEQ ID NO: 156)
JF16544 FCHPLTLSNNKQCNR (SEQ ID NO: 157)
JF16545 LSHCAVLLLRVCSGS (SEQ ID NO: 158)
JF16546 KIHCHPLRLGTCLVG (SEQ ID NO: 159)
JF16547 ETCAHPLDMRMCRHN (SEQ ID NO: 160)
JF16548 PLCYPLILMSSCWLG (SEQ ID NO: 161)
JF16549 YGICHPAPDLPCMQI (SEQ ID NO: 162)
JF16550 TACHPLYNVEHLCEI (SEQ ID NO: 163)
JF16551 TACNKSVCVAGCCLL (SEQ ID NO: 164)
JF16552 LHPLCSYMKSCMKNN (SEQ ID NO: 165)
JF16553 THCHCMVYFCPCRWS (SEQ ID NO: 166)

M2F3.1

JF16554 PKCPHPLHLANCYAS (SEQ ID NO: 167)
JF16555 KTCYHPTPVIAXNSY (SEQ ID NO: 168)
JF16556 AKCLPPLIQYCRCIK (SEQ ID NO: 169)
JF16557 HACQHPLQLHTCKHN (SEQ ID NO: 170)
JF16558 LCHPLVLSAWESCSN (SEQ ID NO: 171)
JF16559 WPLCSFGKSFCAQNA (SEQ ID NO: 172)
JF16560 ECQSFEHFLTNNCHS (SEQ ID NO: 173)
JF16561 SCKHPLVMPNLKCTR (SEQ ID NO: 174)
JF16562 YPCHPLQLSIPHCTK (SEQ ID NO: 175)
JF16563 ICHPLTHTMEYMCMN (SEQ ID NO: 176)
JF16564 TLCHPLTFSVPTCTN (SEQ ID NO: 177)
JF16565 PLCQPNRLLQACGNT (SEQ ID NO: 178)
JF16566 TLCRHPLALDGCQNN (SEQ ID NO: 179)
JF16567 QPMCYQPAHPLCNTI (SEQ ID NO: 180)
JF16568 SNCHPLLFQHYHCML (SEQ ID NO: 181)
JF16569 EKCYHPLTLAHCQNH (SEQ ID NO: 182)
JF16571 NKCFVHPLAMPNCNS (SEQ ID NO: 183)
JF16572 VNNCLLMTRAHCTSY (SEQ ID NO: 184)
JF16573 LPCWAFAVNPLHCGD (SEQ ID NO: 185)

M2F4.1

JS7503 VNNCLLMTRAHCTSY (SEQ ID NO: 186)

JS7504 SSCPHPLGLTGCNDK (SEQ ID NO: 187)

JS7505 NKCFVHPLAMPNCNS (SEQ ID NO: 188)

JS7506 FVGCHSVYVSGCLRA (SEQ ID NO: 189)

JS7507 NMCHPPHNIYSICNM (SEQ ID NO: 190)

JS7509 LTCHLLPGLTLH-TK (SEQ ID NO: 191)

JS7510 RTCHPLPGLTLHCTK (SEQ ID NO: 192)

JS7511 HPLCFESMKNCFPNY (SEQ ID NO: 193)

JS7513 TTCHPLSFTHNYCIT (SEQ ID NO: 194)

JS7515 RDCGFDAVRADCLFG (SEQ ID NO: 195)

JS7516 RTCSTHPLTMPQCNY (SEQ ID NO: 196)

JS7517 MKCHPLQLTGNTCSM (SEQ ID NO: 197)

JS7518 SGCPHPLQLITCSTA (SEQ ID NO: 198)

JS7519 KCFPAFHDGPLACAS (SEQ ID NO: 199)

JS7520 LKCQHPLPMSHCQPQ (SEQ ID NO: 200)

JS7521 AFCGFSVIHPLCSGA (SEQ ID NO: 201)

JS7522 SVHCAVLKLDGCLGW (SEQ ID NO: 202)

JS7523 TLPCHPIMVLGCTPM (SEQ ID NO: 203)

JS7525 HYPCMKYNPLNCSMS (SEQ ID NO: 204)

JS7526 LKCPHPLSLNGCTLK (SEQ ID NO: 205)

JS7527 VYSCMANNPLDCFTQ (SEQ ID NO: 206)

JS7528 PICHPLVTLMSYCNK (SEQ ID NO: 207)

JS7529 DWCSFWAGQSVWCTS (SEQ ID NO: 208)

JS7530 STCHPLTPFHDKCRY (SEQ ID NO: 209)

JS7531 PVCPPLVTLMSYCNK (SEQ ID NO: 210)

JS7532 STCHPLPTLMPYCNS (SEQ ID NO: 211)

JS7533 FPLCGIGPAFCDTTV (SEQ ID NO: 212)

JS7534 PTCHPLVLSVPCPKI (SEQ ID NO: 213)

JS7537 GPLCDYFVFYSCRGS (SEQ ID NO: 214)

JS7538 HTCYHPLKLGQCEMF (SEQ ID NO: 215)

JS7539 RTCIHPLPLHQCHKP (SEQ ID NO: 216)

JS7540 ACHPINFNSIVYCNN (SEQ ID NO: 217)
JS7542 SHPCSVVNLPGCEPD (SEQ ID NO: 218)

[000542] Masks were truncated and alanine scanned to generate families of activatable antibodies with different masking efficiencies. The sequences are shown below in Table 10. The "a" indicates the position of the alanine incorporated as part of the scan. It is equivalent to "A".

Table 10. Truncation and Alanine Scanning of Masking Peptides

7614	LCHPLVLSAWESCSS (SEQ ID NO: 219)
7614.4	LCaPLVLSAWESCSS (SEQ ID NO: 220)
7614.5	LCHaLVLSAWESCSS (SEQ ID NO: 221)
7614.6	LCHPaVLSAWESCSS (SEQ ID NO: 222)
7614.7	LCHPLaLSAWESCSS (SEQ ID NO: 223)
7614.8	LCHPLVaSAWESCSS (SEQ ID NO: 224)
7614.9	LCHPLVLSAaESCSS (SEQ ID NO: 225)
7614.10	LCHPLVLSAWaSCSS (SEQ ID NO: 226)
7614.11	CHPLVLSAWESC (SEQ ID NO: 227)
7614.12	HPLVL (SEQ ID NO: 228)
7614.13	HPL (SEQ ID NO: 229)

16522	LEGWCLHPLCLWGAG (SEQ ID NO: 230)
16522.14	LEGaCLHPLCLWGAG (SEQ ID NO: 231)
16522.15	LEGWCaHPLCLWGAG (SEQ ID NO: 232)
16522.16	LEGWCLaPLCLWGAG (SEQ ID NO: 233)
16522.17	LEGWCLHaLCLWGAG (SEQ ID NO: 234)
16522.18	LEGWCLHPaCLWGAG (SEQ ID NO: 235)
16522.19	LEGWCLHPLCaWGAG (SEQ ID NO: 236)
16522.20	LEGWCLHPLCLaGAG (SEQ ID NO: 237)
16522.21	CLHPLC (SEQ ID NO: 238)

[000543] These masking peptides were used to generate anti-CD166 activatable antibodies of the disclosure. The sequences for certain of these anti-CD166 activatable antibodies are shown below in Table 11. In some embodiments, these anti-CD166 activatable antibodies include cleavable moiety 2001 (ISSGLLSGRSDNH; SEQ ID NO: 70), cleavable moiety 3001 (AVGLLAPPGGLSGRSDNH; SEQ ID NO: 76), cleavable moiety 2007 (ISSGLLSGRSDIH; SEQ ID NO: 342), cleavable moiety 2008 (ISSGLLSGRSDQH; SEQ ID NO: 343), cleavable moiety 2011 (ISSGLLSGRSDNP; SEQ ID NO: 346), cleavable moiety 2012 (ISSGLLSGRSANP; SEQ ID NO: 347), cleavable moiety 2013 (ISSGLLSGRSANI; SEQ ID NO: 348), cleavable moiety 3007 (AVGLLAPPGGLSGRSDIH; SEQ ID NO: 350), cleavable moiety 3008 (AVGLLAPPGGLSGRSDQH; SEQ ID NO: 351), cleavable moiety 3011 (AVGLLAPPGGLSGRSDNP; SEQ ID NO: 354), cleavable moiety 3012 (AVGLLAPPGGLSGRSANP; SEQ ID NO: 355), or cleavable moiety 3013 (AVGLLAPPGGLSGRSANI; SEQ ID NO: 356), as indicated.

[000544] While certain sequences shown below include the spacer sequence of SEQ ID NO: 305, those of ordinary skill in the art appreciate that the activatable anti-CD166 antibodies of the disclosure can include any suitable spacer sequence, such as, for example, a spacer sequence selected from the group consisting of QGQSGQG (SEQ ID NO: 305), QGQSGQ (SEQ ID NO: 88), QGQSG (SEQ ID NO: 306), QGQS (SEQ ID NO: 307), QGQ (SEQ ID NO: 308), QG (SEQ ID NO: 309), GQSGQG (SEQ ID NO: 359), QSGQG (SEQ ID NO: 360), SGQG (SEQ ID NO: 361), GQG (SEQ ID NO: 362), G or Q. In some embodiments, the activatable anti-CD166 antibodies of the disclosure can have no spacer sequence joined to its N-terminus.

Table 11. Anti-CD166 Activatable Antibody Sequences

Anti-CD166 Activatable Antibody Heavy Chain (HuCD166_HcC):
Amino Acid sequence

QITLKESGPTLVKPTQTLTLTCTFSGFSLSTYGMGVGWIRQPPGKALEWLANIWWSEDKHYSPSLKS
RLTITKDTSKNQVVLTITNVDPVDTATYYCVQIDYGNDYAFTYWGQGTLVTVSSASTKGPSVFPLAP
SSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQT
YICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP

IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL
DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 239)

Nucleotide sequence

CAGATCACCCTGAAAGAGTCCGGCCCCACCCTGGTGAAACCCACCCAGACCCTGACCCTGACATGCA
CCTTCTCCGGCTTCAGCCTGTCCACCTACGGCATGGGCGTGGGCTGGATCAGGCAGCCTCCTGGCAA
GGCCCTGGAATGGCTGGCCAACATCTGGTGGTCCGAGGACAAGCACTACTCCCCCAGCCTGAAGTCC
CGGCTGACCATCACCAAGGACACCTCCAAGAACCAGGTGGTGCTGACAATCACAAACGTGGACCCCG
TGGACACCGCCACCTACTACTGCGTGCAGATCGACTACGGCAACGACTACGCCTTCACCTACTGGGG
CCAGGGCACACTGGTGACAGTGTCCTCCGCCTCCACCAAGGGCCCCTCCGTGTTCCCTCTGGCCCCT
TCCAGCAAGTCCACCTCTGGCGGCACAGCTGCCCTGGGCTGCCTGGTGAAAGACTACTTCCCCGAGC
CCGTGACCGTGTCCTGGAACTCTGGCGCCCTGACCAGCGGAGTGCACACCTTCCCTGCCGTGCTGCA
GTCCTCCGGCCTGTACTCCCTGTCCTCCGTGGTGACCGTGCCCTCCAGCTCTCTGGGCACCCAGACC
TACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAGGTGGACAAGAAGGTGGAACCCAAGTCCT
GCGACAAGACCCACACCTGTCCCCCCTGCCCTGCCCCTGAACTGCTGGGCGGACCTTCCGTGTTTCT
GTTCCCCCCAAAGCCTAAGGACACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTG
GACGTGTCCCACGAGGACCCTGAAGTGAAGTTCAATTGGTACGTGGACGGCGTGGAAGTGCACAACG
CCAAGACCAAGCCCAGAGAGGAACAGTACAACTCCACCTACCGGGTGGTGTCTGTGCTGACCGTGCT
GCACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAGGCCCTGCCTGCCCCC
ATCGAAAAGACCATCTCCAAGGCCAAGGGCCAGCCCCGCGAGCCTCAGGTGTACACACTGCCCCCTA
GCCGGGAAGAGATGACCAAGAATCAGGTGTCCCTGACCTGTCTGGTGAAAGGCTTCTACCCCTCCGA
TATCGCCGTGGAATGGGAGTCCAACGGCCAGCCCGAGAACAACTACAAGACCACCCCCCCTGTGCTG
GACTCCGACGGCTCATTCTTCCTGTACTCCAAGCTGACCGTGGACAAGTCCCGGTGGCAGCAGGGCA
ACGTGTTCTCCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCT
GAGCCCCGGCAAG (SEQ ID NO: 241)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2001 (SEQ ID NO: 310)] Amino Acid sequence

[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSDNHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 242)

[spacer (SEQ ID NO: 319)] [HuCD166Lc1_7614.6_2001 (SEQ ID NO: 311)] Nucleotide Sequence

[CAGGGCCAGTCTGGACAGGGC][CTGTGTCACCCTGCCGTGCTGTCTGCCTGGGAGTCCTGTTCCT
CCGGCGGTGGCTCCTCTGGCGGCTCCATCTCCTCTGGCCTGCTGTCCGGCAGATCCGACAACCACGG

CGGAGGCAGCGACATCGTGATGACCCAGTCCCCCCTGTCCCTGCCCGTGACACCTGGCGAGCCTGCC
TCCATCAGCTGCCGGTCCTCCAAGTCCCTGCTGCACTCCAACGGCATCACCTACCTGTACTGGTATC
TGCAGAAGCCCGGCCAGTCCCCTCAGCTGCTGATCTACCAGATGTCCAACCTGGCCTCCGGCGTGCC
CGACAGATTCTCCGGCTCTGGCTCCGGCACCGACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAG
GACGTGGGCGTGTACTACTGCGCCCAGAACCTGGAACTGCCCTACACCTTCGGCCAGGGCACCAAGC
TGGAAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTCATCTTCCCACCCTCCGACGAGCAGCTGAA
GTCCGGCACCGCCTCCGTCGTGTGCCTGCTGAACAACTTCTACCCTCGCGAGGCCAAGGTGCAGTGG
AAGGTGGACAACGCCCTGCAGTCCGGCAACTCCCAGGAATCCGTCACCGAGCAGGACTCCAAGGACA
GCACCTACTCCCTGTCCTCCACCCTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGTGTACGC
CTGCGAAGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGTCCTTCAACCGCGGCGAGTGC]
(SEQ ID NO: 243)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_2001 (SEQ ID NO: 312)] Amino Acid Sequence
[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSDNHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 244)

[spacer (SEQ ID NO: 319)] [huCD166Lc1_7614.8_2001 (SEQ ID NO: 313)] Nucleotide Sequence
[CAGGGCCAGTCTGGACAGGGC][CTGTGTCACCCTCTGGTGGCCTCTGCCTGGGAGTCCTGTTCCT
CCGGCGGTGGCTCCTCTGGCGGCTCCATCTCCTCTGGCCTGCTGTCCGGCAGATCCGACAACCACGG
CGGAGGCAGCGACATCGTGATGACCCAGTCCCCCCTGTCCCTGCCCGTGACACCTGGCGAGCCTGCC
TCCATCAGCTGCCGGTCCTCCAAGTCCCTGCTGCACTCCAACGGCATCACCTACCTGTACTGGTATC
TGCAGAAGCCCGGCCAGTCCCCTCAGCTGCTGATCTACCAGATGTCCAACCTGGCCTCCGGCGTGCC
CGACAGATTCTCCGGCTCTGGCTCCGGCACCGACTTCACCCTGAAGATCTCCCGCGTGGAAGCCGAG
GACGTGGGCGTGTACTACTGCGCCCAGAACCTGGAACTGCCCTACACCTTCGGCCAGGGCACCAAGC
TGGAAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTCATCTTCCCACCCTCCGACGAGCAGCTGAA
GTCCGGCACCGCCTCCGTCGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGG
AAGGTGGACAACGCCCTGCAGTCCGGCAACTCCCAGGAATCCGTCACCGAGCAGGACTCCAAGGACA
GCACCTACTCCCTGTCCTCCACCCTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGTGTACGC
CTGCGAAGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGTCCTTCAACCGCGGCGAGTGC]
(SEQ ID NO: 245)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3001 (SEQ ID NO: 314)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC (SEQ ID NO: 246)

[spacer (SEQ ID NO: 319)] [huCD166Lc1_7614.6_3001 (SEQ ID NO: 315)] Nucleotide Sequence

[CAGGGACAGTCTGGCCAGGGC][CTGTGTCACCCTGCTGTGCTGTCTGCCTGGGAGTCCTGTTCCA
GCGGCGGAGGCTCCTCTGGCGGCTCTGCTGTGGGCCTGCTGGCTCCACCTGGCGGCCTGTCCGGCAG
ATCTGACAACCACGGCGGCTCCGACATCGTGATGACCCAGTCCCCCCTGTCCCTGCCCGTGACTCCT
GGCGAGCCTGCCTCCATCTCCTGCCGGTCCTCCAAGTCCCTGCTGCACTCCAACGGCATCACCTACC
TGTACTGGTATCTGCAGAAGCCCGGCCAGTCCCCTCAGCTGCTGATCTACCAGATGTCCAACCTGGC
CTCCGGCGTGCCCGACAGATTCTCCGGCTCTGGCTCCGGCACCGACTTCACCCTGAAGATCTCCCGG
GTGGAAGCCGAGGACGTGGGCGTGTACTACTGCGCCCAGAACCTGGAACTGCCCTACACCTTCGGCC
AGGGCACCAAGCTGGAAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTCATCTTCCCACCCTCCGA
CGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTCTGCCTGCTGAACAACTTCTACCCCCGCGAGGCC
AAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCCCAGGAATCCGTCACCGAGCAGG
ACTCCAAGGACAGCACCTACTCCCTGTCCTCCACCCTGACCCTGTCCAAGGCCGACTACGAGAAGCA
CAAGGTGTACGCCTGCGAAGTGACCCACCAGGGACTGAGCAGCCCCGTGACCAAGTCCTTCAACCGG
GGCGAGTGC] (SEQ ID NO: 247)

[spacer (SEQ ID NO: 309)] [huCD166Lc1_7614.8_3001 (SEQ ID NO: 316)] Amino Acid Sequence

[QG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNHGGSDIVMTQSPLSLPVTPGEP
ASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVEA
EDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
] (SEQ ID NO: 248)

[spacer (SEQ ID NO: 320)] [huCD166Lc1_7614.8_3001 (SEQ ID NO: 317)] Nucleotide Sequence

[[CAGGGCC][TGTGTCACCCTCTGGTGGCCTCTGCCTGGGAGTCCTGTTCCTCCGGCGGAGGCTCC
TCTGGCGGCTCTGCTGTGGGCCTGCTGGCTCCACCTGGCGGCCTGTCCGGCAGATCTGACAACCACG
GCGGCTCCGACATCGTGATGACCCAGTCCCCCCTGTCCCTGCCCGTGACTCCTGGCGAGCCTGCCTC
CATCTCCTGCCGGTCCTCCAAGTCCCTGCTGCACTCCAACGGCATCACCTACCTGTACTGGTATCTG
CAGAAGCCCGGCCAGTCCCCTCAGCTGCTGATCTACCAGATGTCCAACCTGGCCTCCGGCGTGCCCG
ACAGATTCTCCGGCTCTGGCTCCGGCACCGACTTCACCCTGAAGATCTCCCGGGTGGAAGCCGAGGA

CGTGGGCGTGTACTACTGCGCCCAGAACCTGGAACTGCCCTACACCTTCGGCCAGGGCACCAAGCTG
GAAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTCATCTTCCCACCCTCCGACGAGCAGCTGAAGT
CCGGCACCGCCTCCGTCGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAA
GGTGGACAACGCCCTGCAGTCCGGCAACTCCCAGGAATCCGTGACCGAGCAGGACTCCAAGGACAGC
ACCTACTCCCTGTCCTCCACCCTGACCCTGTCCAAGGCCGACTACGAGAAGCACAAGGTGTACGCCT
GCGAAGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGTCCTTCAACCGGGGCGAGTGC]
(SEQ ID NO: 478)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3001 (SEQ ID NO: 316)] Amino Acid Sequence

[QGQSGQG] [LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNHGGSDIVMTQSPLSLPV
TPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKI
SRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR
EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC] (SEQ ID NO: 303)

[spacer (SEQ ID NO: 319)] [huCD166Lc1_7614.8_CM2 (SEQ ID NO: 317)] Nucleotide Sequence
[CAGGGCCAGTCTGGCCAGGGC] [CTGTGTCACCCTCTGGTGGCCTCTGCCTGGGAGTCCTGTTCCT
CCGGCGGAGGCTCCTCTGGCGGCTCTGCTGTGGGCCTGCTGGCTCCACCTGGCGGCCTGTCCGGCAG
ATCTGACAACCACGGCGGCTCCGACATCGTGATGACCCAGTCCCCCCTGTCCCTGCCCGTGACTCCT
GGCGAGCCTGCCTCCATCTCCTGCCGGTCCTCCAAGTCCCTGCTGCACTCCAACGGCATCACCTACC
TGTACTGGTATCTGCAGAAGCCCGGCCAGTCCCCTCAGCTGCTGATCTACCAGATGTCCAACCTGGC
CTCCGGCGTGCCCGACAGATTCTCCGGCTCTGGCTCCGGCACCGACTTCACCCTGAAGATCTCCCGG
GTGGAAGCCGAGGACGTGGGCGTGTACTACTGCGCCCAGAACCTGGAACTGCCCTACACCTTCGGCC
AGGGCACCAAGCTGGAAATCAAGCGGACCGTGGCCGCTCCCTCCGTGTTCATCTTCCCACCCTCCGA
CGAGCAGCTGAAGTCCGGCACCGCCTCCGTCGTGTGCCTGCTGAACAACTTCTACCCCCGCGAGGCC
AAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCCCAGGAATCCGTGACCGAGCAGG
ACTCCAAGGACAGCACCTACTCCCTGTCCTCCACCCTGACCCTGTCCAAGGCCGACTACGAGAAGCA
CAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGAGCAGCCCCGTGACCAAGTCCTTCAACCGG
GGCGAGTGC (SEQ ID NO: 304)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2001 VL domain (SEQ ID NO: 364)] Amino Acid sequence
[QGQSGQG] [LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSDNHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 363)

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[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_2001 VL domain (SEQ ID NO: 366)] Amino Acid Sequence

[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSDNHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 365)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3001 VL domain (SEQ ID NO: 368)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 367)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3001 VL domain (SEQ ID NO: 370)] Amino Acid Sequence

[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNHGGSDIVMTQSPLSLPV
TPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKI
SRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 369)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2007 (SEQ ID NO: 372)] Amino Acid sequence

[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSDIHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 371)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2007 VL domain (SEQ ID NO: 374)] Amino Acid sequence

[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSDIHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 373)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3007 (SEQ ID NO: 376)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDIHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP

REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 375)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3007 VL domain (SEQ ID NO: 378)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDIHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 377)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2008 (SEQ ID NO: 380)] Amino Acid sequence

[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSDQHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 379)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2008 VL domain (SEQ ID NO: 382)] Amino Acid sequence

[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSDQHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 381)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3008 (SEQ ID NO: 384)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDQHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 383)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3008 VL domain (SEQ ID NO: 386)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDQHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 385)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2011 (SEQ ID NO: 388)] Amino Acid sequence
[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSDNPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 387)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2011 VL domain (SEQ ID NO: 390)] Amino Acid sequence
[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSDNPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 389)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3011 (SEQ ID NO: 392)] Amino Acid Sequence
[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 391)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3011 VL domain (SEQ ID NO: 394)] Amino Acid Sequence
[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 393)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2012 (SEQ ID NO: 396)] Amino Acid sequence
[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSANPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 395)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2012 VL domain (SEQ ID NO: 398)] Amino Acid sequence

[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSANPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 397)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3012 (SEQ ID NO: 400)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 399)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3012 VL domain (SEQ ID NO: 402)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 401)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2013 (SEQ ID NO: 404)] Amino Acid sequence

[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSANIGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 403)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.6_2013 VL domain (SEQ ID NO: 406)] Amino Acid sequence

[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSISSGLLSGRSANIGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 405)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3013 (SEQ ID NO: 408)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANIGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP

REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 407)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.6_3013 VL domain (SEQ ID NO: 410)] Amino Acid Sequence

[[QGQSGQG][LCHPAVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANIGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 409)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2007 (SEQ ID NO: 412)] Amino Acid sequence

[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSDIHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 411)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2007 VL domain (SEQ ID NO: 414)] Amino Acid sequence

[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSDIHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 413)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3007 (SEQ ID NO: 416)] Amino Acid Sequence

[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDIHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 415)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3007 VL domain (SEQ ID NO: 418)] Amino Acid Sequence

[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDIHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 417)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2008 (SEQ ID NO: 420)] Amino Acid sequence
[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSDQHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 419)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2008 VL domain (SEQ ID NO: 422)] Amino Acid sequence
[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSDQHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 421)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3008 (SEQ ID NO: 424)] Amino Acid Sequence
[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDQHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 423)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3008 VL domain (SEQ ID NO: 426)] Amino Acid Sequence
[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDQHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 425)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2011 (SEQ ID NO: 428)] Amino Acid sequence
[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSDNPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 427)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2011 VL domain (SEQ ID NO: 430)] Amino Acid sequence

[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSDNPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 429)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3011 (SEQ ID NO: 432)] Amino Acid Sequence

[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 431)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3011 VL domain (SEQ ID NO: 434)] Amino Acid Sequence

[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 433)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2012 (SEQ ID NO: 436)] Amino Acid sequence

[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSANPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 435)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2012 VL domain (SEQ ID NO: 438)] Amino Acid sequence

[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSANPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 437)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3012 (SEQ ID NO: 440)] Amino Acid Sequence

[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP

REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 439)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3012 VL domain (SEQ ID NO: 442)] Amino Acid Sequence

[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 441)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2013 (SEQ ID NO: 444)] Amino Acid sequence

[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSANIGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 443)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614.8_2013 VL domain (SEQ ID NO: 446)] Amino Acid sequence

[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSISSGLLSGRSANIGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 445)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3013 (SEQ ID NO: 448)] Amino Acid Sequence

[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANIGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 447)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614.8_3013 VL domain (SEQ ID NO: 450)] Amino Acid Sequence

[[QGQSGQG][LCHPLVASAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANIGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 449)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614_2001 (SEQ ID NO: 452)] Amino Acid sequence
[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSISSGLLSGRSDNHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 451)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614_2001 VL domain (SEQ ID NO: 454)] Amino Acid sequence
[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSISSGLLSGRSDNHGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 453)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614_3001 (SEQ ID NO: 456)] Amino Acid Sequence
[[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 455)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614_3001 VL domain (SEQ ID NO: 458)] Amino Acid Sequence
[[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNHGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 457)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614_2011 (SEQ ID NO: 460)] Amino Acid sequence
[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSISSGLLSGRSDNPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 459)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614_2011 VL domain (SEQ ID NO: 462)] Amino Acid sequence

[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSISSGLLSGRSDNPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 461)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614_3011 (SEQ ID NO: 464)] Amino Acid Sequence

[[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 463)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614_3011 VL domain (SEQ ID NO: 466)] Amino Acid Sequence

[[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSDNPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 465)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614_2012 (SEQ ID NO: 468)] Amino Acid sequence

[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSISSGLLSGRSANPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE
C] (SEQ ID NO: 467)

[spacer (SEQ ID NO: 305)] [HuCD166Lc1_7614_2012 VL domain (SEQ ID NO: 470)] Amino Acid sequence

[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSISSGLLSGRSANPGGGSDIVMTQSPLSLPVTPGE
PASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLKISRVE
AEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 469)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614_3012 (SEQ ID NO: 472)] Amino Acid Sequence

[[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP

REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKS
FNRGEC] (SEQ ID NO: 471)

[spacer (SEQ ID NO: 305)] [huCD166Lc1_7614_3012 VL domain (SEQ ID NO: 474)] Amino Acid Sequence
[[QGQSGQG][LCHPLVLSAWESCSSGGGSSGGSAVGLLAPPGGLSGRSANPGGSDIVMTQSPLSLP
VTPGEPASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQMSNLASGVPDRFSGSGSGTDFTLK
ISRVEAEDVGVYYCAQNLELPYTFGQGTKLEIK] (SEQ ID NO: 473)

EXAMPLE 3. Generation of Activatable Anti-CD166 Antibodies

[000545] The studies provided herein were designed to generate activatable anti-CD166 antibodies of the disclosure.

[000546] Anti-CD166 activatable antibodies were generated with different masking efficiencies (i.e., a measurement of the ability of the MM of the activatable antibody to block binding of the AB of the activatable antibody to its target). The peptides 16522 and 7614 were mutated by truncation and alanine scanning as described in Example 2, and these masking peptide variants were used to generate families of anti-CD166 activatable antibodies with a range of fold masking. The ability of the anti-CD166 antibody CD166 M9 vK1/HcB (VH of SEQ ID NO: 121, VL of SEQ ID NO: 123) and various anti-CD166 activatable antibodies to bind human CD166 was evaluated using a CD166 binding ELISA (Figure 3A, 3B). The anti-CD166 activatable antibodies tested included the variable heavy chain sequence of SEQ ID NO: 121, the variable light chain sequence of SEQ ID NO: 123, a cleavable moiety (CM) comprising the amino acid sequence ISSGLLSGRSDNH (SEQ ID NO: 70) referred to herein as substrate 2001, and one of the masking moieties shown in Table 10. The full sequences are shown above in Table 11.

[000547] Using a standard ELISA protocol, human CD166 protein is absorbed to ELISA plates and subsequently incubated with the indicated concentration of antibody or activatable antibody. Bound antibody or activatable antibody was detected with an anti-human FAB-peroxidase secondary. A summary of the exemplary apparent *in vitro* dissociation constants (Kd) of the activatable antibodies of the present invention to CD166 polypeptide as determined by ELISA is shown below in Table 17, as well as the respective increase in Kd relative to the parent anti-CD166 M9 antibody (vk-1/HcB). Except for the parental anti-CD166 M9 antibody, the mask for each activatable antibody is as indicated as

described herein, and the substrate sequence was 2001 (ISSGLLSGRSDNH (SEQ ID NO: 70)).

Table 17: Apparent ELISA Dissociation Constants of Activatable Anti-CD166 Antibodies

Antibody Construct	Apparent Kd (nM)	Fold Increase in Kd
CD166 M9 vk1/HcB	4.4	1
CD166-16522-2001	423.7	95
CD166-7614-2001	998.6	225
CD166-7614.5-2001	495.0	112
CD166-7614.6-2001	265.6	60
CD166-7614.7-2001	618.6	139
CD166-7614.8-2001	82.2	19
CD166-7614.9-2001	534.0	120
CD166-7614.10-2001	685.2	154
CD166-7614.11-2001	544.0	123
CD166-7614.12-2001	331.9	75
CD166-7614.13-2001	77.7	18
CD166-16522.14-2001	422.1	95
CD166-16522.15-2001	447.7	101
CD166-16522.16-2001	47.4	11
CD166-16522.17-2001	61.6	14
CD166-16522.18-2001	57.7	13
CD166-16522.19-2001	49.2	11
CD166-16522.20-2001	700.3	158
CD166-16522.21-2001	144.7	33

EXAMPLE 4. Activation of Activatable Anti-CD166 Antibodies

[000548] The studies provided herein were designed to evaluate activation of activatable anti-CD166 antibodies.

[000549] Figure 4 is a graph depicting the ability of various anti-CD166 activatable antibodies of the disclosure to bind human CD166 when proteolytically activated. As shown in Figure 4, anti-CD166 activatable antibodies recover antibody binding when proteolytically activated. The binding of the anti-CD166 antibody (huM9 HcC/vk-1; VH of SEQ ID NO: 122, VL of SEQ ID NO: 123), various anti-CD166 activatable antibodies of the disclosure, and uPA activated anti-CD166 activatable antibodies was evaluated using a CD166 binding ELISA. The anti-CD166 activatable antibodies tested included the variable heavy chain sequence of SEQ ID NO: 122, the variable light chain sequence of SEQ ID NO: 123, either a cleavable moiety (CM1, substrate 2001) comprising the amino acid sequence ISSGLLSGRSDNH (SEQ ID NO: 70) or a cleavable moiety (CM2, substrate 3001) comprising the amino acid sequence AVGLLAPPGGLSGRSDNH (SEQ ID NO: 76), and one of the masking moieties shown in Table 10. The full sequences are shown above in Table 11.

[000550] Using a standard ELISA protocol, human CD166 protein is absorbed to ELISA plates and subsequently incubated with the indicated concentration of antibody or activatable antibody. Bound antibody or activatable antibody was detected with an anti-human FAB-peroxidase secondary.

[000551] In an exemplary study, the binding affinity of anti-CD166 antibodies (anti-CD166 HcC/vk-1 of the present disclosure and anti-CD166 activatable antibodies (anti-CD166-7614.6-3001) of the present disclosure to human cells (HCC1806 human breast cancer cells) and human CD166 were determined. Under these conditions, apparent exemplary binding affinities (K_d) by ELISA are 96.2 nM and 1.3 nM for the anti-CD166 activatable antibody and the anti-CD166 antibody of the present disclosure, respectively. In the exemplary HCC1806 cell binding assay as measured by flow cytometry, apparent binding affinities (Kd) are 372 nM and 3.2 nM for the anti-CD166 activatable antibody and the anti-CD166 antibody of the present disclosure, respectively. These exemplary results demonstrated that the anti-CD166 activatable antibody, in an uncleaved state, demonstrated a lower binding affinity to isolated CD166 polypeptide and CD166 on cells, as compared to the anti-CD166 antibody of the present disclosure.

[000552] Figure 5 is a graph depicting the ability of various conjugated anti-CD166 activatable antibodies of the disclosure to effect cell killing of HCC1806 cells when proteolytically activated with uPA. These conjugated anti-CD166 activatable antibodies are also referred to herein as "CD166 AADC" or CD166 Activatable Antibody Drug

Conjugates. The conjugated anti-CD166 activatable antibodies tested included the variable heavy chain sequence of SEQ ID NO: 122, the variable light chain sequence of SEQ ID NO: 123, a cleavable moiety (CM1, substrate 2001) comprising the amino acid sequence ISSGLLSGRSDNH (SEQ ID NO: 70), one of the masking moieties shown in Table 10, and the maytansinoid DM4 conjugated to the activatable antibody via an SPDB linker. The full sequences of the activatable antibodies are shown above in Table 11. All conjugated activatable antibodies disclosed herein were produced by TCRS (The Chemistry Research Solution).

[000553] As shown in Figure 5, various conjugated anti-CD166 activatable antibodies of the disclosure behave like isotype-DM4 when masked, but when proteolytically activated with uPA, these conjugated anti-CD166 activatable antibodies show similar cell killing to that of the huCD166 ADC. The ability of drug conjugates to kill HCC1806 cells was evaluated by adding the indicated concentration of ADC or AADC and incubating the cells for 3 days. Cell viability was measured using the CellTiter Glo assay. Similar results were observed when an activatable antibody conjugated to a nucleic acid damaging agent was tested in such cell killing assays.

[000554] The next studies were designed to evaluate the activation and binding of anti-CD166 activatable antibodies in tumor samples upon exposure to one or more proteases.

[000555] Xenograft tumor samples and healthy tissue samples were analyzed using immunohistochemistry (IHC) analysis and an *in situ* imaging assay that is described in PCT Publication No. WO 2014/107599, the contents of which are hereby incorporated by reference in their entirety. Briefly, this *in situ* assay uses an activatable anti-CD166 antibody of the disclosure, the corresponding parental antibody, and a modified version of the activatable anti-CD166 antibody in which the CM is replaced with a non-cleavable linker, referred to herein generally as anti-CD166 NSUB modified antibody. The parental antibody is used as a positive control, while the non-cleavable version of the activatable anti-CD166 antibody is used as a negative control. Xenograft frozen tissue sections are then placed on the glass slide, rinsed two times with PBS-T followed by PBS, followed by 30 min pretreatment of tissue with broad spectrum protease inhibitors cocktail or buffer only. A detectable label such as Alexa Fluor-680® is then conjugated to each of the activatable anti-CD166 antibody and the anti-CD166 NSUB modified antibody. The detectably labeled activatable anti-CD166 antibody and the anti-CD166 NSUB modified antibody are then applied on the tissue and incubated for one hour in the dark (to prevent bleaching of

fluorescence). After incubation with 1 μg/ml of the incubated tumor sections were rinsed three times with PBS-T followed by PBS and counterstained with nuclear marker DAPI for 1 minute. Fluorescence microscopy analysis is then used to detect positive staining. Positive staining of the activatable anti-CD166 antibody that is abolished by the pretreatment of the tissue sections with protease inhibitors indicates that the binding of the activatable anti-CD166 antibody to the tissue sample is a result of the proteolytic event. Positive staining of the activatable anti-CD166 antibody should also be abolished when the tissue is pretreated with an excess of unlabeled ("cold") parental antibody. Furthermore, incubation of the tumor tissue should reveal positive staining for parental antibody that is not affected by pretreatment of tissue with protease inhibitors, but is abolished when the tissue is pretreated with unlabeled parental antibody. No signal should be detected for the anti-CD166 NSUB modified antibody detected on the tissue pretreated, on the tissue not pretreated with protease inhibitors, or on the tissue pretreated with unlabeled parental antibody.

[000556] Figures 6A-6D are a series of images demonstrating that the activatable anti-CD166 antibody is activated (i.e., cleaved) in colon cancer tissue samples, and the activatable anti-CD166 antibody is not activated in healthy tissue samples. Figures 6A and 6C depict the results of IHC analysis on the tumor and healthy tissue samples, and Figures 6B and 6D depict the results of the *in situ* imaging assay on the tumor and healthy tissue samples.

[000557] Figures 7A-7D are a series of images demonstrating that the activatable anti-CD166 antibody is activated (i.e., cleaved) in lung cancer tissue samples, and the activatable anti-CD166 antibody is not activated in healthy tissue samples. Figures 7A and 7C depict the results of IHC analysis on the tumor and healthy tissue samples, and Figures 7B and 7D depict the results of the *in situ* imaging assay on the tumor and healthy tissue samples.

[000558] Figures 8A-8D are a series of images demonstrating that the activatable anti-CD166 antibody is not activated in healthy tissue samples. Figures 8A and 8C depict the results of IHC analysis on the healthy tissue samples, and Figures 8B and 8D depict the results of the *in situ* imaging assay on the healthy tissue samples.

EXAMPLE 5. Potency of Conjugated Anti-CD166 Antibodies

[000559] This example demonstrates that a conjugated anti-CD166 antibody of the disclosure ("CD166 ADC") displays in vitro killing activity as compared to a control antibody conjugate ("Control ADC").

[000560] Figure 9 is a series of graphs depicting the potency of a conjugated anti-CD166 antibody of the disclosure against a breast cancer cell line, a prostate cancer cell line, a pancreatic cancer cell line, a head and neck squamous cell cancer cell line, and a CD166 cell line as a negative control.

EXAMPLE 6. Efficacy of Conjugated Activatable Anti-CD166 Antibodies in Tumor Models

[000561] This example demonstrates the efficacy of a conjugated activatable anti-CD166 antibody of the disclosure ("CD166 AADC" or CD166 Activatable Antibody Drug Conjugate) in various cancer models.

[000562] Figure 10 is a graph depicting the efficacy of an AADC that includes an activatable anti-CD166 antibody of the disclosure conjugated to the maytansinoid DM4, as compared to an isotype DM4-conjugated control, and an ADC that includes a DM4-conjugated version of the parental antibody for the activatable anti-CD166 antibody. Efficacy is measured as mean tumor volume measured at various time points post administration (5 mg/kg IV on days 1 and 8) in a breast cancer model.

[000563] As shown in Figure 10, the efficacy of the AADC is equivalent to the efficacy seen with the ADC.

[000564] Figure 11 is a graph depicting the efficacy of the CD166 activatable anti-CD166 antibodies of the disclosure, as compared to the isotype DM4-conjugated control, and the CD166 ADC DM4 conjugated parental anti-CD166 antibody. Efficacy is measured as mean tumor volume measured at various time points post administration (5 mg/kg IV on days 1 and 8) in the H292 non-small cell lung cancer (NSCLC) model.

[000565] H292 xenograft tumors were treated with isotype-DM4 control, huCD166-DM4 ADC, huCD166_7614.6_CM2-DM4 AADC, or huCD166_7614.6_CM1-DM4 ADDC, where CM1 and CM2 are the 2001 and 3001 substrates described herein, respectively. Tumors were grown to an average of 150 mm^3, then the mice were

randomized into groups of eight and dosed on days 1 and 8 with the indicated test articles. Mean tumor volume ± SEM is plotted.

[000566] As shown in Figure 11, the efficacy of both of the AADCs tested is equivalent to the efficacy seen with the ADC.

[000567] Figure 12 is a graph depicting the efficacy of the CD166 AADC DM4 conjugated activatable anti-CD166 antibody, also referred to herein as huCD166_7614.6_CM2-DM4 AADC (where CM2 is the 3001 substrate), as compared to the isotype DM4-conjugated control, and the CD166 ADC DM4 conjugated parental anti-CD166 antibody. Efficacy is measured as mean tumor volume measured at various time points post administration (5 mg/kg IV on days 1 and 8) in the H1975 non-small cell lung cancer (NSCLC) model.

[000568] As shown in Figure 12, the efficacy of the AADC is equivalent to the efficacy seen with the ADC.

EXAMPLE 7. Tolerability Analysis of Activatable Anti-CD166 Antibodies

[000569] Anti-CD166 antibodies of the disclosure were characterized for their species specificity towards binding to human CD166 and other closely related proteins.

[000570] As shown in Figure 13, anti-CD166 antibodies of the disclosure bind human and cynomolgus monkey CD166 with equal affinity. None of the anti-CD166 antibodies of the disclosure tested bound to rat or mouse CD166. The K_d of binding of the anti-CD166 antibody to human CD166 and cynomolgus CD166 in these exemplary binding studies are 1.3 nM for both.

[000571] Next, CD166 expression levels were analyzed in various normal human and cynomolgus monkey tissue types. As shown in Table 3 below, CD166 expression levels were nearly identical the human and cynomolgus monkey tissue samples analyzed:

Table 3. CD166 Expression Levels in Normal Human and Cynomolgus Tissue Samples

Tissue Type	Cynomolgus	Human
Breast	++	++
Brain	-	-
Colon	++	++
Esophagus	-	-

Heart	-	-
Kidney	+	+
Liver	++	++
Lung	++	+
Nerve	-	-
Ovary	++	+
Pancreas	++	++
Prostate	+++	+++
Skin	N/A	-/+
Small Intestine	++	++
Salivary Gland	++	++
Spleen	-	-
Stomach	+++	+++
Striated / Skeletal Muscle	-	-
Testis	-	-
Uterus	++	++

[000572] Significant CD166 expression was detected in both human and cynomolgus liver tissue samples.

[000573] In initial pharmacokinetic studies, the conjugated anti-CD166 activatable antibodies of the disclosure were found to avoid antigen sink (i.e., rapid clearance of antibodies due to the abundance of naturally expressed CD166 antigen throughout the body).

[000574] Figure 14 demonstrates the results of a tolerability study in cynomolgus monkeys using 5 mg/kg administration of a conjugated anti-CD166 activatable antibody of the disclosure. These studies were performed using huCD166_7614.6_CM2-DM4 AADC (where CM2 is the 3001 substrate). 5 mg/kg was selected as that is the therapeutic dose used for other DM4 antibody conjugates that include a SPDB linkage.

[000575] The pharmacokinetics of the huCD166-7614.6-CM1 DM4 drug conjugate (where CM1 is the 2001 substrate) and the un-conjugated huCD166 antibody were evaluated in cynomolgus monkeys after a single 5 mg/kg or 3 mg/kg dose, respectively.

Total serum levels of human IgG were measured using an anti-human IgG sandwich ELISA. Consistent with avoiding on-target sinks, the CD166 AADC showed significantly more exposure than the antibody. Monkeys treated with the huCD166-7614.6-CM1 DM4 drug conjugate had no observable toxicities. After 21 days, there were no clinical observations, no signs of on-target toxicity, and no sign of liver toxicity. As shown in Figure 14, the clearance of a DM4 anti-CD166 antibody conjugate was compared to the parental antibody. Antigen binding was below the level of quantification.

[000576] Figure 15 is a graph that demonstrates that the conjugated anti-CD166 activatable antibody is well tolerated at the projected therapeutic dosage. These studies were performed using huCD166_7614.6_CM2-DM4 AADC (where CM2 is the 3001 substrate).

[000577] Thus, unlike traditional ADC therapy, there is no evidence of liver damage in the cynomolgus monkeys following administration of a conjugated anti-CD166 activatable antibody of the disclosure.

EXAMPLE 8. Protease-Dependent Activation of Anti-CD166 Activatable Antibodies

[000578] The exemplary studies provided herein were designed to evaluate the protease-dependent activation of activatable anti-CD166 activatable antibodies and conjugated activatable antibodies of the disclosure.

[000579] Figures 16A, 16B, and 16C are graphs depicting the ability of various anti-CD166 conjugated anti-CD166 activatable antibodies of the disclosure to bind human CD166 on HCC1806 cells in the presence or absence of protease-dependent activation. These conjugated anti-CD166 activatable antibodies are also referred to herein as "CD166 AADC" or "CD166 Activatable Antibody Drug Conjugates". As shown in Figure 16A, conjugated anti-CD166 activatable antibodies are blocked from binding to CD166 on HCC1806 cells. In contrast, as shown in Figures 16B and 16C, conjugated anti-CD166 activatable antibodies recover antibody binding activity that is similar to the binding activity of unmasked conjugated anti-CD166 antibody and unmasked anti-CD166 antibody when the AADCs were proteolytically activated with matriptase (MT-SP1) or matrix metalloprotease 14 (MMP-14).

[000580] The binding of the anti-CD166 antibody (VH of SEQ ID NO: 122, VL of SEQ ID NO: 123), various conjugated and unconjugated anti-CD166 activatable antibodies of the disclosure, and protease-activated conjugated anti-CD166 activatable antibodies of

the disclosure were evaluated using flow cytometry-based binding assay. The conjugated and unconjugated anti-CD166 activatable antibodies tested included the variable heavy chain sequence of SEQ ID NO: 122, the variable light chain sequence of SEQ ID NO: 123, either a cleavable moiety (CM1, substrate 2001) comprising the amino acid sequence ISSGLLSGRSDNH (SEQ ID NO: 70) or a cleavable moiety (CM2, substrate 3001) comprising the amino acid sequence AVGLLAPPGGLSGRSDNH (SEQ ID NO: 76), and one of the masking moieties shown in Table 10. The full sequences are shown above in Table 11. The conjugated anti-CD166 antibodies and conjugated anti-CD166 activatable antibodies included the maytansinoid DM4 conjugated to the activatable antibody via an SPDB linker. In a typical assay, HCC1806 cells were incubated with the indicated concentrations of anti-CD166 antibody, activatable antibody, conjugated antibody, or activatable antibody in PBS+2%FBS for 1 hr on ice. After washing 2X with PBS + 2% FBS, cells were incubated with a goat anti-human IgG secondary antibody, conjugated to AlexaFluor 647 (Jackson ImmunoResearch), for 30-45 min on ice. Cells were then washed 2X with PBS +2% FBS and fixed with 1% formaldehyde. Bound antibody was detected using a Guava EasyCyte cytometer and the median fluorescence intensity (MFI) of the cell population was measured.

[000581] As shown in Figure 16A, conjugated anti-CD166 activatable antibodies are blocked from binding to CD166 on HCC1806 cells. As shown in Figures 16B and 16C, various conjugated and unconjugated anti-CD166 activatable antibodies of the disclosure behave like each other when masked, but when the conjugated anti-CD166 activatable antibodies were proteolytically activated with a protease, they showed similar binding to that of the unmasked huCD166 parental antibody and the unmasked huCD166 ADC.

EXAMPLE 9. Binding of Activatable Anti-CD166 and Antibody Drug Conjugates to Human Tissues

[000582] The exemplary studies in this Example show the binding properties of anti-CD166 antibody drug conjugates (ADCs) and activatable anti-CD166 antibody drug conjugates (AADCs) of the present disclosure to human tissues.

[000583] In this study, frozen tissue sections derived from normal human prostate, ovary, breast, pancreas, and right atrium (Cat. Nos. T1234201, T1234086, T1234183, T1234188, and T1234127, respectively; BioChain, Newark, CA) were prepared and

blocked using standard protocols, and then incubated with 0.4 µg/mL with an anti-CD166 ADC of the present disclosure (anti-CD166-spdb-DM4), an anti-CD166 AADC of the present disclosure (anti-CD166-7614.6-3001-spdb-DM4), an activated anti-CD166 AADC of the present disclosure (anti-CD166-7614.6-3001-spdb-DM4 incubated with purified uPA for 16 hours at 37°C), or an isotype control ADC (chKTI- spdb-DM4, a chimeric human IgG1 anti-soybean trypsin inhibitor antibody, conjugated to spdb-DM4). After incubation with the test articles, sections were treated with 2.5 µg/mL mouse anti-DM4 monoclonal antibody (Immunogen). Detection of the DM4 payload was achieved by incubation with an anti-mouse antibody conjugated to an HRP polymer (EnvisionTM+ System-HRP Labeled polymer anti-mouse, Dako, K4006) followed by addition of a 3,3'-diaminobenzidine substrate (DAB Plus, Dako, K3467). Tissues were counterstained with hematoxylin and images were acquired on an Olympus VS120 Virtual Slide Scanner.

[000584] The exemplary results of this study showed that anti-CD166 AADC of the present disclosure (anti-CD166-7614.6-3001-spdb-DM4) displayed a lack of immunostaining on each of the five human tissue sections consistent with its masked state, and similar to that observed with the isotype control ADC. Activation of the anti-CD166 AADC of the present disclosure by uPA restored binding of the activated anti-CD166 AADC in two of the highest expressing tissues, prostate and breast. The staining intensity and distribution of the uPA-activated anti-CD166 AADC of the present disclosure were similar to that of the parental anti-CD166 antibody drug conjugate (anti-CD166-spdb-DM4).

EXAMPLE 10. Binding of Anti-CD166 Antibodies to Human Cancers

[000585] This Example shows that CD166 is expressed in a variety of patient-derived tumors by immunohistochemical (IHC) staining using an anti-CD166 antibody.

[000586] In this study, formalin-fixed paraffin-embedded tumor samples (FFPE) in tissue microarrays (US Biomax) were prepared and blocked using standard protocols, and then incubated with 5 µg/mL anti-CD166 rabbit monoclonal antibody EPR2759[2] (Abcam, ab109215). Detection of anti-CD166 antibody was performed by incubation with 5 µg/mL biotinylated-conjugated donkey anti-rabbit IgG antibody (Jackson Immunoresearch, 711-065-152), followed by addition of the ABC-HRP Elite Standard (Vector Laboratories, PK-6100) to form the avidin-biotin-HRP complex, followed by addition of a 3,3'-

diaminobenzidine substrate (DAB Plus, Dako, K3467). Tissues were counterstained with hematoxylin and images were acquired on an Olympus VS120 virtual slide scanner.

[000587] Each tissue core was assigned a IHC score of "negative" (no staining), "weak" (intensity 1+ in ≤ 70% tumor cells or 2+ in ≤ 30% tumor cells), "moderate" (intensity 1+ in > 70% of tumor cells or 2+ in >30% to ≤ 70% tumor cells or 3+ in ≤ 30% tumor cells), or "strong" (intensity 2+ in > 70% of tumor cells or 3+ in >30% tumor cells), and the percent of tested samples showing "moderate" or "strong" IHC staining for anti-CD166 are show in Table 4 below.

Table 4: IHC Assay of CD166 Expression In Patient-Derived Cancers

Tissue (Total No. of Samples)	Cancer Type	% Samples with Moderate or Strong IHC Score
Prostrate (119)	Adenocarcinoma	98.3
Breast (392)	Ductal carcinoma	87.5
Lung (213)	Non-small cell lung cancer	83.1
Head & Neck (122)	Squamous cell carcinoma	81.1
Endometrial (147)	Adenocarcinoma	75.5
Ovarian (129)	Adenocarcinoma	70.5
Biliary (177)	Cholangiocarcinoma	56.5

EXAMPLE 11. Binding of Anti-CD166 Antibodies to Human and Cynomolgus Cells

[000588] The exemplary studies provided herein were designed to evaluate anti-CD166 activatable antibodies of the disclosure for binding to human and cynomolgus cells in a flow cytometry assay.

[000589] Figure 17 is a graph depicting the ability of an anti-CD166 antibody (anti-huCD166) of the disclosure to bind human H292 cells or cynomolgus primary kidney epithelial cells, as measured by flow cytometry. As shown in Figure 17, anti-CD166 antibodies of the present disclosure demonstrated comparable binding affinities to CD166 on the cell surface of both human and cynomolgus cells.

[000590] The binding of the anti-CD166 antibody (huM9 vk-1/HcC; VH of SEQ ID NO: 122, VL of SEQ ID NO: 123) of the present disclosure to human H292 cells and cynomolgus primary kidney epithelial cells was evaluated using flow cytometry-based

binding assay. In a typical assay, H292 cells or cynomolgus primary kidney epithelial cells were incubated with the indicated concentrations of anti-CD166 antibody in PBS+2%FBS for 1 hr on ice. After washing 2X with PBS + 2% FBS, cells were incubated with a goat anti-human IgG secondary antibody, conjugated to AlexaFluor 647 (Jackson ImmunoResearch), for 30-45 min on ice. Cells were then washed 2X with PBS +2% FBS and fixed with 1% formaldehyde. Bound antibody was detected using a Guava EasyCyte cytometer and the mean fluorescence intensity (MFI) of the cell population was measured.

[000591] As shown in Figure 17, an anti-CD166 antibody of the present disclosure bound to human and cynomolgus cells with comparable affinity (EC_{50} of 3.1 nM to human cells, and EC_{50} of 1.7 nM to cynomolgus cells).

EXAMPLE 12. Inhibition of Cell Binding to CD6 by Anti-CD166 Antibodies

[000592] In this exemplary study, anti-CD166 antibodies of the present disclosure demonstrate the ability to block the adhesion of human lymphoma cells to immobilized CD6, the receptor for which CD166 is the ligand.

[000593] Figure 18 is a graph depicting the ability of an anti-CD166 antibody (anti-huCD166; vk-1/HcC) of the present disclosure to block the adhesion of HuT-78 human lymphoma cells to immobilized human CD166. In this assay, CD166-expressing HuT-78 cells are fluorescently labeled and incubated with recombinant CD6 protein immobilized on plastic plates. After several washes, bound HuT-78 cells are detected by measuring fluorescence and reported as a percentage of total fluorescence (before washing). MAB656 is an anti-CD166 mouse monoclonal antibody reported to inhibit cell adhesion in this assay (R&D Systems), presumably by disrupting CD166 interaction with its receptor, CD6. In this exemplary assay, the anti-CD166 antibody of the present disclosure and MAB656 showed similar levels of inhibition of cell adhesion to CD6. Both antibodies showed nearly an EC50 of approximately 3.7 nM. An isotype control antibody showed no inhibition of cell adhesion.

EXAMPLE 13. Protease-Dependent Activation of Anti-CD166 Activatable Antibodies

[000594] The exemplary studies provided herein were designed to evaluate the protease-dependent activation of anti-CD166 activatable antibodies and conjugated activatable antibodies of the disclosure.

[000595] Figure 19A is a graph depicting the *in vitro* binding to CD166 in an ELISA assay of an anti-CD166 antibody (anti-huCD166 HcC/vk-1, "CD166 Ab"), an anti-CD166 antibody drug conjugate (anti-CD166-spdb-DM4, "CD166-ADC"), an anti-CD166 activatable antibody (anti-CD166-7614.6-3001, "CD166 Pb"), an activatable antibody drug conjugate (anti-CD166-7614.6-3001-spdb-DM4, "CD166-AADC"). As indicated, assays were also performed with protease-activated anti-CD166 activatable antibody drug conjugate, using either urokinase (uPA), matriptase (MT-SP1), or matrix metalloprotease 14 (MMP14) as indicated. Using a standard ELISA protocol, human CD166 protein was absorbed to ELISA plates and subsequently incubated with the indicated concentration of antibody. Bound antibody was detected with a horseradish peroxidase-conjugated anti-human IgG secondary antibody.

[000596] Figures 19B and 19C are graphs depicting the binding to human HCC1806 or H292 cells in a flow cytometry assay of an anti-CD166 antibody (anti-huCD166 HcC/vk-1, "CD166 Ab"), an anti-CD166 antibody drug conjugate (anti-CD166-spdb-DM4, "CD166-ADC"), an anti-CD166 activatable antibody (anti-CD166-7614.6-3001, "CD166 Pb"), an activatable antibody drug conjugate (anti-CD166-7614.6-3001-spdb-DM4, "CD166-AADC"). As indicated, assays were also performed with protease-activated anti-CD166 activatable antibody drug conjugate, using either urokinase (uPA), matriptase (MT-SP1), or matrix metalloprotease 14 (MMP14) as indicated. As a control, and IgG1 isotype antibody conjugated to spdb-DM4 ("IgG1 isotype-ADC") was also tested. In a typical assay, H292 cells or HCC1806 cells were incubated with the indicated concentrations of anti-CD166 antibody in PBS+2%FBS for 1 hr on ice. After washing 2X with PBS + 2% FBS, cells were incubated with a goat anti-human IgG secondary antibody, conjugated to AlexaFluor 647 (Jackson ImmunoResearch), for 30-45 min on ice. Cells were then washed 2X with PBS +2% FBS and fixed with 1% formaldehyde. Bound antibody was detected using a Guava EasyCyte cytometer and the median fluorescence intensity (MFI) of the cell population was measured.

Table 14: Binding of Anti-CD166 Antibody Constructs to CD166 and Human Cells

Antibody Construct	CD166 ELISA Kd (nM)	HCC1806 Flow Cytomtry Kd (nM)	H292 Flow Cytomtry Kd (nM)
CD166-AADC	2.9	>300	>200

CD166-AADC, uPA cleaved	0.18	9.9	5.5
CD166-AADC, matriptase cleaved	0.17	9.9	5.3
CD166-AADC, MMP-14 cleaved	0.16	12	6.9
CD166-ADC	0.15	6.1	3.3
CD166-Pb	3.05	>300	>200

[000597] As shown by the exemplary results in Table 14, the uncleaved CD166-AADC demonstrated an increased apparent Kd as compared to the unmasked CD166-ADC in ELISA and flow cytometry assays. Activation of the CD166-AADC by all three of the proteases appeared to restore the binding affinity of the anti-CD166 activatable antibody drug conjugate to a level comparable to that the unmasked CD166-ADC.

[000598] Figures 20A and 20B are graphs depicting the *in vitro* cytotoxicity to human HCC1806 or H292 cells in of an anti-CD166 antibody (anti-huCD166 HcC/vk-1, "CD166 Ab"), an anti-CD166 antibody drug conjugate (anti-CD166-spdb-DM4, "CD166-ADC"), an anti-CD166 activatable antibody (anti-CD166-7614.6-3001, "CD166 Pb"), an activatable antibody drug conjugate (anti-CD166-7614.6-3001-spdb-DM4, "CD166-AADC"). As indicated, assays were also performed with protease-activated anti-CD166 activatable antibody drug conjugate, using either urokinase (uPA), matriptase (MT-SP1), or matrix metalloprotease 14 (MMP14) as indicated. As a control, and IgG1 isotype antibody conjugated to spdb-DM4 ("IgG1 isotype-ADC") was also tested. In a typical assay, H292 cells or HCC1806 cells were incubated with the indicated concentrations of antibody, activatable antibody, activatable antibody drug conjugated, or activatable antibody drug conjugate treated with protease, and incubating the cells for 3 to 5 days. Cell viability was measured using the CellTiter Glo assay. The results of these cytotoxicity assays is summarized below in Table 15.

Table 15: *In vitro* Cytotoxicity of Anti-CD166 Antibody Constructs to Human Cells

Antibody Construct	H292 EC50 (nM)	HC1806 EC50 (nM)
CD166-AADC	2.2	0.9

CD166-AADC, uPA cleaved	0.5	0.3
CD166-AADC, matriptase cleaved	0.5	0.2
CD166-AADC, MMP-14 cleaved	0.5	0.2
CD166-ADC	0.3	0.1

[000599] These exemplary results of Table 15 and Figures 20A and 20B demonstrate that activatable anti-CD166 antibody drug conjugates (CD166-AADC) of the present disclosure that are proteolytically-activated with protease that recognize the substrate sequence (CM) show a several-fold increase in cytotoxicity to human cells as compared to the corresponding uncleaved anti-CD166 AADC. Furthermore, the activated anti-CD166 AADC demonstrate a cytotoxicity that is comparable to the unmasked anti-CD166 ADC.

EXAMPLE 14. Immunological Risk Assay Of Anti-CD166 Activatable Antibodies

[000600] The exemplary studies provided herein were designed to evaluate the ability of activatable anti-CD166 antibody drug conjugates (CD166-AADC) of the present disclosure to trigger an immunological response in an *in vitro* assay.

[000601] In these exemplary studies, human peripheral blood mononuclear cells (PBMCs) were tested in *in vitro* assays for cytokine release and proliferation in response to treatment with activatable anti-CD166 antibody drug conjugate (anti-CD166-7614.6-3001-spdb-DM4, "CD166-AADC"), an anti-CD166 antibody drug conjugate (anti-CD166 (vk-1/HcC)-spdb-DM4, "CD166-ADC"), an anti-CD3 positive control (OKT3 antibody), and an isotype control antibody drug conjugate ("Isotype") at the following concentrations:

No.	Antibody (nM)	No.	Antibody (nM)
1	Untreated	10	Isotype (0.67 nM)
2	Anti-CD3 (0.67 nM)	11	Isotype (6.7 nM)
3	Anti-CD3 (6.7 nM)	12	Isotype (67 nM)
4	Anti-CD3 (67 nM)	13	Isotype (670 nM)
5	Anti-CD3 (670 nM)	14	CD166-ADC (0.67 nM)
6	CD166-AADC (0.67 nM)	15	CD166-ADC (6.7 nM)
7	CD166-AADC (6.7 nM)	16	CD166-ADC (67 nM)

8	CD166-AADC (67 nM)	17	CD166-ADC (670 nM)
9	CD166-AADC (670 nM)		

[000602] In this exemplary study, five normal, healthy donors in two separate formats, wet coat and soluble test article, were evaluated. A standard panel of Th1 and Th2 cytokines including IL-2, IL-4, IL-6, IL-10, TNFα, and IFNγ were measured at 24 hr post-treatment. As shown in Figure 21A-21D, dose titration of either CD166-AADC or the corresponding CD166-ADC showed no significant cytokine release from human PBMCs, as compared with a positive control(anti-CD3, OKT3) and negative (isotype ADC) control antibodies. Additionally, unlike the positive control OKT3 antibody, neither CD166-AADC nor CD166-ADC induced PBMC proliferation, assessed at day 8 post-treatment.

EXAMPLE 16. Antibody-Dependent Cell Cytotoxicity of Conjugated Activatable Anti-CD166 Antibodies

[000603] The exemplary studies provided herein were designed to evaluate the *in vitro* antibody-dependent cell cytotoxicity (ADCC) to human cells of anti-CD166 activatable antibody drug conjugates of the present disclosure.

[000604] Figure 22 is a graph depicting an *in vitro* ADCC assay on human ovarian adenocarcinoma cells (SKOV3) using an anti-CD166 activatable antibody drug conjugate of the present disclosure (anti-huCD166 (HcC/vk-1)-7614.6-3001-spdb-DM4, "CD166-AADC"), an anti-CD166 antibody drug conjugate (anti-CD166-spdb-DM4, "CD166-ADC"), an anti-EGFR positive control, and an IgG1 isotype control-spdb-DM4 ("IgG1 isotype-ADC")

[000605] ADCC activities in this exemplary assay were evaluated using an ADCC reporter bioassay (Promega). In this assay, Jurkat T cells stably transfected with FcRγIIIa and an NF-AT-inducible luciferase reporter were incubated with CD166-expressing SKOV3 cells in the presence of CD166-AADC or control antibodies. Luciferase activity is stimulated as a function of antibody binding to target cells, FcRγIIIa receptor engagement and downstream signaling in effector cells. In SKOV3 cells, which express high levels of CD166 and EGFR, incubation with CD166-AADC or the CD166-ADC showed similar activity to an IgG1 isotype control ADC, and lower than observed with the positive control anti-EGFR antibody. Dose titration of CD166-AADC or CD166-ADC in the assay. These data suggest that CD166-AADC, as well as CD166-ADC, have limited potential for ADCC.

EXAMPLE 17. Efficacy of Conjugated Activatable Anti-CD166 Antibodies Against Cell- And Patient-Derived Xenograft Tumor Models

[000606] The exemplary studies provided herein were designed to evaluate the *in vivo* efficacy of anti-CD166 activatable antibody drug conjugates of the present disclosure against cell- and patient-derived xenografts in a mouse tumor model.

[000607] In these exemplary studies, multiple human cell line-derived and patient-derived xenograft (PDX) models representing various cancer types were tested. Two cell line derived xenograft models (H292 non-small cell lung carcinoma (NSCLC) cells and HCC1806 triple negative breast carcinoma cells), and two patient-derived xenograft (PDX) models for ovarian carcinoma (CTG-0791) and cholangiocarcinoma (CTG-0941). For each model, tumor-bearing mice were randomized into groups and treatment begun with an anti-CD166 activatable antibody drug conjugate of the present disclosure (anti-huCD166 (HcC/vk-1)-7614.6-3001-spdb-DM4, "CD166-AADC"), an isotype control SPDB-DM4 conjugate, or vehicle control as indicated. Test articles were administered intravenously on study day 0 and 7, at 3 or 5 mg/kg as indicated.

[000608] As depicted in Figure 23, treatment with CD166-AADC, at or below the expected therapeutic dose in humans, led to tumor regressions and durable responses in the majority of mice. Other similar exemplary studies are summarized in Table 16 below.

Table 16: Efficacy of Anti-CD166 Activatable Antibody Drug Conjugates

Tumor Model	Cancer Type	Anti-Tumor Response With CD166-AADC
H292	NSCLC	Yes
H1975	NSCLC	Yes
CTG-0166	NSCLC (squamous)	Yes
HCC1806	Triple negative breast cancer	Yes
CTG-0791	Ovarian	Yes
CTG-0941	Cholangiocarcinoma	Yes

EXAMPLE 18. *In vitro* Cytotoxicity of Conjugated Activatable Anti-CD166 Antibodies Against Endometrial Cancer-Derived Cell Lines

[000609] The exemplary studies provided herein were designed to evaluate the *in vitro* efficacy of anti-CD166 activatable antibody drug conjugates of the present disclosure against endometrial cancer-derived cell lines.

[000610] As shown in Figures 24A-24C, these exemplary studies showed the *in vitro* cytotoxicity to human uterine endometrial cancer-derived cell lines (HEC-1-A, AN3-CA, and KLE cell lines) of an anti-CD166 antibody drug conjugate (anti-CD166-spdb-DM4, "CD166-ADC"), and activatable antibody drug conjugates (anti-CD166-7614.6-3001-spdb-DM4, "CD166-7614.6-AADC"; and anti-CD166-7614.8-3001-spdb-DM4, "CD166-7614.8-AADC"). An isotype control-ADC (chKTI-spdb-DM4) was used as a control. In a typical assay, the cells were incubated with the indicated concentrations of antibody drug conjugate, or activatable antibody drug conjugate, and incubating the cells for 3 to 5 days. Cell viability was measured using the CellTiter Glo assay. These exemplary results showed that the anti-CD166-AADCs and anti-CD166-ADCs of the present disclosure demonstrated cytotoxicity against all cell lines as compared to the negative control. These exemplary results also showed that the uncleaved anti-CD166-AADCs showed a lower cytotoxicity than anti-CD166-ADCs of the present disclosure due to their lower affinity to the target due to the mask substrate. The relative susceptibility of the cell lines to the anti-CD166 articles of the present disclosure appeared to correlate to the level of CD166 expression in each cell. As shown in Figure 24D, to determine the relative amount of surface-expressed CD166 on each endometrial cell line, a flow cytometry assay using anti-CD166 of the present disclosure was performed.

EXAMPLE 18. *In vitro* Cytotoxicity of Conjugated Anti-CD166 Antibody Drug Conjugates Against Various Cancer-Derived Cell Lines

[000611] The exemplary studies provided herein were designed to evaluate the *in vitro* efficacy of anti-CD166 antibody drug conjugates of the present disclosure against endometrial cancer-derived cell lines.

[000612] In these exemplary studies, the *in vitro* cytotoxicity of anti-CD166 antibody drug conjugates (anti-CD166 (vk-1/HcC)-spdb-DM4, "CD166-ADC") were tested against multiple human cancer-derived cell lines. In a typical assay, the cells were incubated with

concentrations of CD166-ADC for 3 to 5 days at various concentrations (from 0.1 nM to 50 nM). Cell viability was measured using the CellTiter Glo assay. The cytotoxicity of the CD166-ADC was compared to a negative isotype control (chKTI-spdb-DM4. In the case of the PC3 prostate cancer cell line and the SAS head and neck squamous cell carcinoma cell lines, the tested article was anti-CD166-vc-MMAD, and the negative control was an isotypic palivizumab-vc-MMAD. The results of these cytotoxicity assays is summarized below in Table 8.

Table 8: *In vitro* Cytotoxicity of Anti-CD166 Antibody Drug Conjugate to Human Cancer Cells

Cell Type	Cancer Type	Cytotoxicity of CD166-ADC?
ZR75-1	Human breast ductal carcinoma (estrogen receptor positive)	Yes
ZR75-30	Human breast ductal carcinoma (estrogen receptor positive)	Yes
MDA-MB-361	Human breast adenocarcinoma (estrogen receptor positive)	Yes
HCC1954	Human breast ductal carcinoma (triple-negative)	Yes
HCC1143	Human breast cancer (triple-negative)	Yes
PC3	Human prostate adenocarcinoma	Yes
SAS	Human head & neck squamous cell carcinoma	Yes

EXAMPLE 19: *In Vivo* Imaging of Activatable Anti-CD166 Antibodies in a Mouse Lung Cancer Xenograft Model

[000613] This Example shows that activatable anti-CD166 antibodies of the present disclosure demonstrate tumor-associated protease-dependent *in vivo* activation and binding to CD166 expressed in a lung cancer (NSCLC) mouse xenograft model by *in vivo* fluorescent imaging.

[000614] Figure 25A shows *in vivo* imaging of live mice with lung tumor xenografts (H292 cells) using fluorescently-conjugated anti-CD166 and activatable anti-CD166

antibodies of the present disclosure. In this study, 7-8 week-old *nu/nu* female mice (n=3) were implanted subcutaneously in the right hind flank with 5 x 10^6 H292 cells, a human non-small cell lung cancer-derived cell line. After the tumors grew to 300 to 380 mm^3, an anti-CD166 antibody (vk-1/HcC) of the present disclosure ("CD166-Ab"), activatable anti-CD166 antibodies with differing mask sequences and substrate sequences of the present disclosure (anti-CD166-7614.6-3001, anti-CD166-7614.8-2001), or a masked anti-CD166 antibody of the present disclosure lacking a CM domain ("CD166 7614.6-NSUB") were administered as a pre-blocking reagent to each of the mice at a dose of 5 mg/kg. As a control, an isotype antibody (palivizumab) was similarly administered. About 48 hours later, the mice were injected with anti-CD166 antibody labeled with AlexaFluor 750 (anti-CD166-AF750). The mice were subjected to *in vivo* fluorescent imaging 24, 72, and 96 hours after administration of the anti-CD166-AF750, using a 745 nm excitation signal and detecting an 800 nm emission signal. Representative mice imaged at 96 hours are depicted. The scale shows the relative magnitude of the detected fluorescent signal. The mean tumor-to-background ratio (TBR) for each test article as measured for the mice is shown in Figure 25B.

[000615] The results of this exemplary study showed that fluorescent signals from the unmasked anti-CD166 antibody of the present disclosure and the activatable anti-CD166 antibodies of the present disclosure were able to bind CD166 in the xenograft, thus blocking the subsequent binding of fluorescently-labeled anti-CD166. In contrast, a correspondingly masked anti-CD166 antibody but which lacked a protease cleavage site (CM) did not block subsequent binding of CD166-AF750 to an extent comparable to the isotype control. Without being bound by any particular theory, this exemplary study demonstrated that activatable anti-CD166 antibodies of the present disclosure can be activated *in vivo* via tumor-associated protease cleavage, thus allowing the activated activatable anti-CD166 antibody to bind CD166 in the xenograft tumor to an extent comparable to the unmasked anti-CD166 antibody of the present disclosure. The masked anti-CD166 antibody lacking a protease cleavage domain (CM) of the present disclosure was not activatable in the same manner, and thus did not appreciably bind to the tumor xenograft.

EXAMPLE 20. Efficacy of Conjugated Activatable Anti-CD166 Antibodies Against Cell-Derived Xenograft Tumor Models

[000616] The exemplary studies provided herein were designed to evaluate the *in vivo* efficacy of anti-CD166 activatable antibody drug conjugates (AADCs) of the present disclosure against cell-derived xenografts in a mouse tumor model.

[000617] In these exemplary studies, anti-CD166 activatable antibody drug conjugates of the present disclosure were tested for efficacy against model mouse tumor xenograft model of lung cancer (H292 human non-small cell lung carcinoma (NSCLC) cells). In this study, tumor-bearing mice were treated with an anti-CD166 antibody drug conjugates and anti-CD166 activatable antibody drug conjugate (AADC) of the present disclosure, including anti-CD166-7614-2001-spdb-DM4 ("CD166-7614-2001-DM4"), anti-CD166-7614.6-2001-spdb-DM4 ("CD166-7614.6-2001-DM4"), anti-CD166-7614.8-3001-spdb-DM4 ("CD166-7614.8-3001-DM4"), anti-huCD166-spdb-DM4 ("CD166-DM4"), and an isotype control (palivizumab-spdb-DM4; "Isotype-DM4"). 5 mg/kg of test articles were administered intravenously on study days 1 and 8 to each mouse, and the mean tumor volume (MTV) of the H292 xenograft was measured on the indicated days.

[000618] As depicted in Figure 26, treatment with the activatable anti-CD166 antibody drug conjugates of the present disclosure resulted in a decrease in MTV over time to a degree comparable to that observed with the unmasked anti-CD166-DM4 drug conjugate. The anti-CD166-DM4 AADC having a mask with a measurably higher effect on decreasing the parental antibody binding affinity to CD166 (see, *e.g.*, Table 17) appeared to have measurably lower efficacy than the anti-CD166 AADCs having lower masks.

Other Embodiments

[000619] 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:

1. An isolated antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166.

2. The isolated antibody of claim 1, wherein the antibody or antigen-binding fragment thereof comprises the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134).

3. The isolated antibody of claim 1 or claim 2, wherein the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126.

4. An isolated antibody or antigen binding fragment thereof that binds to the same epitope on human CD166 and/or cynomolgus monkey CD166 as the isolated antibody of any one of claims 1 to 3.

5. An isolated antibody or antigen binding fragment thereof that cross-competes with the isolated antibody of any one of claims 1 to 4 for binding to human CD166 and/or cynomolgus monkey CD166.

6. An activatable antibody that, in an activated state, binds CD166 comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166;

a masking moiety (MM) that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state; and

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

7. The activatable antibody of claim 6, wherein the MM has a dissociation constant for binding to the AB that is greater than the dissociation constant of the AB to CD166.

8. The activatable antibody of claim 6 or claim 7, wherein the MM does not interfere or compete with the AB for binding to CD166 when the activatable antibody is in a cleaved state.

9. The activatable antibody of any one of claims 6-8, wherein the MM is a polypeptide of no more than 40 amino acids in length.

10. The activatable antibody of any one of claims 6-9, wherein the MM polypeptide sequence is different from that of human CD166.

11. The activatable antibody of any one of claims 6-10, wherein the MM polypeptide sequence is no more than 50% identical to any natural binding partner of the AB.

12. The activatable antibody of any one of claims 6-11, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 135-238.

13. The activatable antibody of any one of claims 6-12, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 219-238.

14. The activatable antibody of any one of claims 6-13, wherein the CM is a substrate for a protease that is active in diseased tissue.

15. The activatable antibody of any one of claims 6-14, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18-87 and 318-358.

16. The activatable antibody of any one of claims 6-15, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 70-87 and 336-358.

17. The antibody of any one of claims 1-5 or the activatable antibody of any one of claims 6-16, wherein the antigen binding fragment thereof is selected from the group consisting of a Fab fragment, a $F(ab')_2$ fragment, a scFv, a scAb, a dAb, a single domain heavy chain antibody, and a single domain light chain antibody.

18. The antibody of any one of claims 1-5 or the activatable antibody of any one of claims 6-17, wherein the AB specifically binds human CD166.

19. The antibody of any one of claims 1-5 or the activatable antibody of any one of claims 6-18, wherein the AB comprises the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134).

20. The antibody of any one of claims 1-5 or the activatable antibody of any one of claims 6-19, wherein the AB comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126.

21. The activatable antibody of any one of claims 6-20, wherein the AB is linked to the CM.

22. The activatable antibody of any one of claims 6-21, wherein the AB is linked directly to the CM.

23. The activatable antibody of any one of claims 6-22, wherein the AB is linked to the CM via a linking peptide.

24. The activatable antibody of any one of claims 6-23, wherein the MM is linked to the CM such that the activatable antibody in an uncleaved state comprises the structural arrangement from N-terminus to C-terminus as follows: MM-CM-AB or AB-CM-MM.

25. The activatable antibody of any one of claims 6-24, wherein the activatable antibody comprises a linking peptide between the MM and the CM.

26. The activatable antibody of any one of claims 6-25, wherein the activatable antibody comprises a linking peptide between the CM and the AB.

27. The activatable antibody of any one of claims 6-26, wherein the activatable antibody comprises a first linking peptide (LP1) and a second linking peptide (LP2), and wherein the activatable antibody in the uncleaved state has the structural arrangement from N-terminus to C-terminus as follows: MM-LP1-CM-LP2-AB or AB-LP2-CM-LP1-MM.

28. The activatable antibody of claim 27, wherein the two linking peptides need not be identical to each other.

29. The activatable antibody of claim 27 or claim 28, wherein each of LP1 and LP2 is a peptide of about 1 to 20 amino acids in length.

30. The activatable antibody of any one of claims 6-29, wherein the activatable antibody comprises the heavy chain comprising an amino acid sequence of SEQ ID NOs: 121, 122, or 239 and a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 123-126, 242, 244, 246, 248, 303, 310, 312, 314, 316, and 363-474.

31. The activatable antibody of any one of claims 6-30, wherein the activatable antibody comprises a combination of amino acid sequences, wherein the combination of amino acid sequences is selected from a single row in Table A,

wherein for a given combination,

(a) the heavy chain of the AB comprises the amino acid sequences of the VH CDR sequences corresponding to the given combination in the single row listed in Table A,

(b) the light chain of the AB comprises the amino acid sequences of the VL CDR sequences corresponding to the given combination in the single row listed in Table A,

(c) the MM comprises the amino acid sequence of the mask sequence (MM) corresponding to the given combination in the single row listed in Table A, and

(d) the CM comprises the amino acid sequence of the substrate sequence (CM) corresponding to the given combination in the single row listed in Table A.

32. The activatable antibody of any one of claims 6-30, wherein the activatable antibody comprises a combination of amino acid sequences, wherein for a given combination of amino acid sequences,

(a) the heavy chain of the AB comprises the amino acid sequences of the VH sequence or VH CDR sequences selected from the group consisting of the VH sequences or VH CDR sequences listed in the corresponding column of Table B,

(b) the light chain of the AB comprises the amino acid sequences of the VL sequence or VL CDR sequences selected from the group consisting of: the VL sequences or VL CDR sequences listed in the corresponding column of Table B,

(c) the MM comprises the amino acid sequence of the mask sequence (MM) selected from the group consisting of: the MM sequences listed in the corresponding column of Table B, and

(d) the CM comprises the amino acid sequence of the substrate sequence (CM) selected from the group consisting of: the CM sequences listed in the corresponding column of Table B.

33. An activatable antibody comprising an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, a MM, and a CM, wherein the activatable antibody comprises:

a heavy chain comprising an amino acid sequence of SEQ ID NOS: 121, 122 or 239; and

a light chain sequence comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 123-126, 242, 244, 246, 248, 303, 310, 312, 314, 316, and 363-474.

34. An activatable antibody comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166;

a MM comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 135-238; and

a CM comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18-87 and 318-358.

35. The activatable antibody of claim 34, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 219-238, and the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 70-87 and 336-358.

36. The activatable antibody of claim 34 or claim 35, wherein the AB comprises the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134).

37. The activatable antibody of any one of claims 34-36, wherein the AB comprises a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 121 or SEQ ID NO: 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126, 363-370, 373, 374, 377, 378, 381, 382, 385, 386, 389, 390, 393, 394, 397, 398, 401, 402, 405, 406, 409, 410, 413, 414,

417, 418, 421, 422, 425, 426, 429, 430, 433, 434, 437, 438, 441, 442, 445, 446, 449, 450, 453, 454, 457, 458, 461, 462, 465, 466, 469, 470, 473, and 474.

38. An activatable anti-CD166 antibody comprising

an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds to the same epitope on human CD166 and/or cynomolgus monkey CD166 as the isolated antibody of any one of claims 1-5;

a masking moiety (MM) that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state; and

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

39. An activatable anti-CD166 antibody comprising

an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically cross-competes with the isolated antibody of any one of claims 1-5 for binding to human CD166 and/or cynomolgus monkey CD166;

a masking moiety (MM) that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state; and

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

40. A conjugated antibody or conjugated activatable antibody comprising the antibody of any one of claims 1 to 5 conjugated to an agent or the activatable antibody of any one of claims 6 to 39 conjugated to an agent.

41. The conjugated antibody or conjugated activatable antibody of claim 40, wherein the agent is a toxin or fragment thereof.

42. The activatable antibody of claim 40 or claim 41, wherein the agent is a microtubule inhibitor.

43. The activatable antibody of claim 40 or claim 41, wherein the agent is a nucleic acid damaging agent.

44. The conjugated antibody or conjugated activatable antibody of claim 40 or claim 41, wherein the agent is selected from the group consisting of a dolastatin or a derivative thereof, an auristatin or a derivative thereof, a maytansinoid or a derivative thereof, a duocarmycin or a derivative thereof, a calicheamicin or a derivative thereof, and a pyrrolobenzodiazepine or a derivative thereof.

45. The conjugated antibody or conjugated activatable antibody of any one of claims 40-42 and 44, wherein the agent is auristatin E or a derivative thereof.

46. The conjugated antibody or conjugated activatable antibody of any one of claims 40-42 and 44, wherein the agent is monomethyl auristatin E (MMAE).

47. The conjugated antibody or conjugated activatable antibody of any one of claims 40-42 and 44, wherein the agent is monomethyl auristatin D (MMAD).

48. The conjugated antibody or conjugated activatable antibody of any one of claims 40-42 and 44, wherein the agent is a maytansinoid selected from the group consisting of DM1 and DM4.

49. The conjugated antibody or conjugated activatable antibody of any one of claims 40-42 and 44, wherein the agent is maytansinoid DM4.

50. The conjugated antibody or conjugated activatable antibody of any one of claims 40, 41, 43, and 44, wherein the agent is a duocarmycin.

51. The conjugated antibody or conjugated activatable antibody of any one of claims 40-50, wherein the agent is conjugated to the AB via a linker.

52. The conjugated antibody or conjugated activatable antibody of any one of claims 40-51, wherein the linker with which the agent is conjugated to the AB comprises an SPDB moiety, a vc moiety, or a PEG2-vc moiety,

53. The conjugated antibody or conjugated activatable antibody of any one of claims 40-51, wherein the linker and toxin conjugated to the AB comprises an SPDB-DM4 moiety, a vc-MMAD moiety, a vc-MMAE moiety, a vc-duocarmycin moiety, or a PEG2-vc-MMAD moiety,

54. The conjugated antibody or conjugated activatable antibody of claim 51, wherein the linker is a cleavable linker.

55. The conjugated antibody or conjugated activatable antibody of claim 51, wherein the linker is a non-cleavable linker.

56. The conjugated antibody or conjugated activatable antibody of any one of claims 40-55, wherein the agent is a detectable moiety.

57. The conjugated antibody or conjugated activatable antibody of claim 56, wherein the detectable moiety is a diagnostic agent.

58. A conjugated activatable antibody that, in an activated state, binds CD166 comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166;

a masking moiety (MM) that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state;

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and

an agent conjugated to the AB.

59. The conjugated activatable antibody of claim 58, wherein the agent is selected from the group consisting of a dolastatin or a derivative thereof, an auristatin or a derivative thereof, a maytansinoid or a derivative thereof, a duocarmycin or a derivative thereof, a calicheamicin or a derivative thereof, and a pyrrolobenzodiazepine or a derivative thereof.

60. The conjugated activatable antibody of claim 58 or 59, wherein the agent is selected from the group consisting of auristatin E, monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE), monomethyl auristatin D (MMAD), maytansinoid DM4, maytansinoid DM1, a duocarmycin, a pyrrolobenzodiazepine, and a pyrrolobenzodiazepine dimer.

61. The conjugated activatable antibody of any one of claims 58-60, wherein the agent is conjugated to the AB via a linker.

62. The conjugated antibody or conjugated activatable antibody of any one of claims 58-61, wherein the linker with which the agent is conjugated to the AB comprises an SPDB moiety, a vc moiety, or a PEG2-vc moiety,

63. The conjugated antibody or conjugated activatable antibody of any one of claims 58-62, wherein the linker and toxin conjugated to the AB comprises an SPDB-DM4 moiety, a vc-MMAD moiety, a vc-MMAE moiety, a vc-duocarmycin moiety, or a PEG2-vc-MMAD moiety,

64. The conjugated activatable antibody of any one of claims 58-63, wherein the antibody or antigen binding fragment thereof comprises the VH CDR1 sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134).

65. The conjugated activatable antibody of any one of claims 58-64, wherein the antibody or antigen binding fragment thereof comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 121 and 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126, 363-370, 373, 374, 377, 378, 381, 382, 385, 386, 389, 390, 393, 394, 397, 398, 401, 402, 405, 406, 409, 410, 413, 414, 417, 418, 421, 422, 425, 426, 429, 430, 433, 434, 437, 438, 441, 442, 445, 446, 449, 450, 453, 454, 457, 458, 461, 462, 465, 466, 469, 470, 473, and 474.

66. The conjugated activatable antibody of any one of claims 58-65, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 135-238.

67. The conjugated activatable antibody of any one of claims 58-66, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 219-238.

68. The conjugated activatable antibody of any one of claims 58-67, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18-87 and 318-358.

69. The conjugated activatable antibody of any one of claims 58-68, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 70-87 and 336-358.

70. The conjugated activatable antibody of any one of claims 58-69, wherein the activatable antibody comprises a combination of amino acid sequences, wherein the combination of amino acid sequences is selected from a single row in Table A,

wherein for a given combination,

(a) the heavy chain of the AB comprises the amino acid sequences of the VH CDR sequences corresponding to the given combination in the single row listed in Table A,

(b) the light chain of the AB comprises the amino acid sequences of the VL CDR sequences corresponding to the given combination in the single row listed in Table A,

(c) the MM comprises the amino acid sequence of the mask sequence (MM) corresponding to the given combination in the single row listed in Table A, and

(d) the CM comprises the amino acid sequence of the substrate sequence (CM) corresponding to the given combination in the single row listed in Table A.

71. The conjugated activatable antibody of any one of claims 58-70, wherein the activatable antibody comprises a combination of amino acid sequences, wherein for a given combination of amino acid sequences,

(a) the heavy chain of the AB comprises the amino acid sequences of the VH sequence or VH CDR sequences selected from the group consisting of: the VH sequence or VH CDR sequences listed in the corresponding column of Table B,

(b) the light chain of the AB comprises the amino acid sequences of the VL sequence or VL CDR sequences selected from the group consisting of: the VL sequence or VL CDR sequences listed in the corresponding column of Table B,

(c) the MM comprises the amino acid sequence of the mask sequence (MM) selected from the group consisting of: the MM sequences listed in the corresponding column of Table B, and

(d) the CM comprises the amino acid sequence of the substrate sequence (CM) selected from the group consisting of: the CM sequences listed in the corresponding column of Table B.

72. The conjugated activatable antibody of any one of claims 58-71, wherein the activatable antibody comprises:

a heavy chain comprising the amino acid sequence of SEQ ID NO: 121, 122 or 239; and

a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 123-126, 242, 244, 246, 248, 303, 310, 312, 314, 316, and 363-474.

73. A conjugated antibody comprising:

(a) an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB comprises:

(i) the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128);

the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134), or

(ii) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 121 and 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126, 363-370, 373, 374, 377, 378, 381, 382, 385, 386, 389, 390, 393, 394, 397, 398, 401, 402, 405, 406, 409, 410, 413, 414, 417, 418, 421, 422, 425, 426, 429, 430, 433, 434, 437, 438, 441, 442, 445, 446, 449, 450, 453, 454, 457, 458, 461, 462, 465, 466, 469, 470, 473, and 474, or

(iii) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 121, 122, and 239, and a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 123-126, 242, 244, 246, 248, 303, 310, 312, 314, 316, and 363-474; and

(b) an agent conjugated to the AB, wherein the agent is selected from the group consisting of auristatin E, monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE), monomethyl auristatin D (MMAD), maytansinoid DM4, maytansinoid DM1, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, and a duocarmycin.

74. A conjugated activatable antibody that, in an activated state, binds to CD166, comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds to mammalian CD166, wherein the AB specifically binds human CD166 and cynomolgus monkey CD166;

a masking moiety (MM) that inhibits the binding of the AB to CD166 when the activatable antibody is in an uncleaved state;

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease; and

an agent conjugated to the AB,

wherein the AB comprises:

(i) the VH CDR1 amino acid sequence GFSLSTYGMGVG (SEQ ID NO: 127); the VH CDR2 amino acid sequence NIWWSEDKH (SEQ ID NO: 128); the VH CDR3 amino acid sequence IDYGNDYAFTY (SEQ ID NO: 129); the VL CDR1 amino acid sequence RSSKSLLHSNGITYLY (SEQ ID NO: 130) or RSSQSLLHSNGITYLY (SEQ ID NO: 131); the VL CDR2 amino acid sequence QMSNLAS (SEQ ID NO: 132) or QMSNRAS (SEQ ID NO: 133); and the VL CDR3 amino acid sequence AQNLELPYT (SEQ ID NO: 134), or

(ii) a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 121 and 122, and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 123-126, 363-370, 373, 374, 377, 378, 381, 382, 385, 386, 389, 390, 393, 394, 397, 398, 401, 402, 405, 406, 409, 410, 413, 414, 417, 418, 421, 422, 425, 426, 429, 430, 433, 434, 437, 438, 441, 442, 445, 446, 449, 450, 453, 454, 457, 458, 461, 462, 465, 466, 469, 470, 473, and 474, or

(iii) a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 121, 122, and 239, and a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 123-126, 242, 244, 246, 248, 303, 310, 312, 314, 316, and 363-474; and

wherein the agent is selected from the group consisting of auristatin E, monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE), monomethyl auristatin D (MMAD), maytansinoid DM4, maytansinoid DM1, a pyrrolobenzodiazepine, a pyrrolobenzodiazepine dimer, and a duocarmycin.

75. The conjugated activatable antibody of claim 74, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 135-238.

76. The conjugated activatable antibody of claim 74 or claim 75, wherein the MM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 219-238.

77. The conjugated activatable antibody of any one of claims 74-76, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18-87 and 318-358.

78. The conjugated activatable antibody of any one of claims 74-77, wherein the CM comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 70-87 and 336-358.

79. The conjugated activatable antibody of any one of claims 74-78, wherein the agent is conjugated to the AB via a linker, and wherein the linker to which the agent is conjugated to the AB comprises an SPDB moiety, a vc moiety, or a PEG2-vc moiety,

80. The conjugated antibody or conjugated activatable antibody of any one of claims 74-78, wherein the linker and toxin conjugated to the AB comprises an SPDB-DM4 moiety, a vc-MMAD moiety, a vc-MMAE moiety, a vc-duocarmycin moiety, or a PEG2-vc-MMAD moiety,

81. A conjugated activatable antibody or conjugated antibody comprising:

an antibody or antigen binding fragment thereof (AB) that, in an activated state, binds CD166; and

a toxin conjugated to the AB via a linker,

wherein the conjugated activatable antibody or the conjugated antibody comprises amino acid sequences, a linker, and a toxin selected from a single row in Table C, wherein for the given combination:

(a) the AB comprises a heavy chain comprising the amino acid sequence of the heavy chain sequence or heavy chain variable domain sequence corresponding to the given combination in the single row listed in Table C,

(b) the AB comprises a light chain comprising the amino acid sequence of the light chain sequence or light chain variable domain sequence corresponding to the given combination in the single row listed in Table C, and

(c) the linker and the toxin comprise the linker and the toxin corresponding to the given combination in the single row listed in Table C.

82. A pharmaceutical composition comprising the antibody of any one of claims 1 to 5, the activatable antibody of any one of claims 6 to 39, or the conjugated antibody or conjugated activatable antibody of any one of claims 40 to 81 and a carrier.

83. The pharmaceutical composition of claim 82 comprising an additional agent.

84. The pharmaceutical composition of claim 83, wherein the additional agent is a therapeutic agent.

85. An isolated nucleic acid molecule encoding the isolated antibody of any one of claims 1 to 5 or the activatable antibody of any one of claims 6 to 81.

86. A vector comprising the isolated nucleic acid molecule of claim 85.

87. A method of producing an antibody or an activatable antibody by culturing a cell under conditions that lead to expression of the antibody or the activatable antibody, wherein the cell comprises the nucleic acid molecule of claim 85 or the vector of claim 86.

88. A method of manufacturing an activatable antibody that, in an activated state, binds CD166, the method comprising:

(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 an activatable antibody of any one of claims 6 to 39; and

(b) recovering the activatable antibody.

89. A method of treating, alleviating a symptom of, or delaying the progression of a disorder or disease in which diseased cells express CD166 comprising administering a therapeutically effective amount of the antibody of any one of claims 1 to 5, the activatable antibody of any one of claims 6 to 39, the conjugated antibody or conjugated activatable antibody of any one of claims 40 to 81, or the pharmaceutical composition of any one of claims 82 to 84 to a subject in need thereof.

90. The method of claim 89, wherein the disorder or disease is cancer.

91. A method of treating, alleviating a symptom of, or delaying the progression of a disorder or disease associated with cells expressing CD166 comprising administering a therapeutically effective amount of the antibody of any one of claims 1 to 5, the activatable antibody of any one of claims 6 to 39, the conjugated antibody or conjugated activatable antibody of any one of claims 40 to 81, or the pharmaceutical composition of any one of claims 82 to 84 to a subject in need thereof.

92. The method of claim 91, wherein the disorder or disease associated with cells expressing CD166 is cancer.

93. The method of claim 90 or claim 92, wherein the cancer is an adenocarcinoma, a bile duct (biliary) cancer, a bladder cancer, a bone cancer, a breast cancer, a Her2-negative breast cancer, a triple-negative breast cancer, an endometrial cancer, an estrogen receptor-positive breast cancer, a carcinoid, a cervical cancer, a cholangiocarcinoma, a colorectal cancer, a colon cancer, an endometrial cancer, a glioma, a head and neck cancer, a head and neck squamous cell cancer, a leukemia, a liver cancer, a lung cancer, a non-small cell lung cancer, a small cell lung cancer, a lymphoma, a melanoma, an oropharyngeal cancer, an ovarian cancer, a pancreatic cancer, a prostate cancer, a metastatic castration-resistant prostate carcinoma, a renal cancer, a sarcoma, a skin cancer, a squamous cell cancer, a stomach cancer, a testis cancer, a thyroid cancer, a urogenital cancer, or a urothelial cancer.

94. The method of claim 90 or claim 92, wherein the cancer is selected from the group consisting of a cholangiocarcinoma, an endometrial cancer, triple negative breast cancer (TNBC), an estrogen receptor-positive breast cancer, non-small cell lung cancer (NSCLC), a prostate carcinoma, small cell lung cancer (SCLC), oropharyngeal cancer, cervical cancer, an ovarian cancer, head and neck squamous cell carcinoma (HNSCC), and prostate cancer.

95. A method of inhibiting or reducing the growth, proliferation, or metastasis of cells expressing mammalian CD166 comprising administering a therapeutically effective amount of the antibody of any one of claims 1 to 5, the activatable antibody of any one of claims 6 to 39, the conjugated antibody or conjugated activatable antibody of any one of claims 40 to 81, or the pharmaceutical composition of any one of claims 82 to 84 to a subject in need thereof.

96. A method of inhibiting, blocking, or preventing the binding of a natural ligand or receptor to mammalian CD166, comprising administering a therapeutically effective amount of the antibody of any one of claims 1 to 5, the activatable antibody of any one of claims 6 to 39, the conjugated antibody or conjugated activatable antibody of any one of claims 40 to 81, or the pharmaceutical composition of any one of claims 82 to 84 to a subject in need thereof.

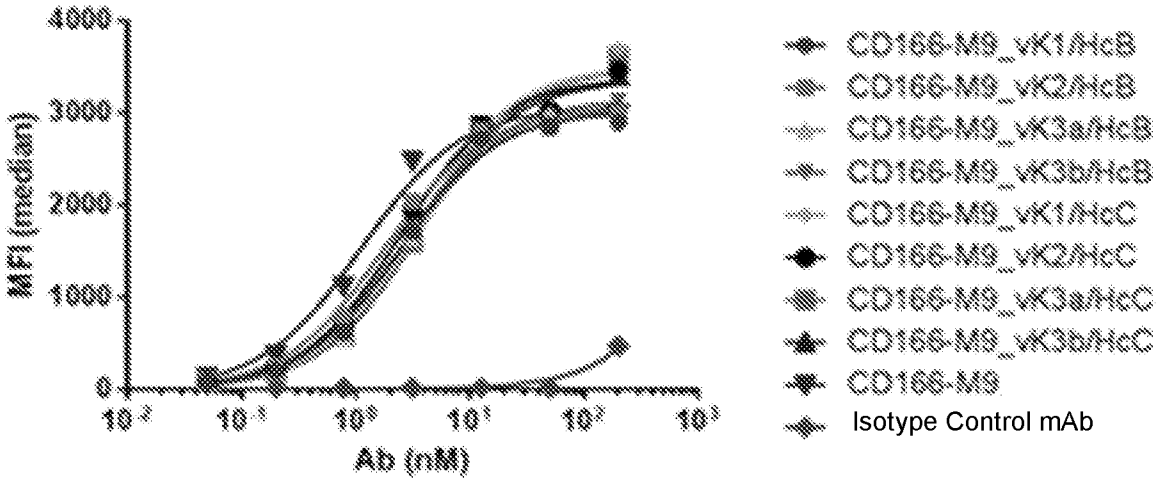
97. The method of claim 96, wherein the natural ligand or receptor is mammalian CD6.

98. The method of any one of claims 89-97, wherein the expression and/or activity of the mammalian CD166 is aberrant.

99. The method of any one of claims 89-98, wherein the method comprises administering an additional agent.

100. The method of claim 99, wherein the additional agent is a therapeutic agent.

FIGURE 1



Ab	Apparent Kd (nM); FACS
CD166-M9_vK1/HcB	2.39
CD166-M9_vK2/HcB	1.79
CD166-M9_vK3a/HcB	2.88
CD166-M9_vK3b/HcB	2.61
CD166-M9_vK1/HcC	3.26
CD166-M9_vK2/HcC	2.50
CD166-M9_vK3a/HcC	3.76
CD166-M9_vK3b/HcC	3.19
CD166-M9_chimera	1.12

FIGURE 2

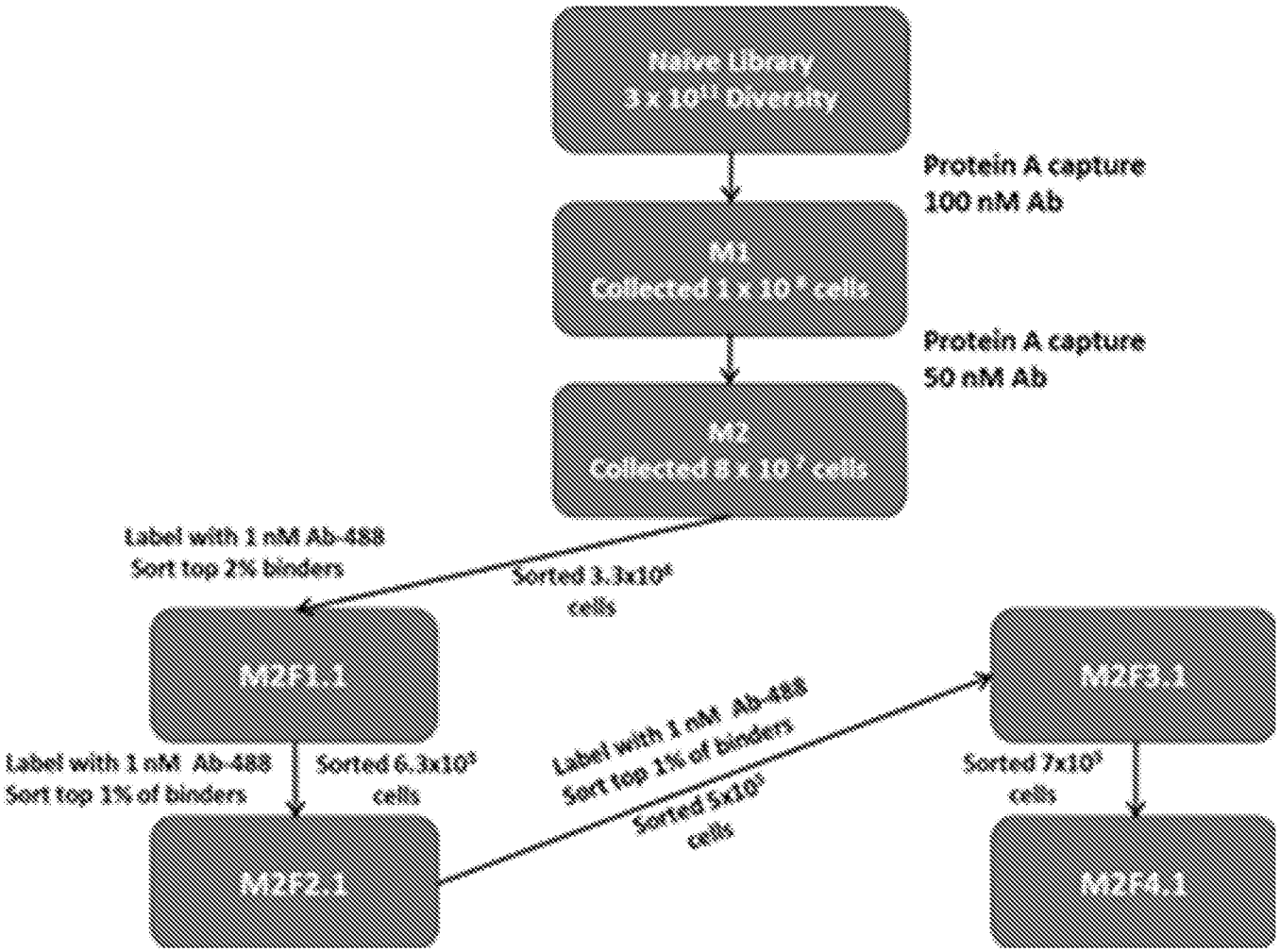

Naive Library
3 x 10^11 Diversity
Protein A capture
100 nM Ab
M1
Collected 1 x 10^9 cells
Protein A capture
50 nM Ab
M2
Collected 8 x 10^7 cells
Label with 1 nM Ab-488
Sort top 2% binders
Sorted 3.3x10^6
cells
M2F1.1
Label with 1 nM Ab-488
Sort top 1% of binders
Sorted 6.3x10^5
cells
M2F2.1
Label with 1 nM Ab-488
Sort top 1% of binders
Sorted 5x10^5
cells
M2F3.1
Sorted 7x10^5
cells
M2F4.1

FIGURE 3A

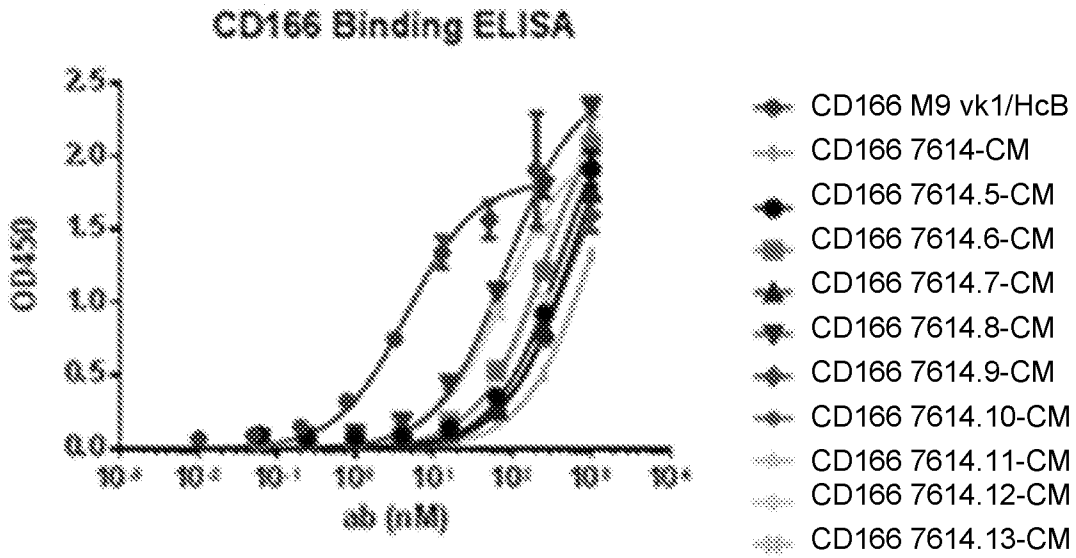


FIGURE 3B

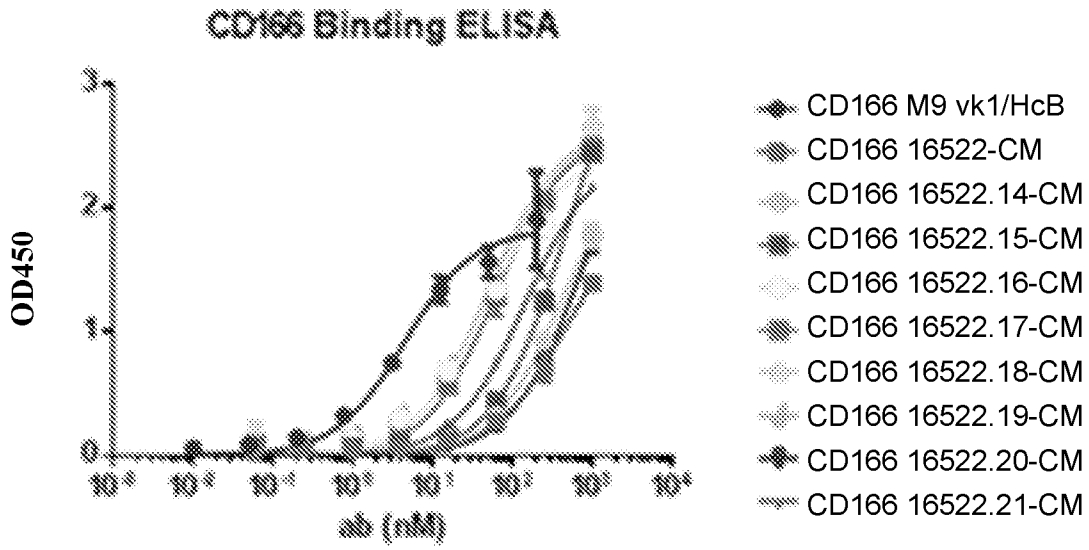


FIGURE 4

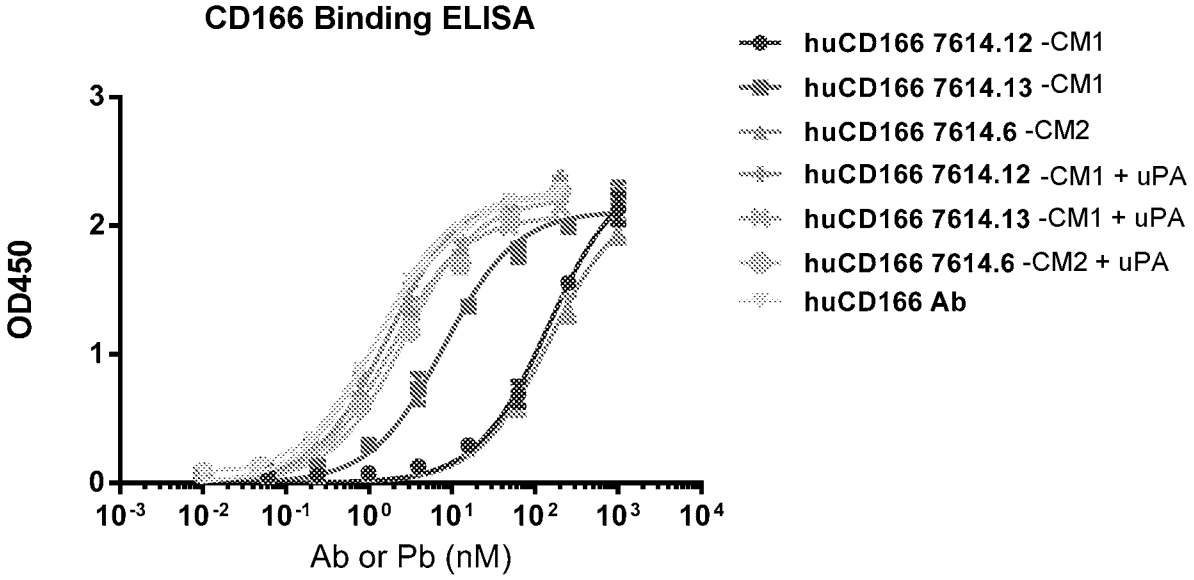


FIGURE 5

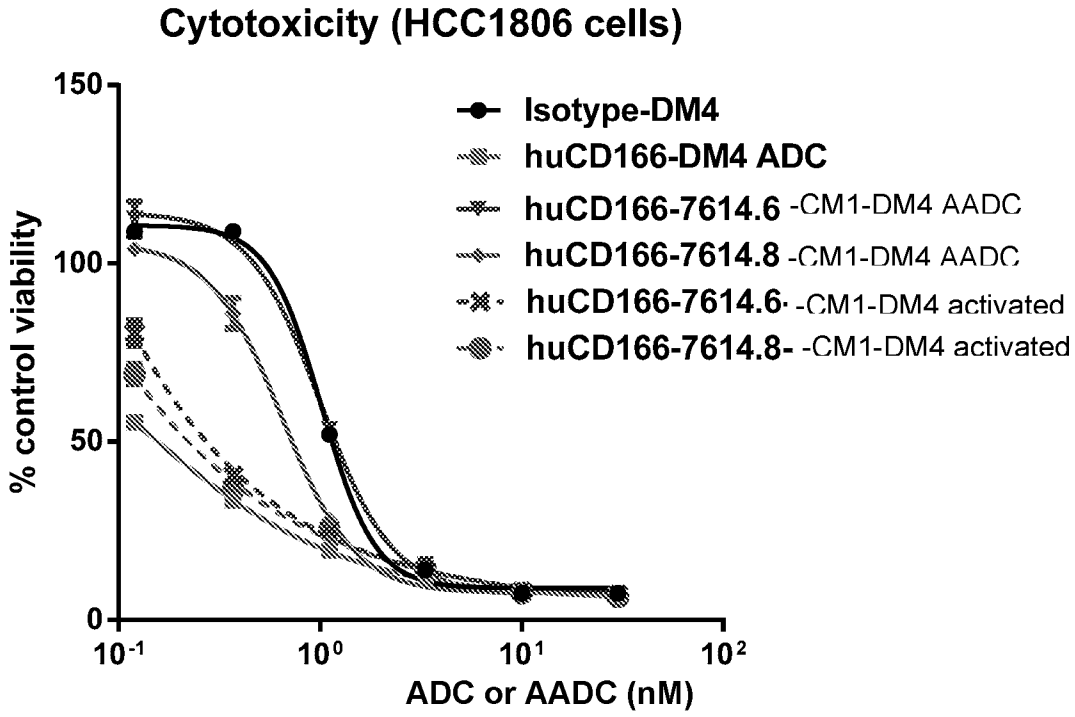
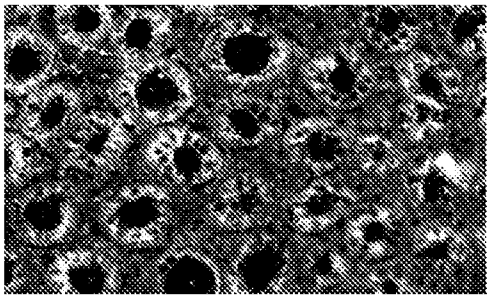


FIGURE 6A

FIGURE 6B

Normal colon



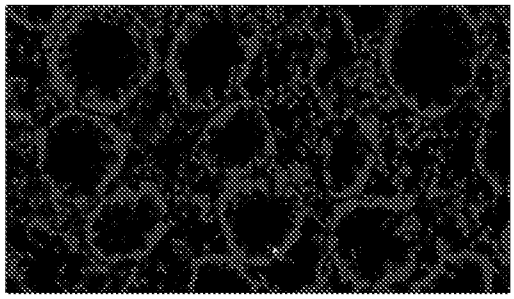
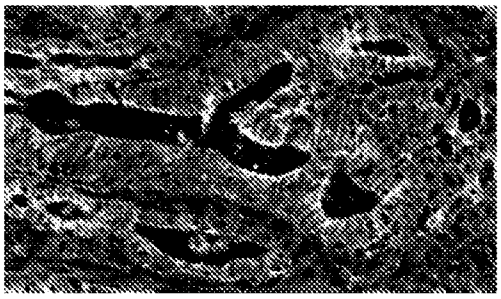


FIGURE 6C

FIGURE 6D

Colon cancer



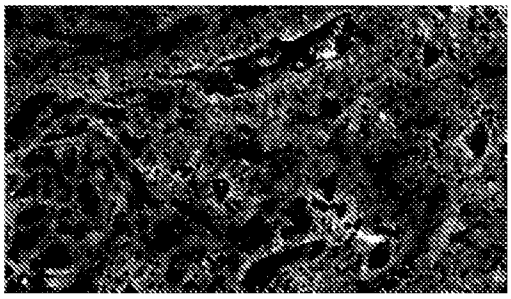
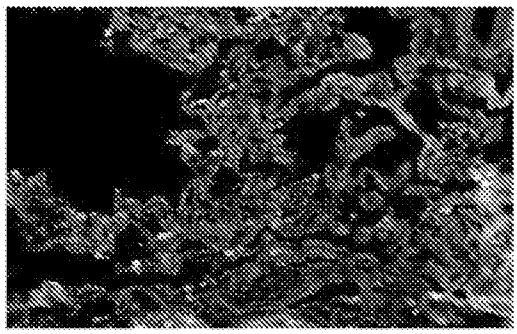


FIGURE 7A

FIGURE 7B

Normal lung



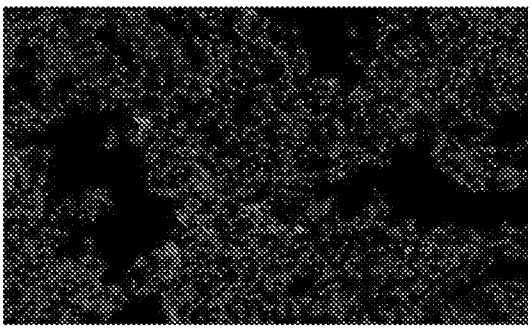
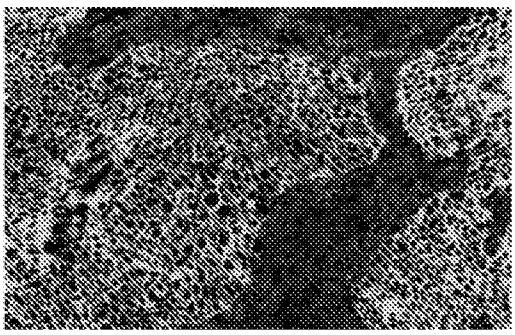
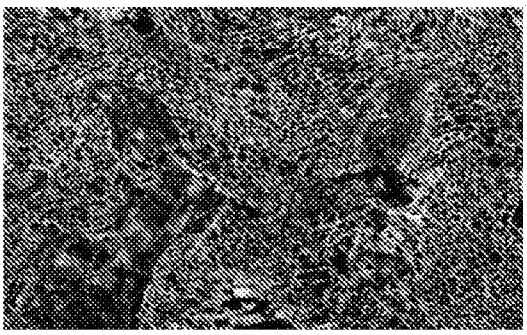


FIGURE 7C

FIGURE 7D

Lung cancer





Normal stomach

FIGURE 8A

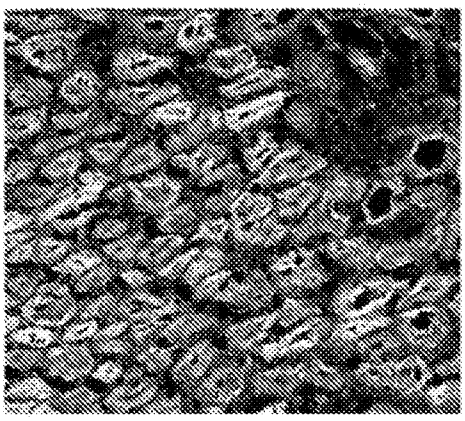
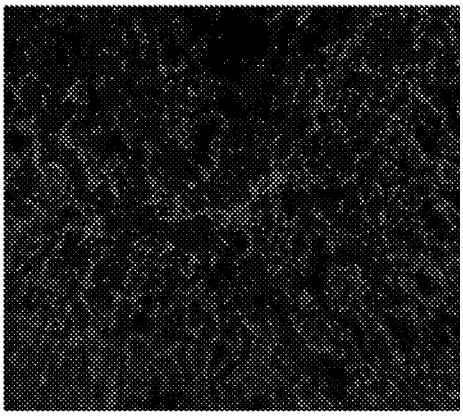


FIGURE 8B



Normal bladder

FIGURE 8C

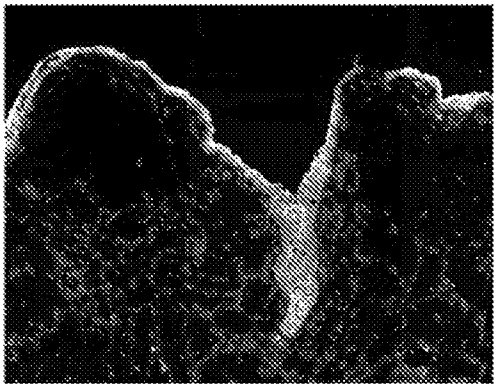


FIGURE 8D

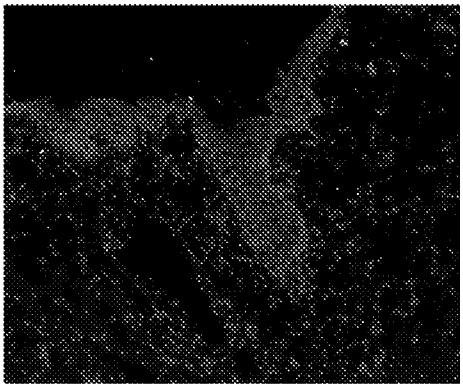


FIGURE 9

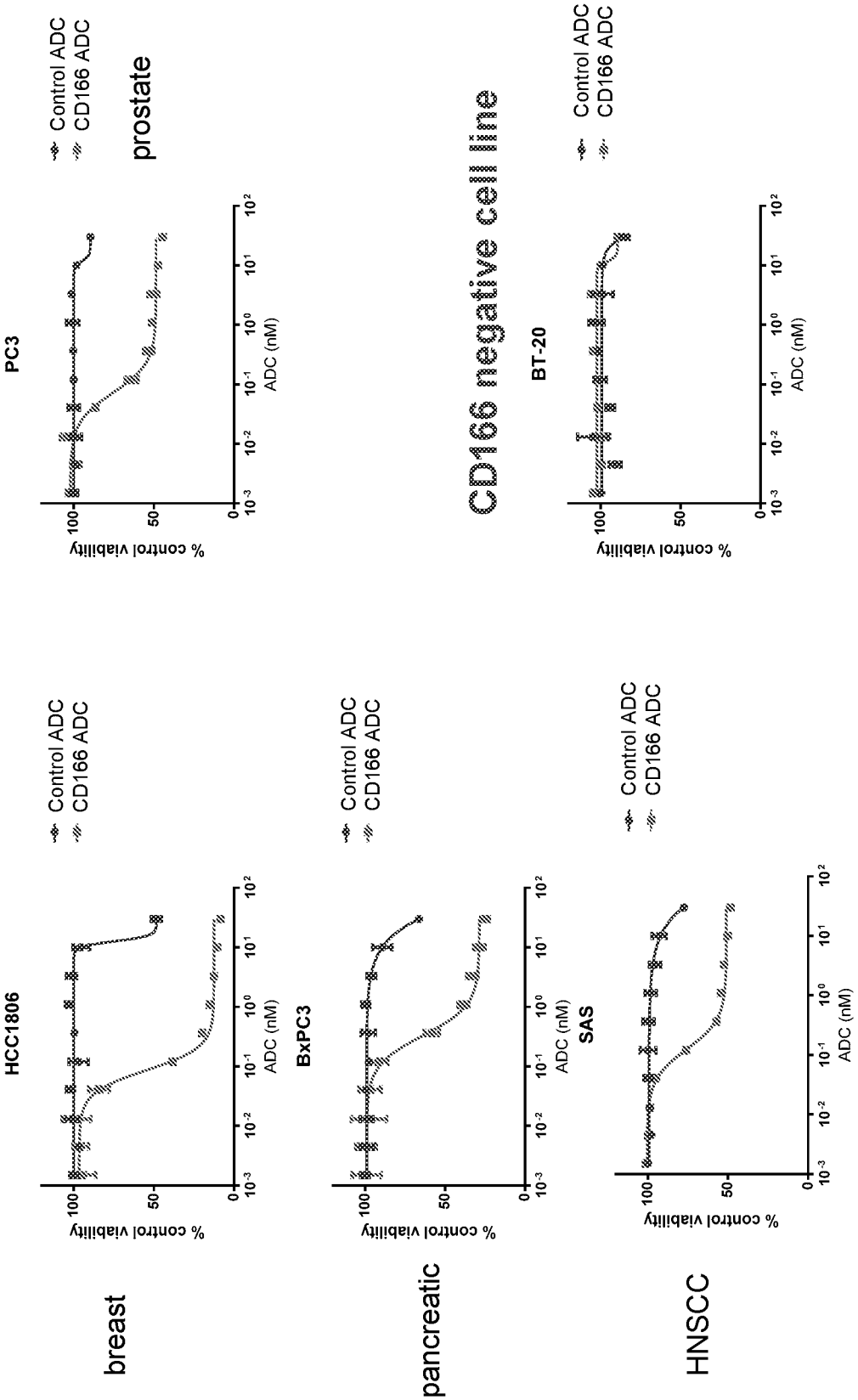
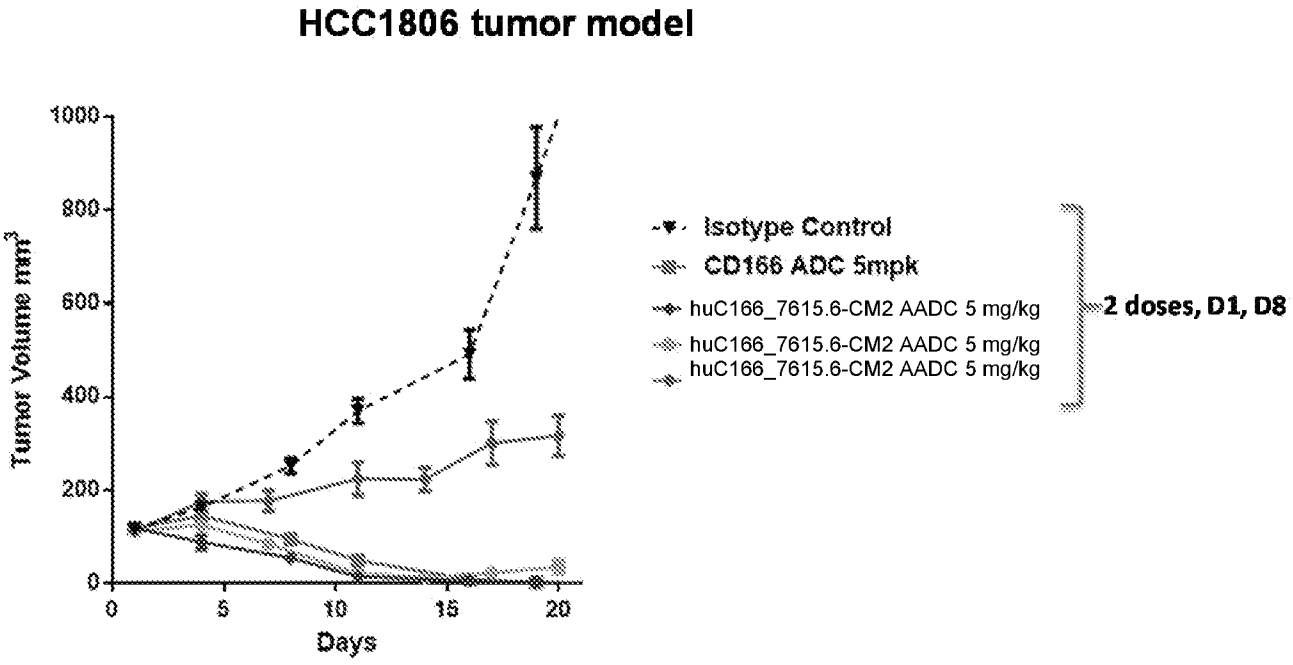

CD166 negative cell line
prostate
PC3
Control ADC
CD166 ADC
% control viability
ADC (nM)
BT-20
breast
HCC1806
BxPC3
pancreatic
SAS
HNSCC

FIGURE 10



CD166 AADC Beats Benchmark Standard For Efficacy

FIGURE 11

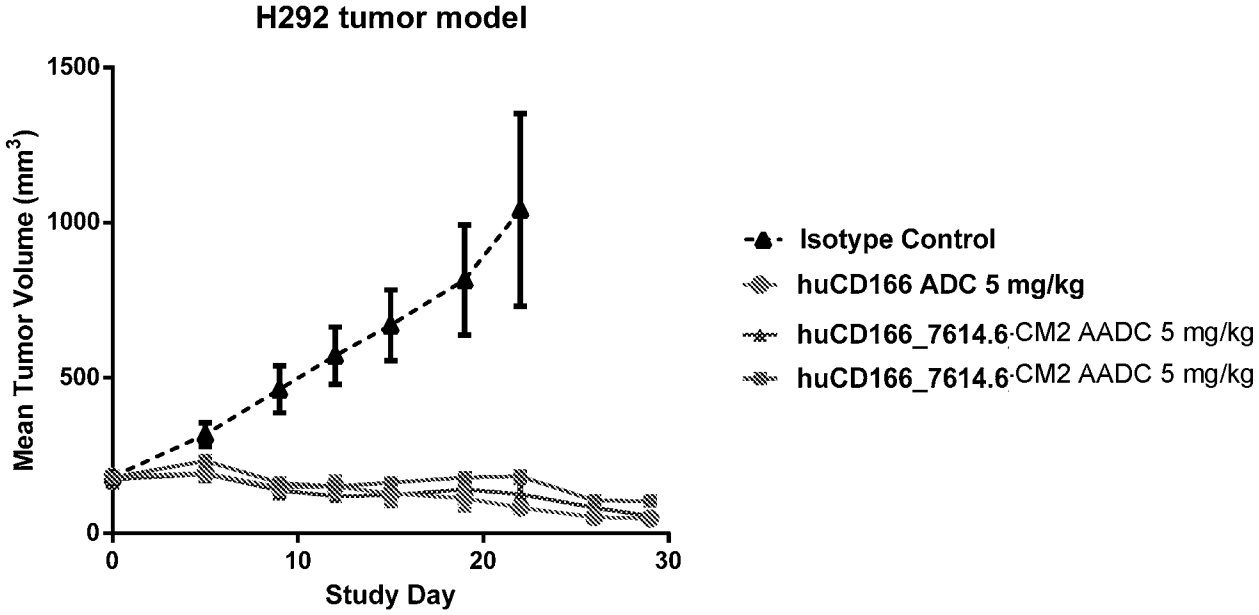


FIGURE 12

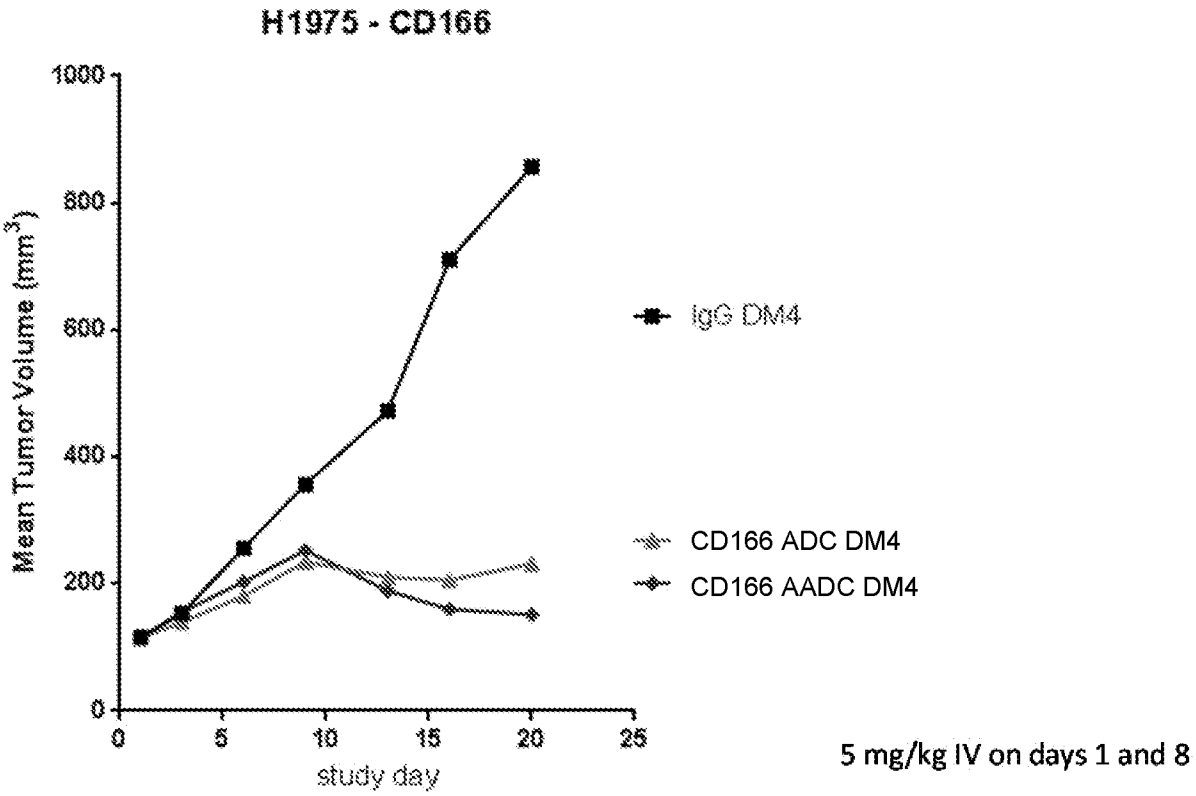


FIGURE 13

ELISA – Cyno and human binding are identical

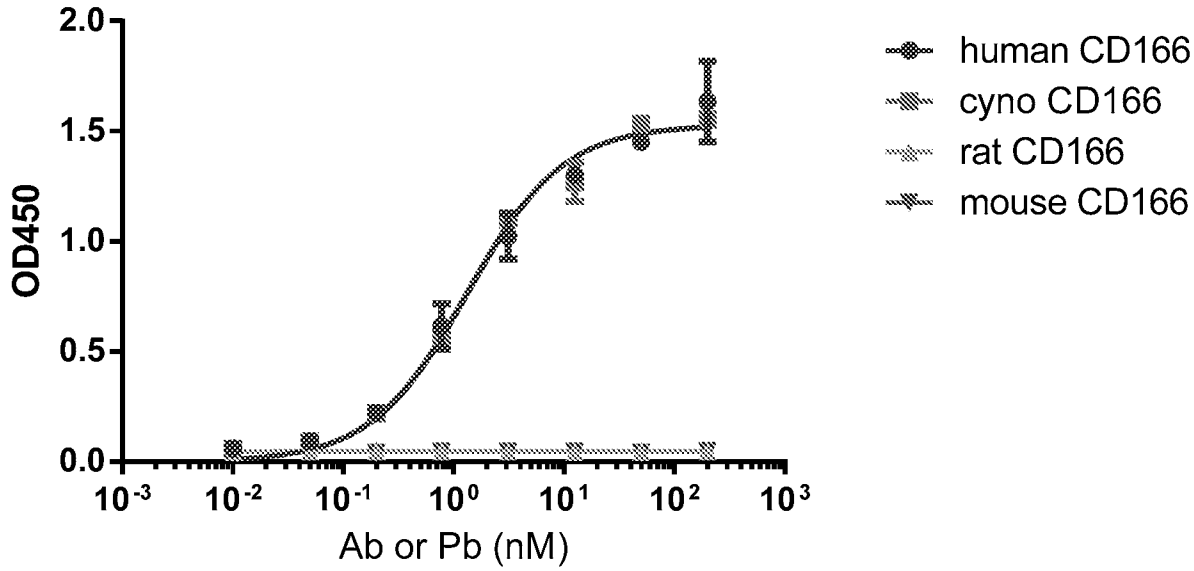


FIGURE 14

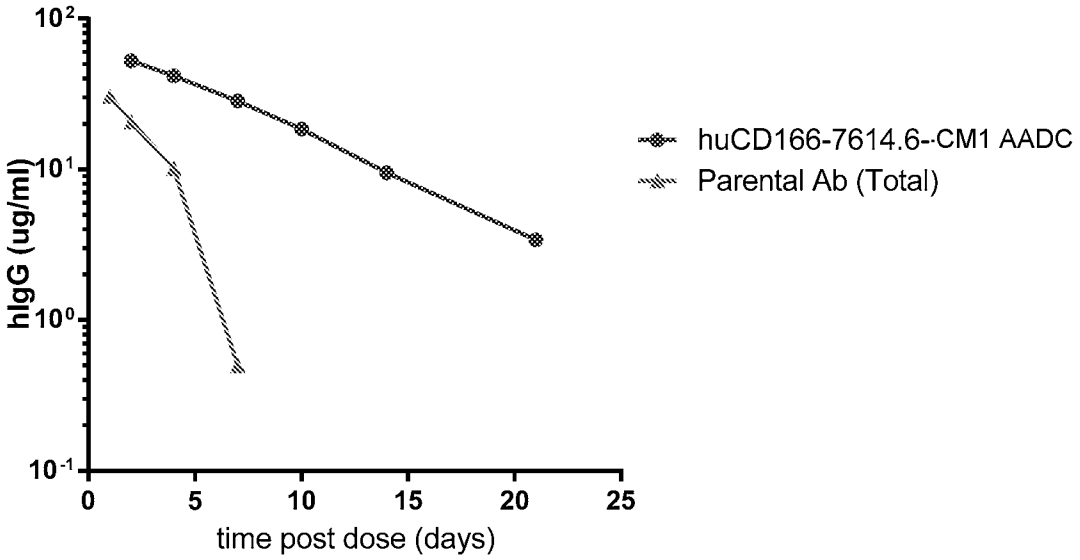

10^2
10^1
10^0
10^{-1}
hIgG (ug/ml)
0
5
10
15
20
25
time post dose (days)
huCD166-7614.6--CM1 AADC
Parental Ab (Total)

FIGURE 15

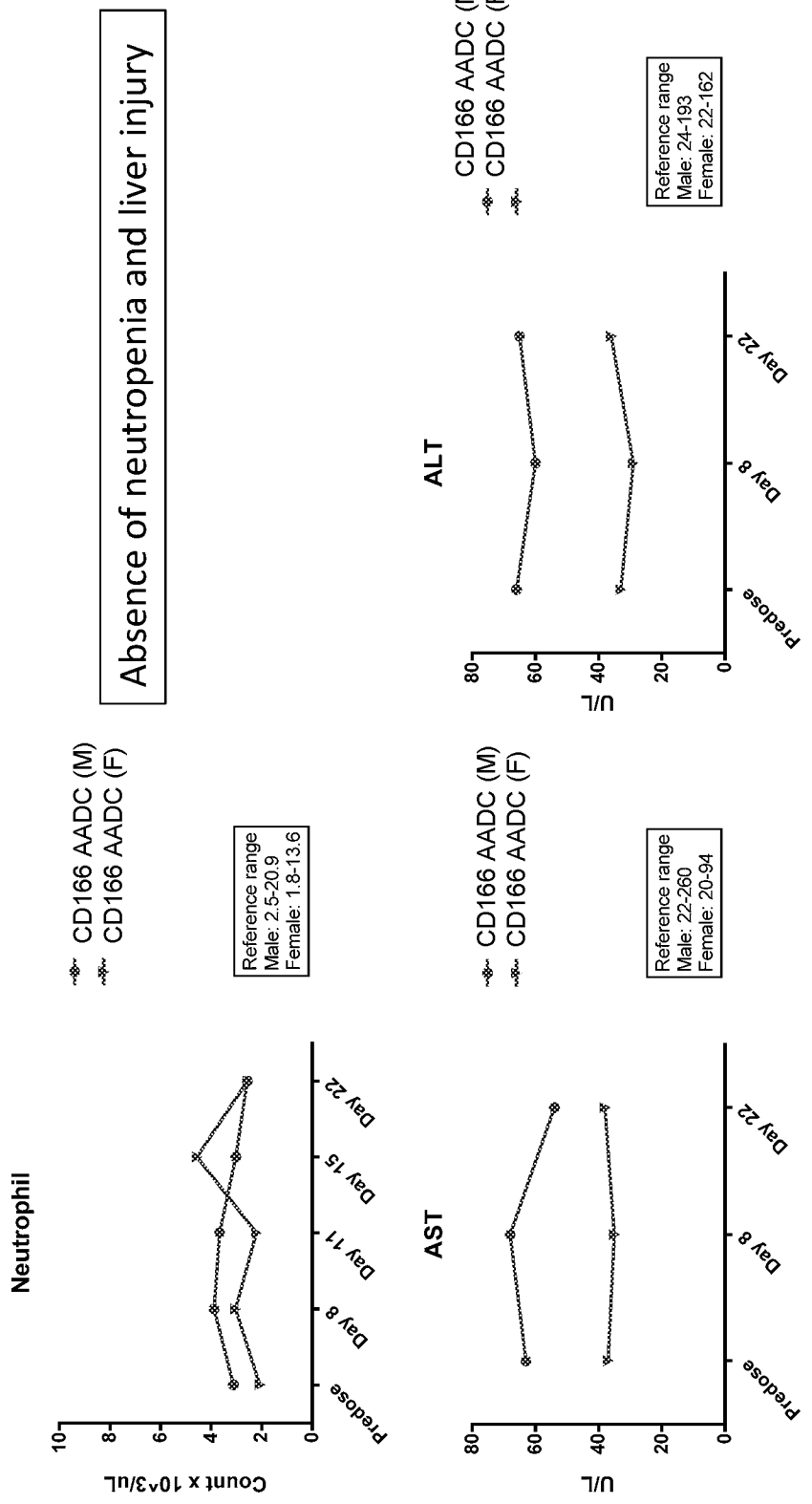

Absence of neutropenia and liver injury
Neutrophil
Count x 10^3/uL
CD166 AADC (M)
CD166 AADC (F)
Reference range
Male: 2.5-20.9
Female: 1.8-13.6
Predose
Day 8
Day 11
Day 15
Day 22
AST
U/L
Reference range
Male: 22-260
Female: 20-94
ALT
Reference range
Male: 24-193
Female: 22-162

FIGURE 16A

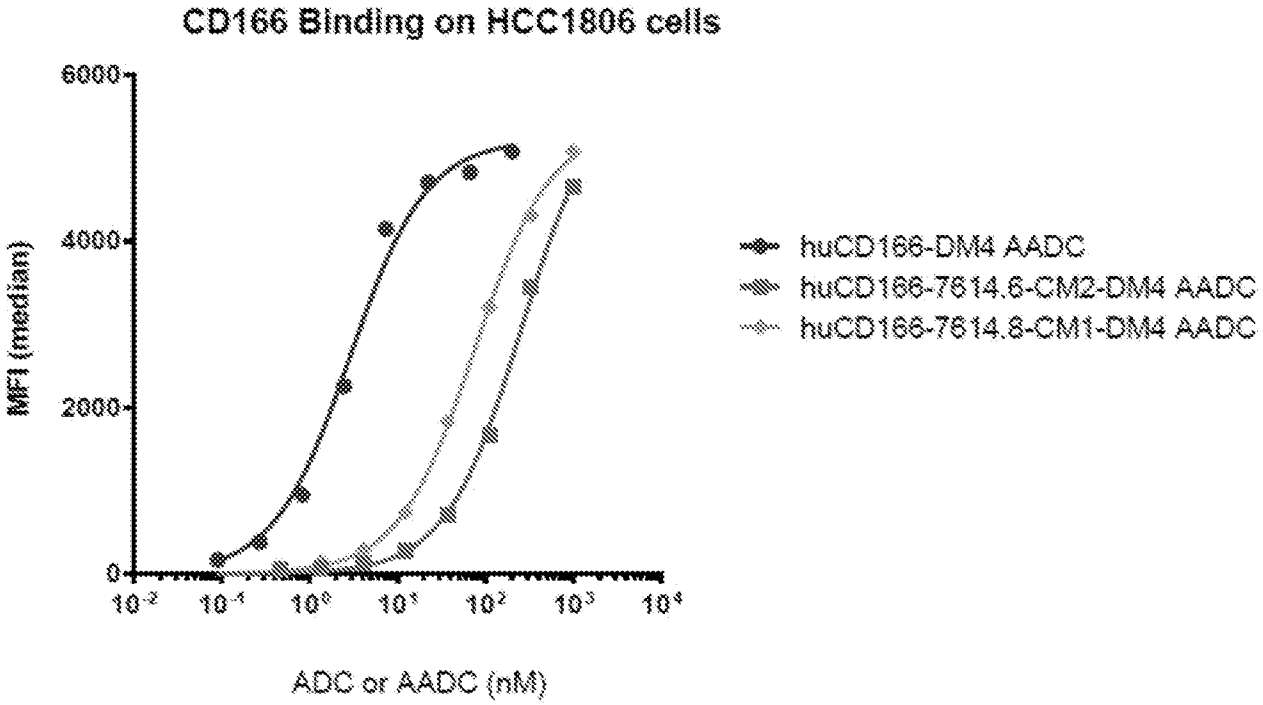


FIGURE 16B

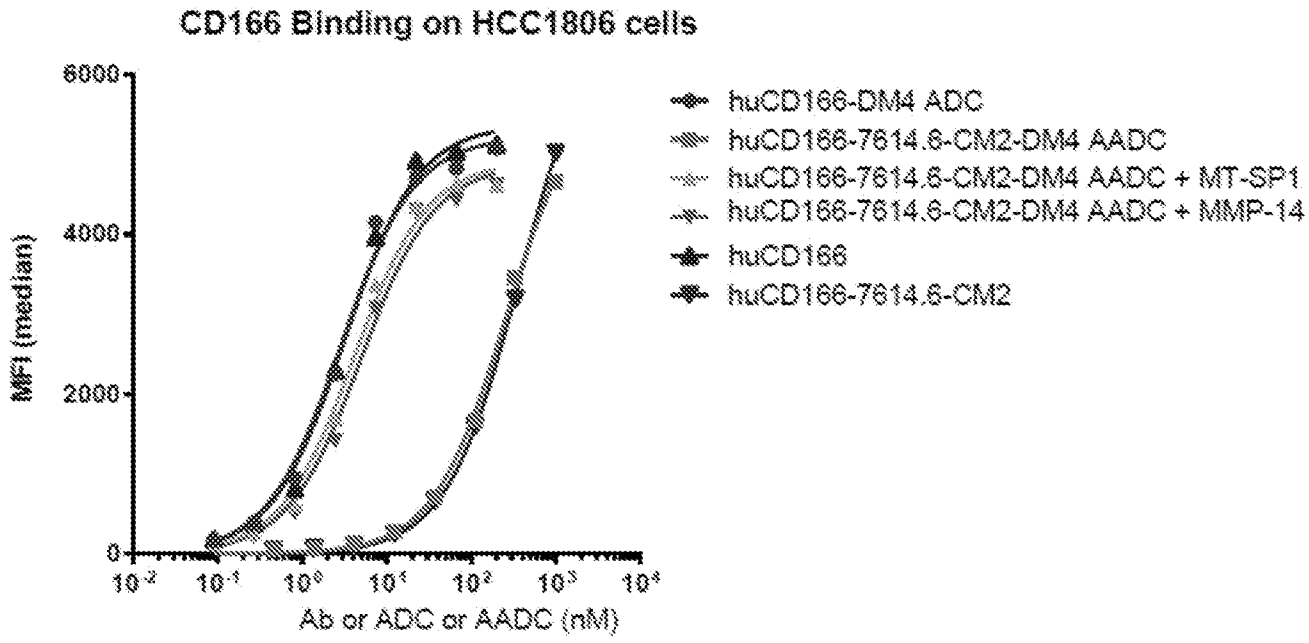


FIGURE 16C

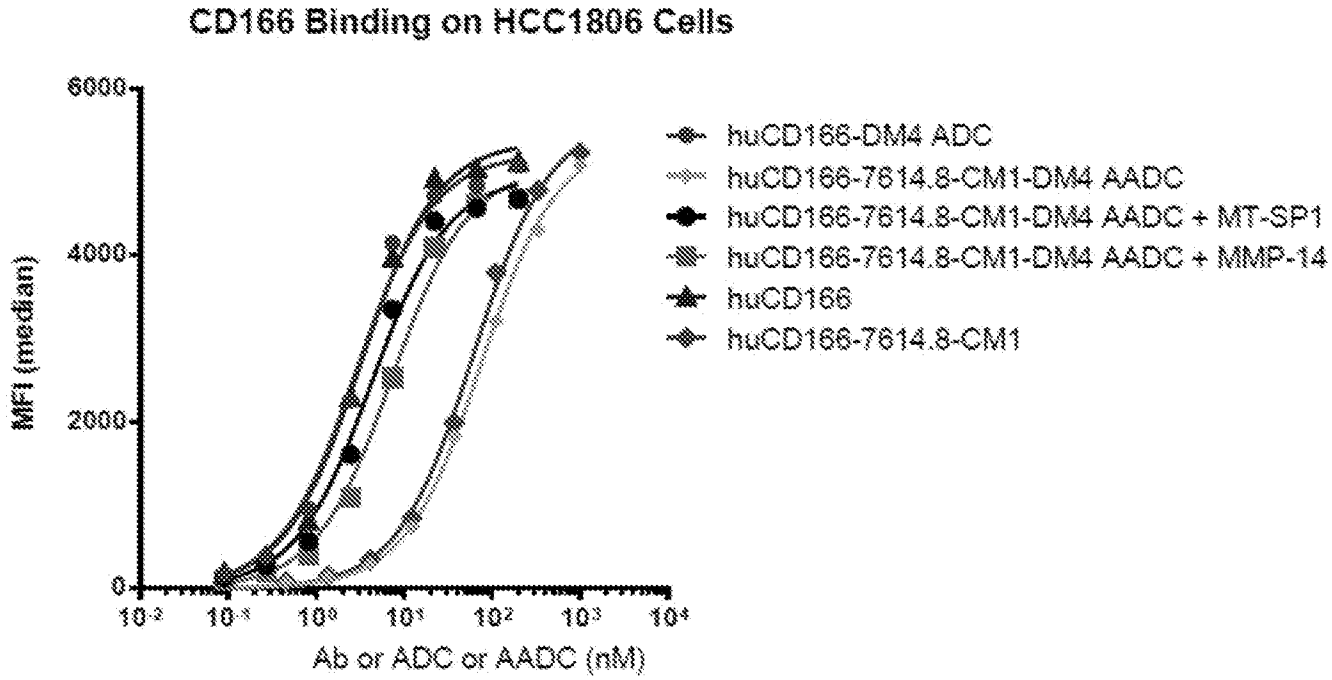

CD166 Binding on HCC1806 Cells
MFI (median)
6000
4000
2000
0
10⁻²
10⁻¹
10⁰
10¹
10²
10³
10⁴
Ab or ADC or AADC (nM)
huCD166-DM4 ADC
huCD166-7614.8-CM1-DM4 AADC
huCD166-7614.8-CM1-DM4 AADC + MT-SP1
huCD166-7614.8-CM1-DM4 AADC + MMP-14
huCD166
huCD166-7614.8-CM1

FIGURE 17

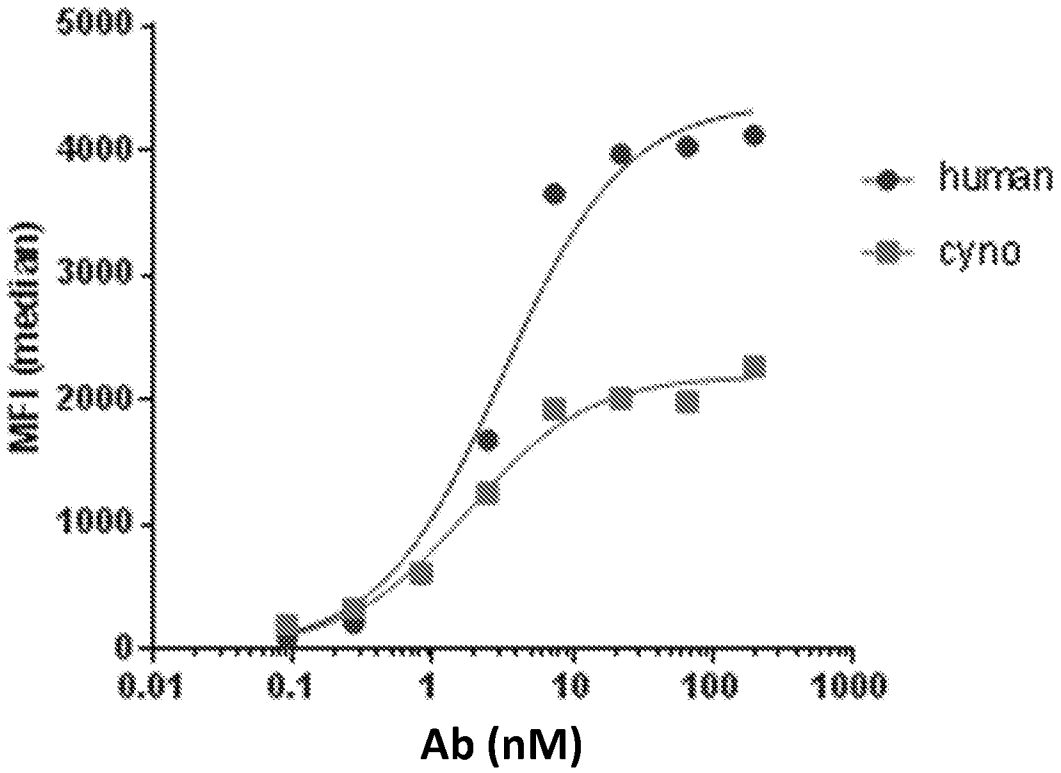

5000
4000
3000
2000
1000
0
MFI (median)
0.01
0.1
1
10
100
1000
Ab (nM)
human
cyno

FIGURE 18

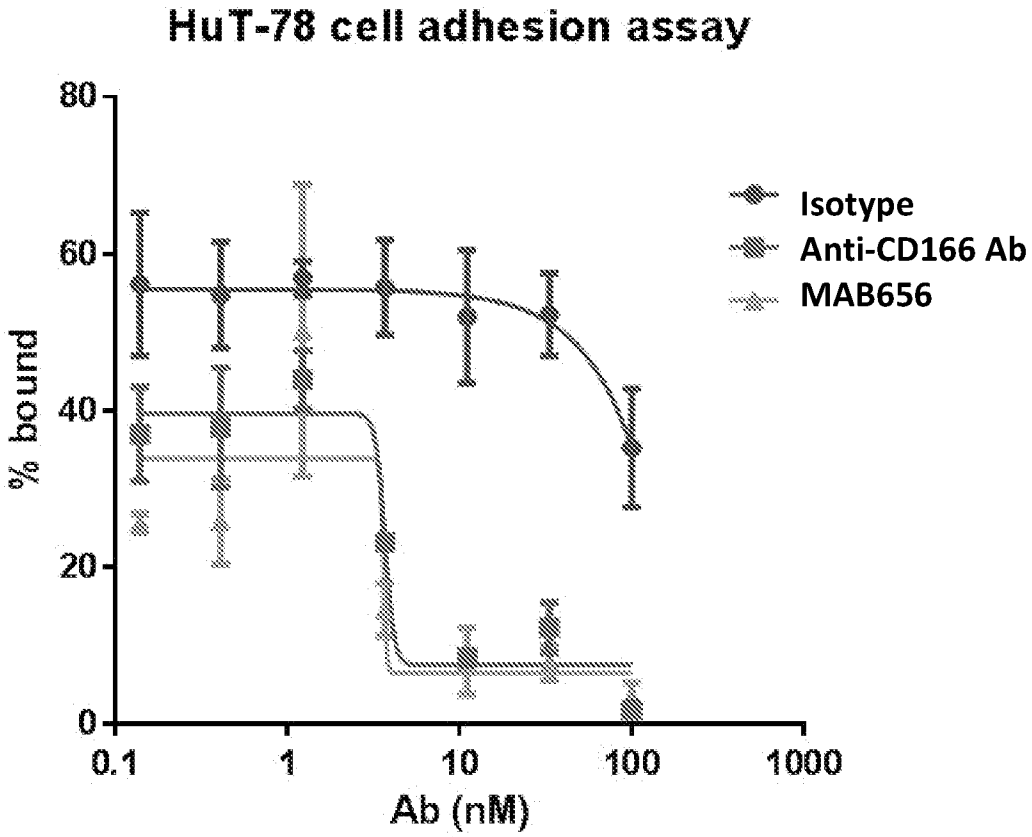
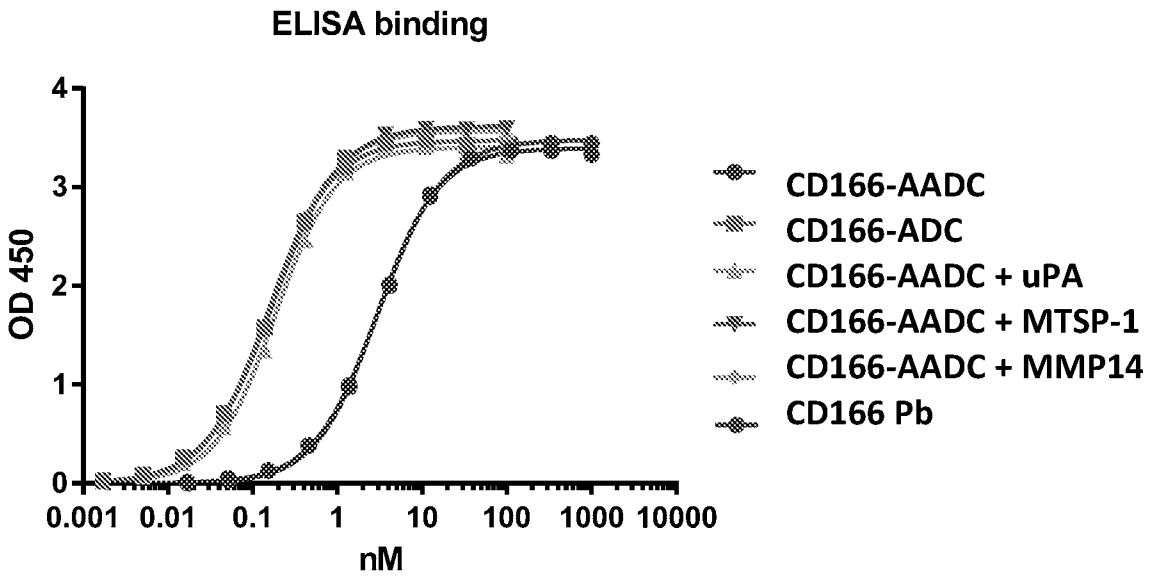

HuT-78 cell adhesion assay
% bound
80
60
40
20
0
0.1
1
10
100
1000
Ab (nM)
Isotype
Anti-CD166 Ab
MAB656

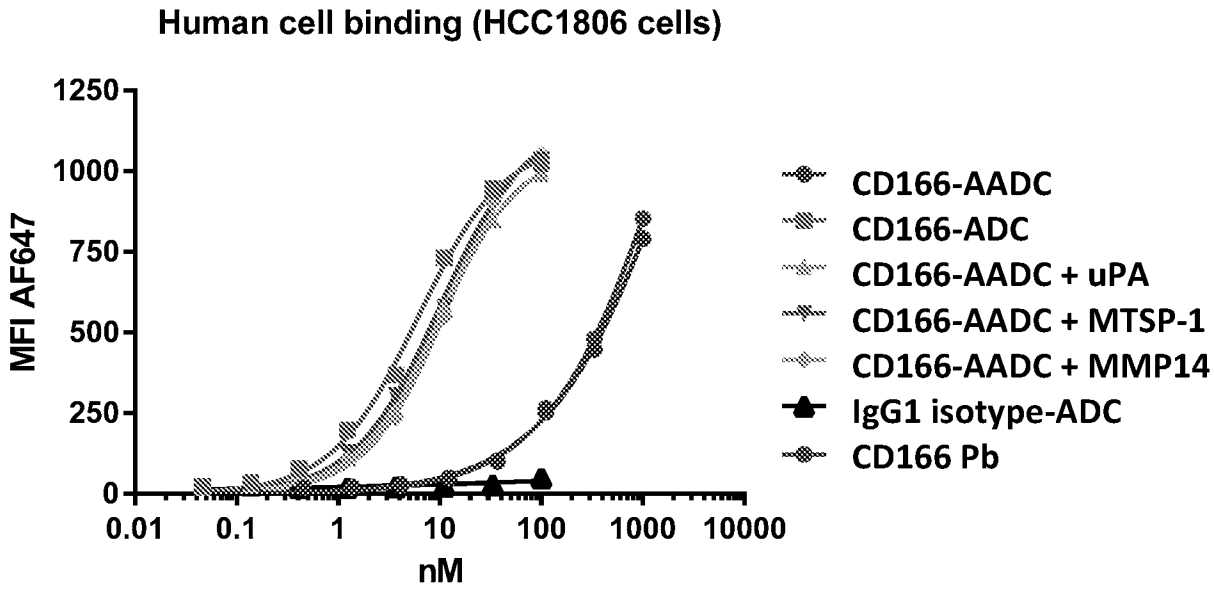
FIGURE 19A

A


ELISA binding
OD 450
0
1
2
3
4
0.001
0.01
0.1
1
10
100
1000
10000
nM
CD166-AADC
CD166-ADC
CD166-AADC + uPA
CD166-AADC + MTSP-1
CD166-AADC + MMP14
CD166 Pb

B

FIGURE 19B



C

FIGURE 19C

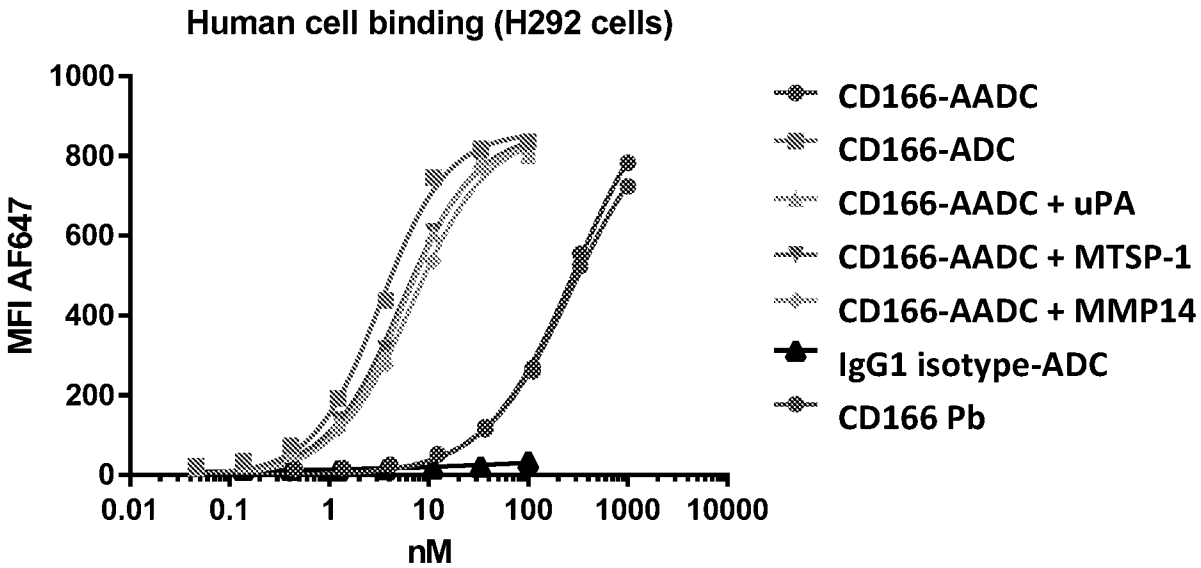


FIGURE 20A

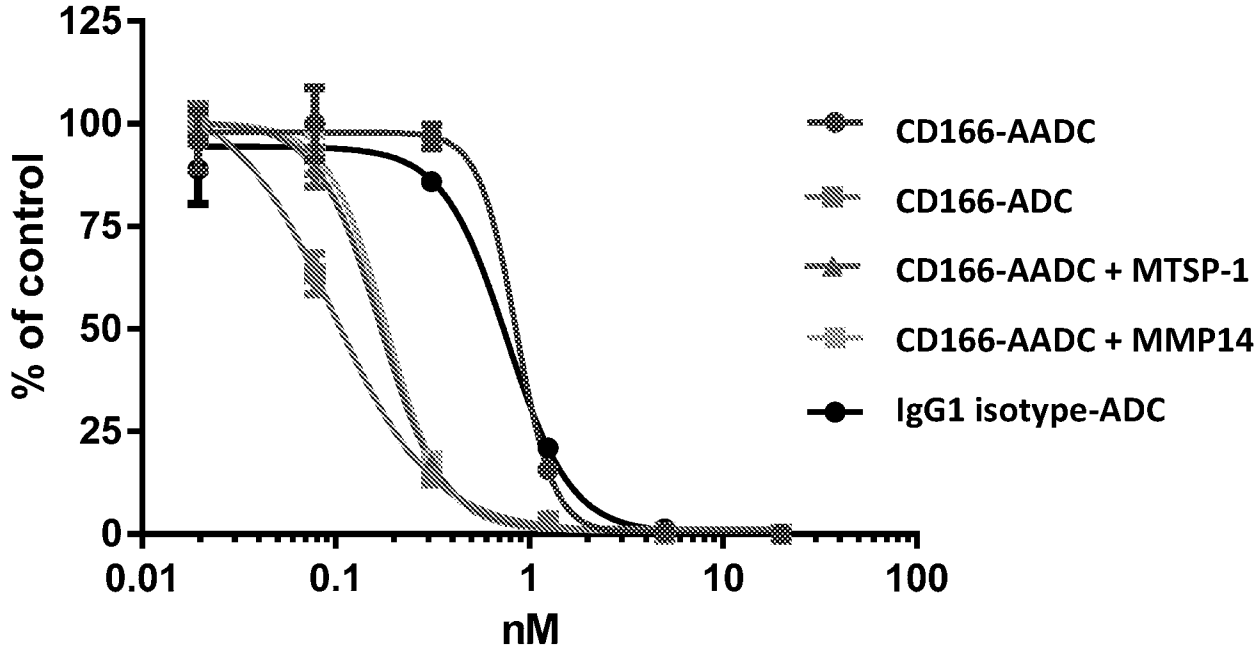


FIGURE 20B

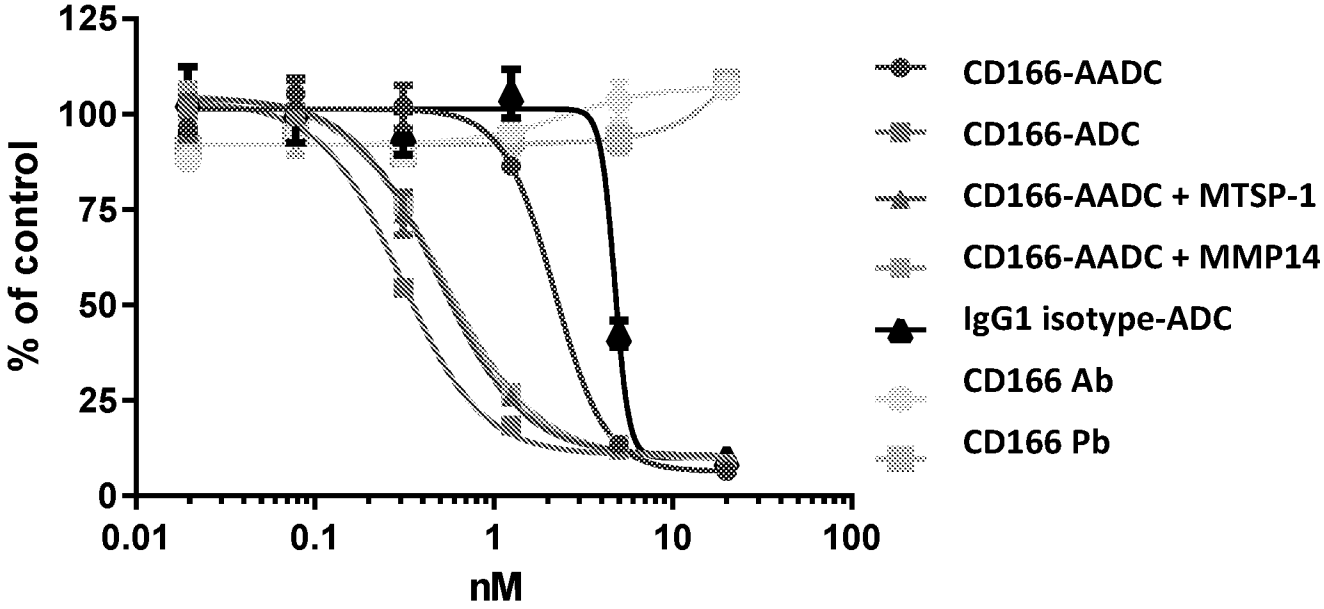


FIGURE 21A
TNFα

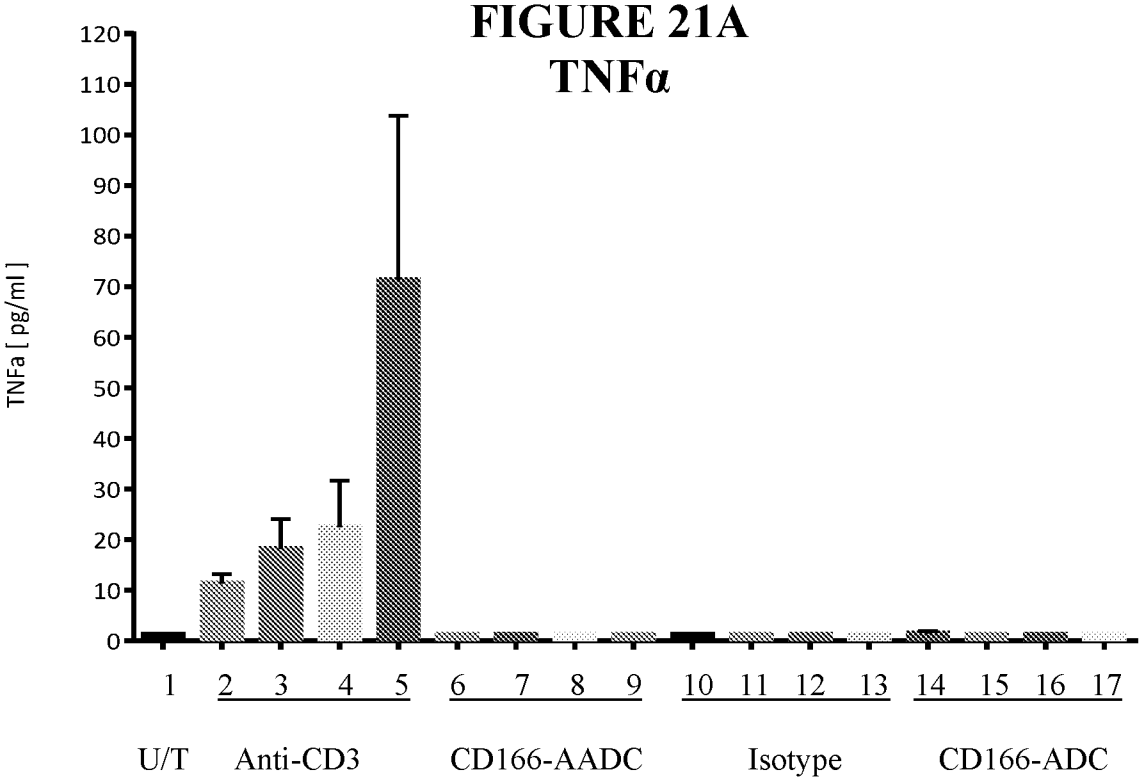


FIGURE 21B
IL-2

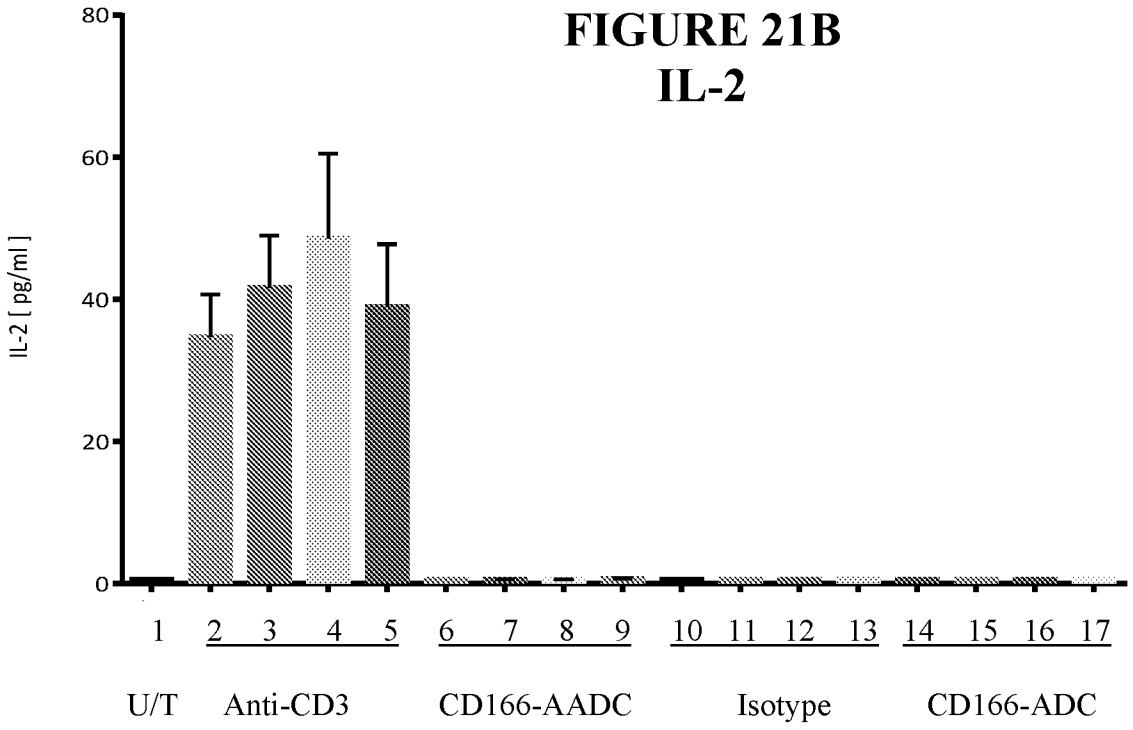


FIGURE 21C
IFNγ

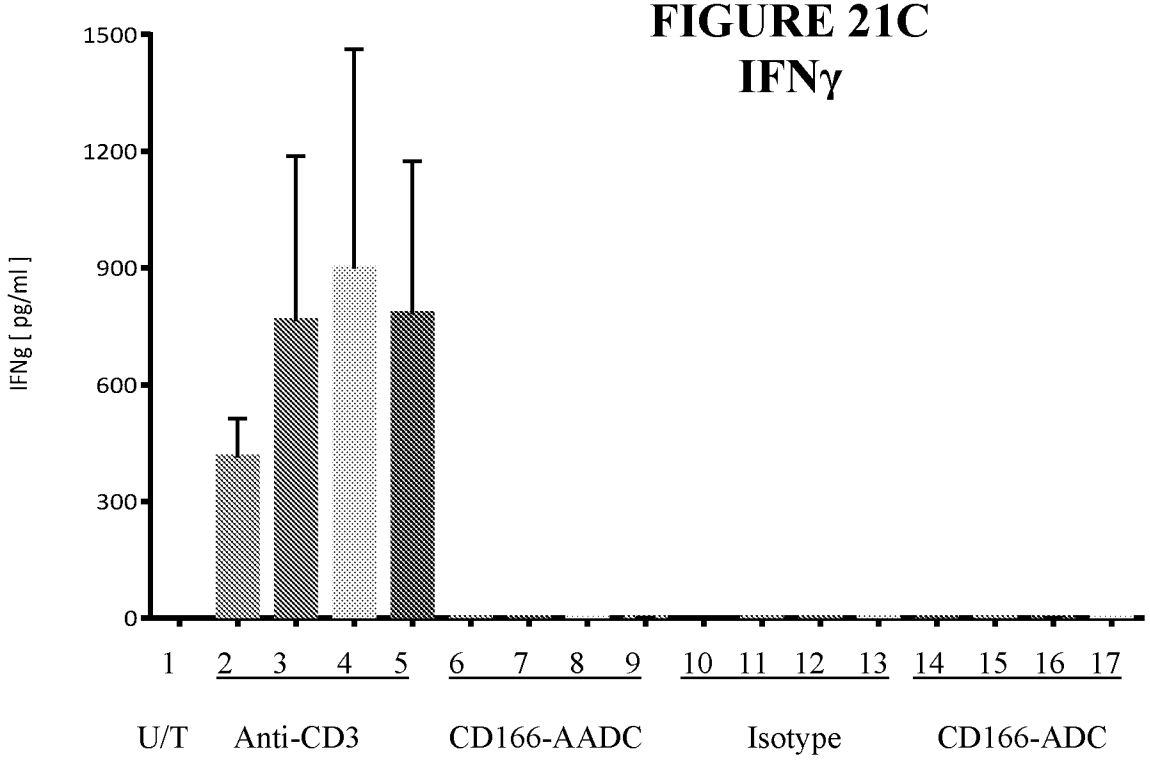


FIGURE 21D
IL-6

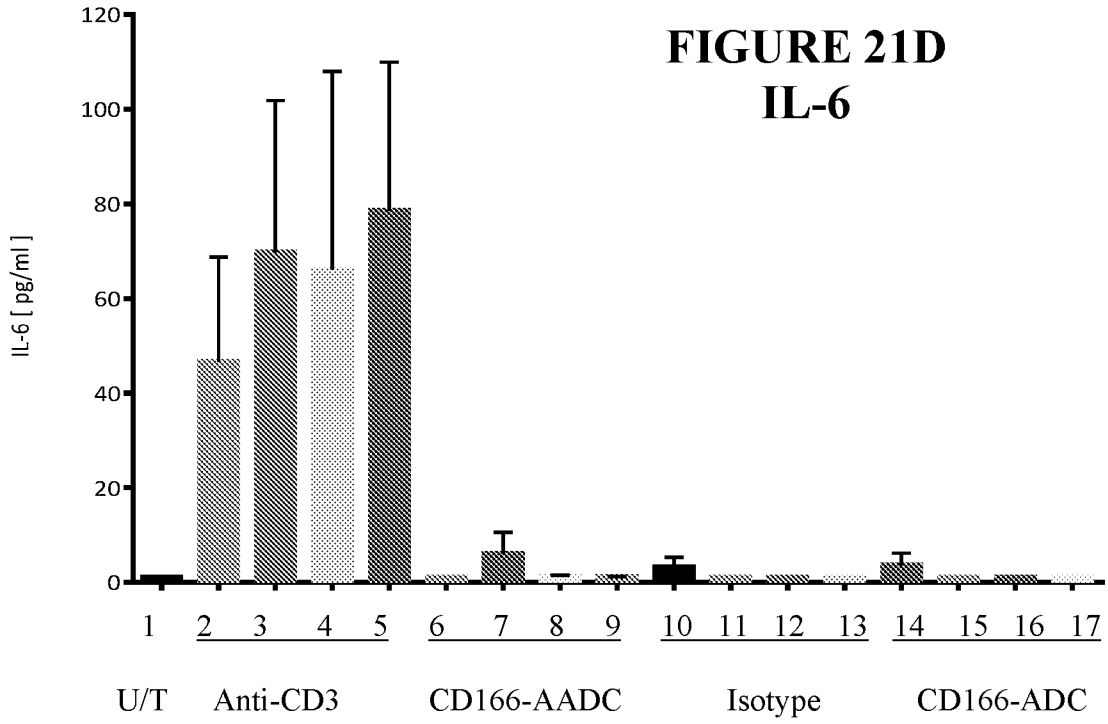


FIGURE 22

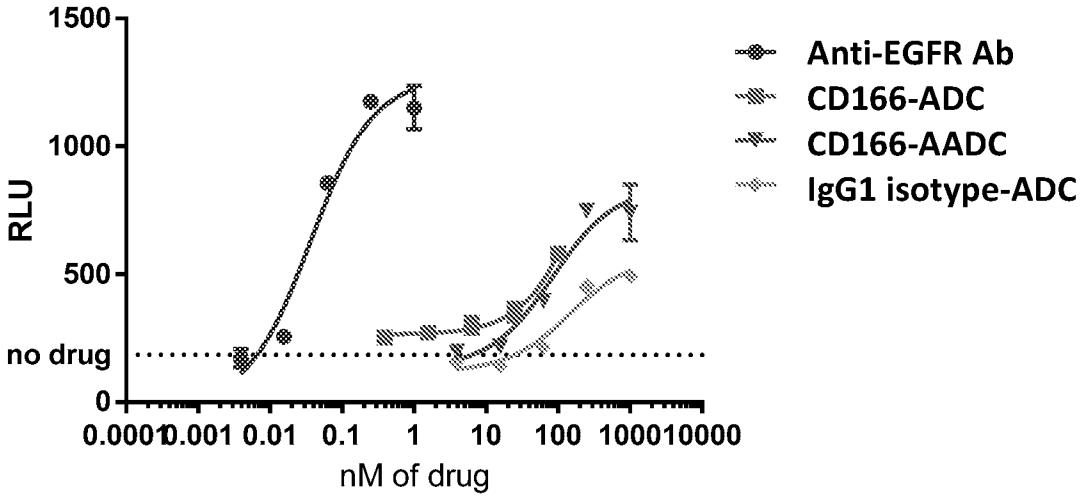

1500
1000
500
no drug
0
RLU
0.0001
0.001
0.01
0.1
1
10
100
10000
nM of drug
Anti-EGFR Ab
CD166-ADC
CD166-AADC
IgG1 isotype-ADC

FIGURE 23

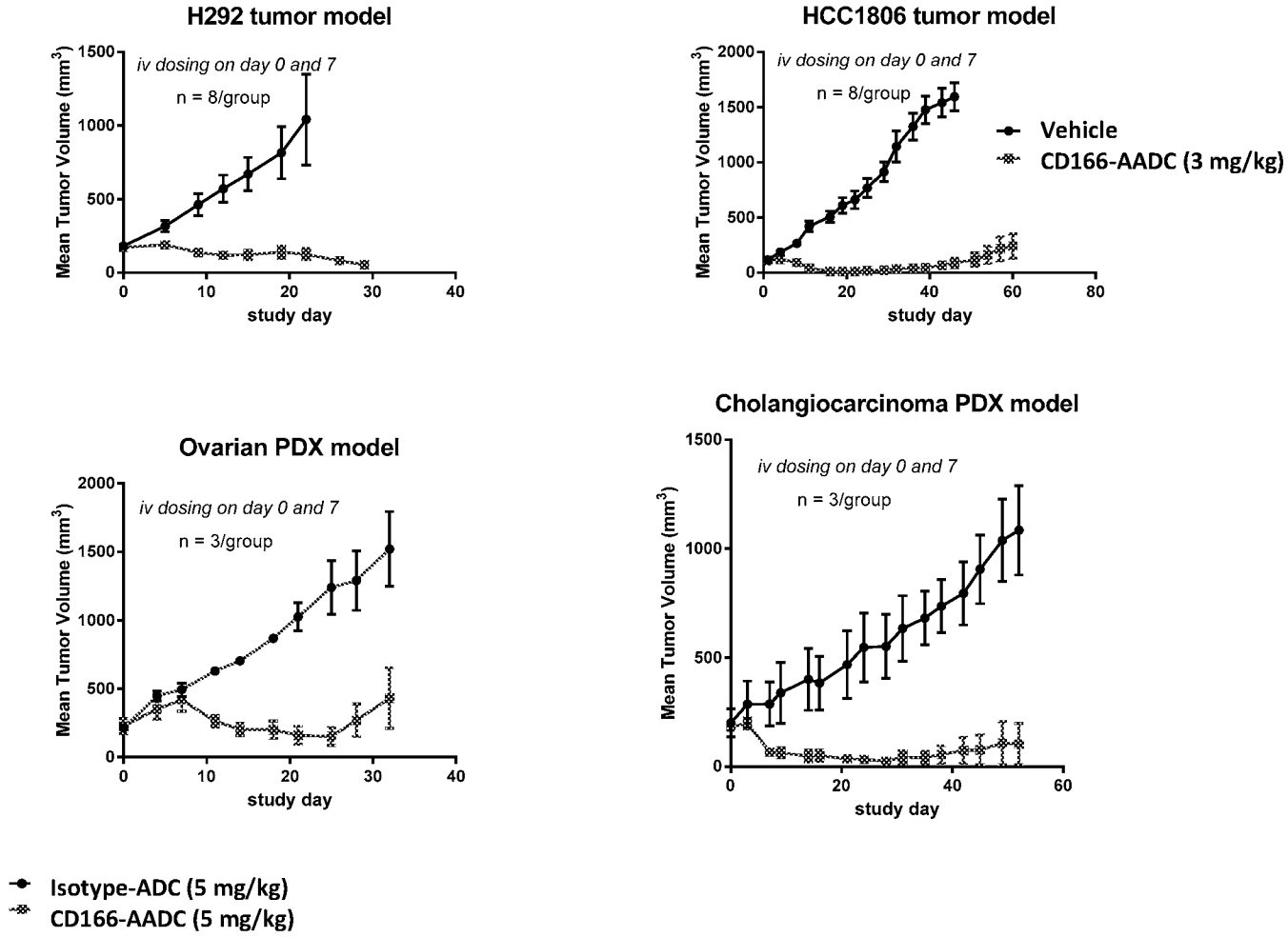

H292 tumor model
iv dosing on day 0 and 7
n = 8/group
Mean Tumor Volume (mm3)
study day
HCC1806 tumor model
iv dosing on day 0 and 7
n = 8/group
Vehicle
CD166-AADC (3 mg/kg)
Mean Tumor Volume (mm3)
study day
Ovarian PDX model
iv dosing on day 0 and 7
n = 3/group
Mean Tumor Volume (mm3)
study day
Cholangiocarcinoma PDX model
iv dosing on day 0 and 7
n = 3/group
Mean Tumor Volume (mm3)
study day
Isotype-ADC (5 mg/kg)
CD166-AADC (5 mg/kg)

FIGURE 24A (HEC-1-A cells)

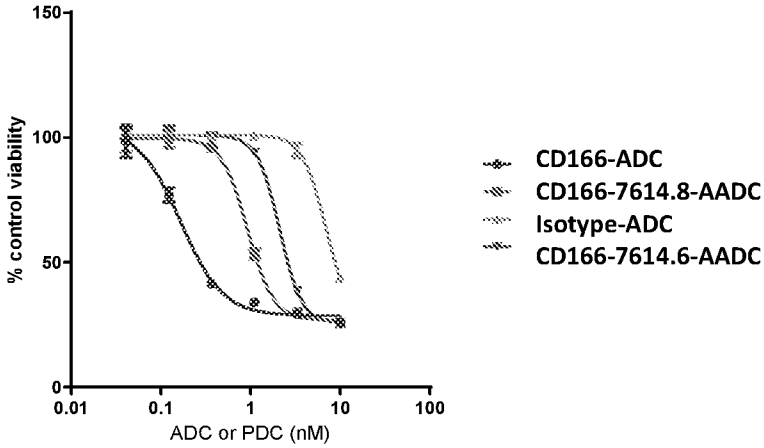


FIGURE 24B (AN3-CA cells)

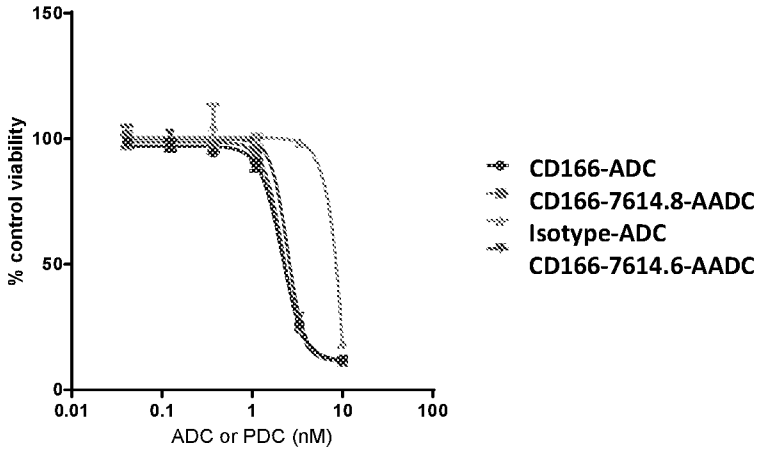


FIGURE 24C (KLE cells)

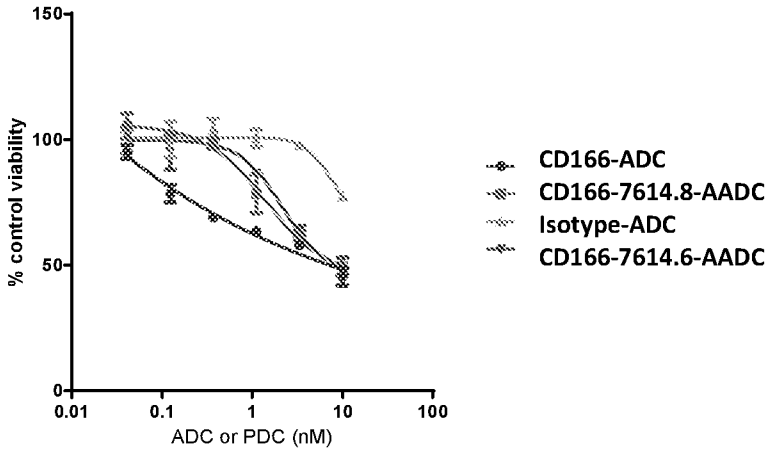


FIGURE 24D

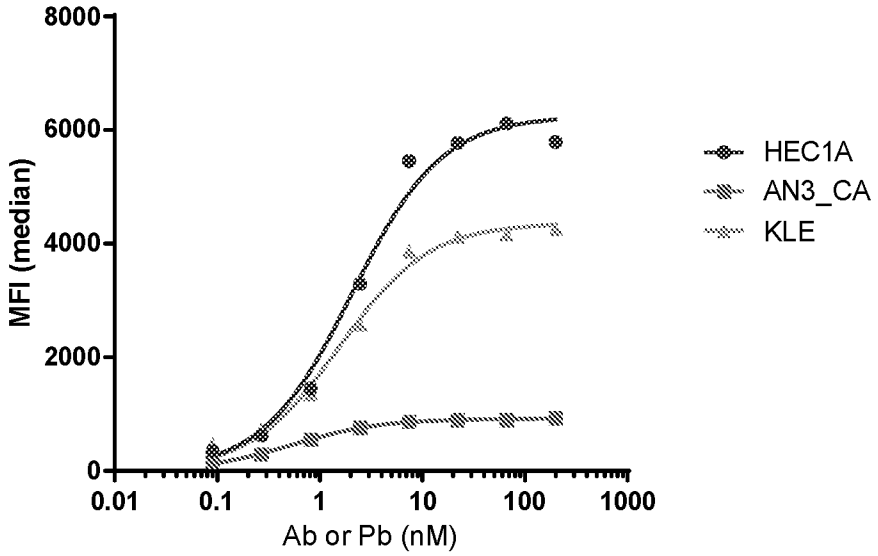

8000
6000
4000
2000
0
MFI (median)
0.01
0.1
1
10
100
1000
Ab or Pb (nM)
HEC1A
AN3_CA
KLE

FIGURE 25A

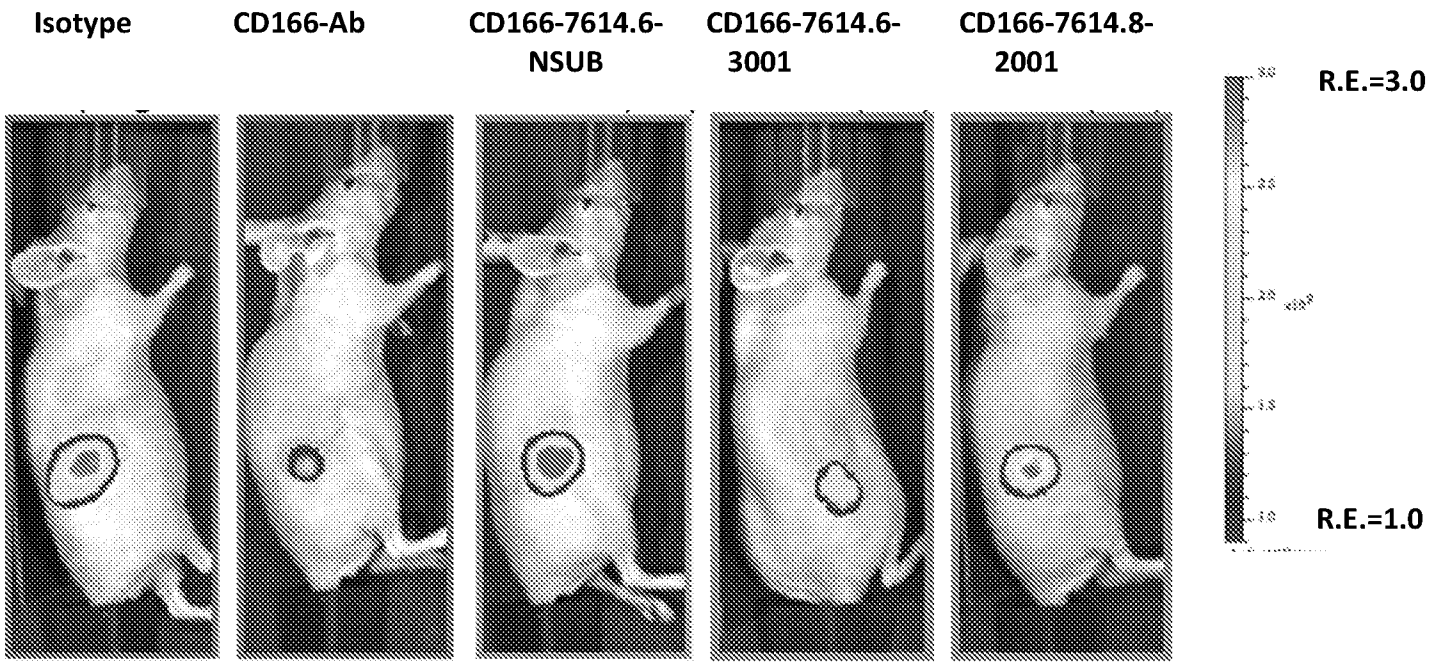


FIGURE 25B

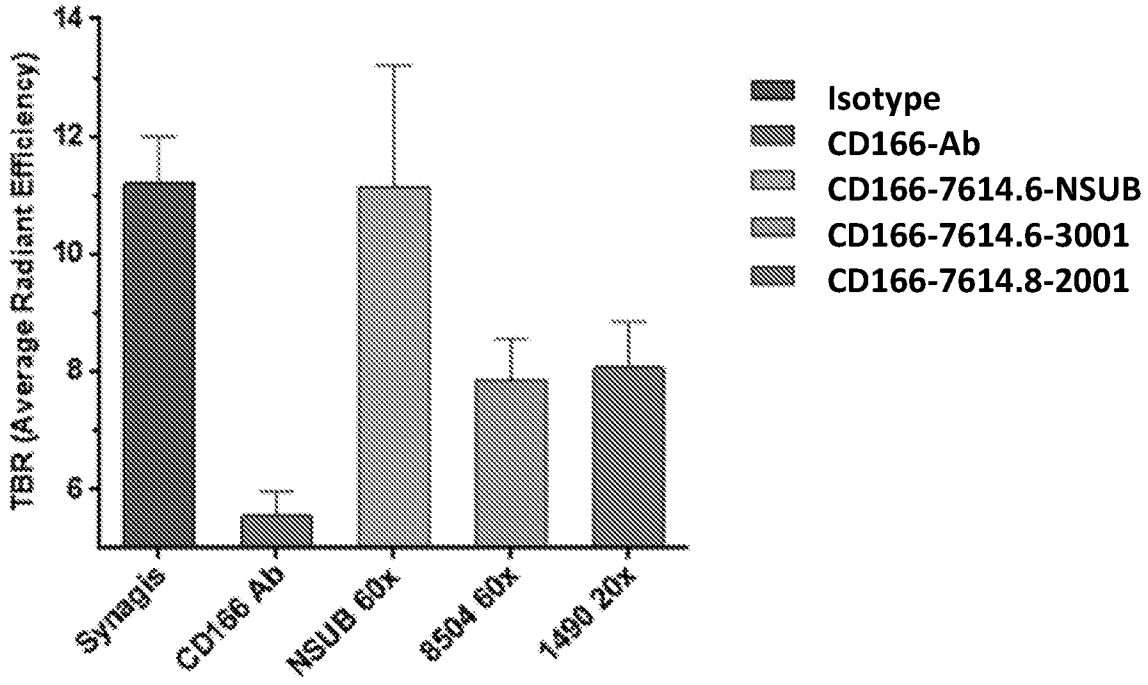


FIGURE 26

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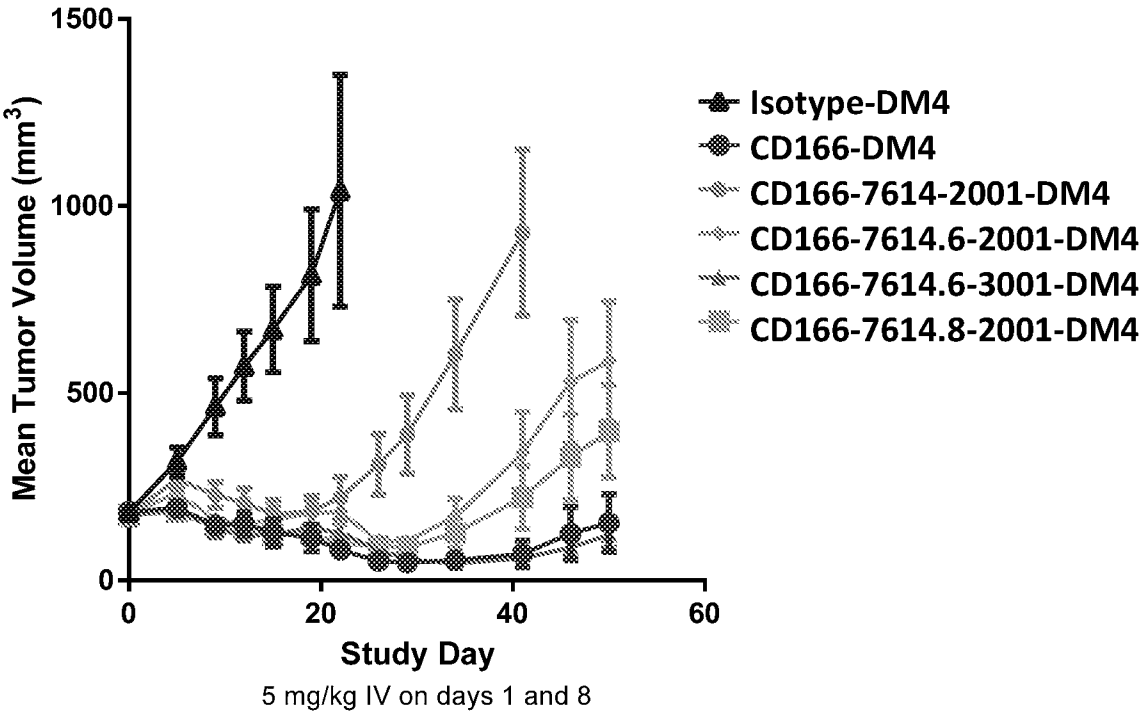

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/030785

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 C07K19/00 A61P35/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KE H ET AL: "Derivation, characterization and gene modification of cynomolgus monkey mesenchymal stem cells", DIFFERENTIATION, SPRINGER VERLAG, DE, vol. 77, no. 3, 1 March 2009 (2009-03-01), pages 256-262, XP025967596, ISSN: 0301-4681, DOI: 10.1016/J.DIFF.2008.09.021 [retrieved on 2008-12-02] page 256, paragraph 2.3 page 258, right-hand column, paragraph 2 ----- -/--	1-11,14, 15, 17-30, 33, 38-65, 68, 72-74, 77, 79-100

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 July 2016	16/08/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Brouns, Gaby

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/081173 A2 (CYTOMX THERAPEUTICS LLC [US]; STAGLIANO NANCY E [US]; WEST JAMES W [US) 15 July 2010 (2010-07-15) paragraphs [0205] - [0250]	6-11,14, 17-30, 33, 38-43, 58,64, 65,72, 82-100
A	WO 2014/197612 A1 (CYTOMX THERAPEUTICS INC [US]; KONRADI ANDREI WILLIAM; SINGH SHWETA) 11 December 2014 (2014-12-11) paragraphs [0148] - [0153]; table 4 examples 7, 8	6-11,14, 15, 17-30, 33, 38-65, 68, 72-74, 77, 79-100
A	MERETE THUNE WIIGER ET AL: "A novel human recombinant single-chain antibody targeting CD166/ALCAM inhibits cancer cell invasion in vitro and in vivo tumour growth", CANCER IMMUNOLOGY, IMMUNOTHERAPY, SPRINGER, BERLIN, DE, vol. 59, no. 11, 16 July 2010 (2010-07-16) , pages 1665-1674, XP019842235, ISSN: 1432-0851	1-100
A	PIAZZA T ET AL: "Internalization and recycling of ALCAM/CD166 detected by a fully human single-chain recombinant antibody", JOURNAL OF CELL SCIENCE, CAMBRIDGE UNIVERSITY PRESS, LONDON, GB, vol. 118, no. 7, 1 April 2005 (2005-04-01) , pages 1515-1525, XP003013680, ISSN: 0021-9533, DOI: 10.1242/JCS.02280 page 1523, right-hand column, last paragraph	1-100
A	WO 2008/117049 A1 (AFFITECH AS [NO]; OWEN DEBORAH JANE [GB]; STASSAR MARIKE [NO]) 2 October 2008 (2008-10-02)	1-100
A	WO 2009/039192 A2 (UNIV CALIFORNINA [US]; LIU BIN [US]; MARKS JAMES D [US]) 26 March 2009 (2009-03-26)	1-100

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	STRASSBERGER VERENA ET AL: "A comprehensive surface proteome analysis of myeloid leukemia cell lines for therapeutic antibody development", JOURNAL OF PROTEOMICS, vol. 99, 30 January 2014 (2014-01-30), pages 138-151, XP028625153, ISSN: 1874-3919, DOI: 10.1016/J.JPROT.2014.01.022 figure 7	40-100
A	WO 2012/155021 A1 (NANOVALENT PHARMACEUTICALS INC [US]; UNIV CALIFORNIA [US]; NAGY JON O) 15 November 2012 (2012-11-15)	40-100
A	ORAN ERSTER ET AL: "Site-specific targeting of antibody activitymediated by disease-associated proteases", JOURNAL OF CONTROLLED RELEASE, ELSEVIER, AMSTERDAM, NL, vol. 161, no. 3, 17 May 2012 (2012-05-17), pages 804-812, XP028409044, ISSN: 0168-3659, DOI: 10.1016/J.JCONREL.2012.05.035 [retrieved on 2012-05-23]	6-100
A	KRISHNA R POLU ET AL: "Probody therapeutics for targeting antibodies to diseased tissue", EXPERT OPINION ON BIOLOGICAL THERAPY, vol. 14, no. 8, 1 August 2014 (2014-08-01) , pages 1049-1053, XP055228738, ASHLEY, LONDON; GB ISSN: 1471-2598, DOI: 10.1517/14712598.2014.920814	1-100
T	Annie Yang Weaver ET AL: "Development of a ProbodyTM Drug Conjugate (PDC) Targeting CD166 for the Treatment of Multiple Cancers", , 4 November 2015 (2015-11-04), XP055291290, Retrieved from the Internet: URL:http://cytomx.com/wp-content/uploads/2015/11/20151104_CD166_AACR_NCI_EORTC_poster_TO_PRINT_FINAL.pdf [retrieved on 2016-07-26]	

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	ANNIE YANG WEAVER ET AL: "Abstract C165: Development of a probody drug conjugate (PDC) against CD166 for the treatment of multiple cancers", MOLECULAR CANCER THERAPEUTICS, vol. 14, no. 12 Supplement 2, 1 December 2015 (2015-12-01), pages C165-C165, XP055291304, US ISSN: 1535-7163, DOI: 10.1158/1535-7163.TARG-15-C165 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2016/030785

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