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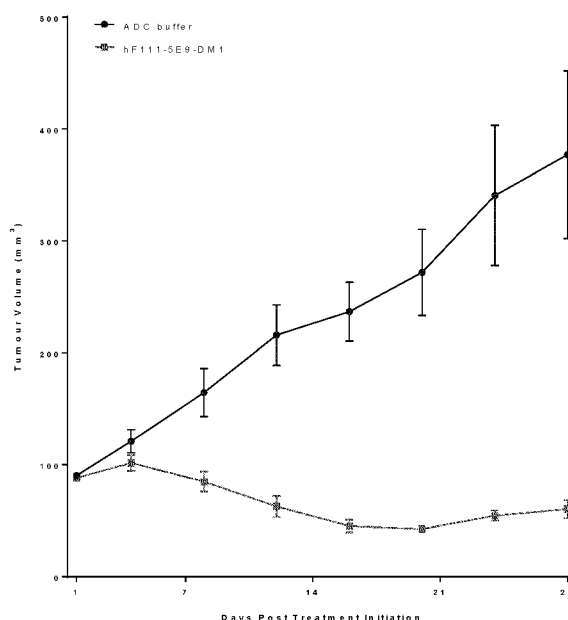


FIG. 18

(57) **Abstract:** AXL-specific antibodies and uses therefor are described, including monoclonal and single domain antibodies. Such antibodies bind to cell surface expressed human AXL at an epitope in an immunoglobulin-like (IgL) domain of the AXL ectodomain. The antibody may be used in an antibody-drug conjugate (ADC), for example in the treatment, detection or staging of cancer. The antibody may be biparatopic.



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AXL-SPECIFIC ANTIBODIES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Patent
5 Application No. 62/557,870 filed September 13, 2017, which is hereby incorporated by
reference.

FIELD

[0002] The present disclosure relates to AXL-specific antibodies and uses thereof.
10 Such antibodies bind to cell surface expressed human AXL and may be used in fusions or in
antibody-drug conjugates.

BACKGROUND

[0003] AXL is a member of the Tyro3- AXL -Mer (TAM) receptor tyrosine kinase
15 subfamily. A schematic of AXL domain structure is shown in the Prior Art illustration of
Figure 1. The extracellular domain of the AXL protein (100) is shown with exons (1 to 20)
indicated. The AXL protein (100) comprises 2 immunoglobulin-like domains (102) and 2
fibronectin type III (FNIII) repeats (104). A transmembrane domain (106) spans the cell
membrane, followed by the intracellular kinase domain (108). Splicing (indicated at 110)
20 yields a slightly shorter transcript.

[0004] Following binding of the AXL extracellular domain to Growth arrest-specific 6
(Gas6), a vitamin K-dependent protein, AXL is capable of transducing signals involved in
several cellular functions including growth, migration, aggregation, and anti-inflammation in
multiple cell types (Holland et al., 2005; Li et al., 2009). AXL was originally identified as an
25 oncogene in chronic myelogenous leukemia (O'Bryan et al., 1991). Subsequently,
expression of AXL has been reported to be upregulated in a variety of cancers including
breast, gastric, prostate, ovarian, and lung (Linger et al., 2008; Paccez et al., 2014).

[0005] AXL has also been implicated in epithelial-to-mesenchymal transition
(Gjerdrum et al., 2010), a process closely linked with invasive motility and metastasis of
30 malignant cells. Moreover, AXL expression has been shown to be negatively associated with
patient survival (Linger et al., 2008; Gjerdrum et al., 2010). Several recent studies have
reported that AXL is overexpressed and activated in several drug-resistant cancer cell lines
(Asiedu et al., 2014; Meyer et al., 2013; Thomson et al., 2011), suggesting that AXL may

play a role in resistance to chemotherapy and other molecularly targeted therapies. AXL expression has also recently been shown to be increased during hypoxia (Rankin et al., 2014), a condition that frequently occurs as solid tumors become larger and more aggressive. Taken together, these data suggest AXL as a promising diagnostic, prognostic, and therapeutic target for cancer.

[0006] Recent studies using gene knock-out or RNAi validate the therapeutic value of AXL inhibition in cell line and mouse models. In addition, several small molecule AXL inhibitors have been developed and marketed including: BOSUTINIB™, a protein-tyrosine kinase inhibitor sold by Pfizer Inc. under the name BOSULIF™, also known as SKI-606, bearing Pfizer Inc. product number PF-5208763 (Lee et al., 2014); CABOZANTINIB™, a small molecule inhibitor of the tyrosine kinases c-Met and VEGFR2 sold by Exelixis under the name COMETRIQ™, product number XL184 (Yakes et al., 2011); and SUNITINIB™, sold by Pfizer Inc. under the name SUTENT™, product number SU11248 (Kitagawa et al., 2013). These drugs target multiple kinases, and inhibit AXL activity less effectively than their primary kinase targets (Feneyrolles et al., 2014).

[0007] Other small molecule drugs are in earlier phases of clinical and preclinical development, including BGB324™ (formerly known as R428) of BergenBio (Bergen, Norway) and Rigel Pharmaceuticals (San Francisco, CA, USA), currently one of the most potent and selective inhibitors of AXL. It was shown to have an IC50 of 14 nM and to be considerably more selective for AXL than other kinases tested (Holland et al., 2010); however, it still shows activity for Tie-2, Flt-4, Flt-1, Ret, Abl as well as for the other members of the TAM family, Tyro-3 and Mer. It blocks AXL's ability to promote angiogenesis and metastasis in preclinical breast animal models (Holland et al., 2010) and entered clinical phase I in 2013 for treatment of aggressive and metastatic cancers. Development of small molecule AXL inhibitors has been challenging in the absence of a three-dimensional structure of the AXL kinase domain, and the potential for off-target toxicities remains a major concern.

[0008] To circumvent some of the drawbacks of small molecules, anti-AXL antibodies may be considered. Li et al. (2009) developed a monoclonal antibody (12A11) against AXL that did not interfere with Gas6 binding, inhibited AXL activation, induced AXL downregulation, and reduced growth of tumor xenografts in a subcutaneous non-small cell lung cancer (NSCLC) model. Subsequently, Ye et al. (2010) developed a monoclonal antibody (YW327.6S2) that recognizes the extracellular domain of both human and murine AXL and blocks Gas6 binding. This antibody has been shown to diminish xenograft tumor

growth in NSCLC models as well as orthotopic metastasis models of breast cancer. Additionally, treatment with YW327.6S2 enhanced the effects of erlotinib and chemotherapy in reducing NSCLC tumor growth as well as potentiated anti-VEGF treatment in breast cancer and NSCLC mouse xenograft models. Leconet et al. (2014) describe anti-AXL monoclonal antibodies that do not interfere with Gas6 binding, inhibit AXL activation, and induced its downregulation from the cell surface. The subject antibodies reduced growth of both subcutaneous and orthotopic pancreatic tumor xenografts in vivo.

[0009] One drawback of therapeutic antibodies that reduce the activity of AXL is that they are likely to encounter therapeutic resistance, of either an innate nature due to intrinsic lack of sensitivity, or of an acquired nature due to bypass mechanisms that arise during selective pressure of treatment. Therefore, there is a need for AXL inhibitors that are active and specific with fewer off-target effects, and with reduced or acceptable toxicity and therapeutic resistance.

[0010] Antibody-drug conjugates are highly complex entities that combine an antibody, typically via a linker, with a drug. An antibody-drug conjugate in development by Genmab A/S (Copenhagen, Denmark) and Seattle Genetics (Washington, USA), HUMAX-AXL-ADC TM, comprises an AXL-specific monoclonal antibody that does not compete with Gas6 binding conjugated to monomethyl auristatin E (MMAE). HUMAX-AXL-ADC TM potently induced tumor regression in NSCLC xenograft models after a single dose of 1 mg/kg, and had similar effects in patient-derived xenograft models in which AXL expression was heterogeneous. Antibodies binding to AXL, which do not compete with Gas6 binding are described in International Patent Publication WO2016/005593 A1 (Breij et al.).

[0011] An antibody-drug conjugate (ADC) approach, in which an anti-AXL antibody is used to deliver a potent cytotoxic drug, increases the patient population that is likely to respond to the drug. An ADC may be of use even in the event of acquired resistance to antibodies.

[0012] There is a need for AXL-specific antibodies for use in disease detection, staging and/or therapy.

SUMMARY

[0013] It is an object of the present disclosure to provide AXL-specific antibodies. AXL-specific monoclonal and single domain antibodies and antigen-binding fragments also referred to as antibodies are described, as well as uses therefor. Such AXL-specific

antibodies bind to cell surface-expressed human AXL. Antibody-drug conjugates and fusions are described.

[0014] The present disclosure provides an isolated or purified antibody that specifically binds to an epitope in an immunoglobulin-like (IgL) domain of human AXL ectodomain (ECD).

[0015] There is also provided an antibody or fragment thereof comprising variable light chain (VL) CDR1, CDR2, and CDR3 sequences selected from the group consisting of: SEQ ID NO:98-100; SEQ ID NO:92-94; SEQ ID NO:110-112; SEQ ID NO:86-88; SEQ ID NO:80-82; SEQ ID NO:104-106; SEQ ID NO:116-118; and sequences substantially identical thereto.

[0016] There is provided herein an antibody or fragment thereof comprising a variable light chain (VL) sequence selected from the group consisting of: SEQ ID NO:38; SEQ ID NO:40; SEQ ID NO:42; SEQ ID NO:44; SEQ ID NO:46; SEQ ID NO:74; SEQ ID NO:76; and sequences substantially identical thereto.

[0017] Further, there is provided an antibody or fragment thereof comprising variable heavy chain (VH) CDR1, CDR2, and CDR3 sequences selected from the group consisting of: SEQ ID NO:95-97; SEQ ID NO:89-91; SEQ ID NO:107-09; SEQ ID NO:83-85; SEQ ID NO:77-79; SEQ ID NO:101-103; SEQ ID NO:113-115; and sequences substantially identical thereto.

[0018] There is provided herein an antibody or fragment thereof comprising a variable heavy chain (VH) sequence selected from the group consisting of: SEQ ID NO:39; SEQ ID NO:41; SEQ ID NO:43; SEQ ID NO:45; SEQ ID NO:47; SEQ ID NO:73; SEQ ID NO:75; and sequences substantially identical thereto.

[0019] Further, there is provided an antibody or fragment thereof comprising a single domain (sd) heavy chain variable (VHH) domain sequence selected from the group consisting of: SEQ ID NOs:119-126; and sequences substantially identical thereto.

[0020] There is also provided an antibody or fragment thereof selected from the group consisting of: VH of SEQ ID NO:39, and VL of SEQ ID NO:38; VH of SEQ ID NO:41, and VL of SEQ ID NO:40; VH of SEQ ID NO:43, and VL of SEQ ID NO:42; VH of SEQ ID NO:45, and VL of SEQ ID NO:44; VH of SEQ ID NO:47, and VL of SEQ ID NO:46; VH of SEQ ID NO:73, and VL of SEQ ID NO:74; VH of SEQ ID NO:75, and VL of SEQ ID NO:76; VHH of SEQ ID NO:119; VHH of SEQ ID NO:120; VHH of SEQ ID NO:121; VHH of SEQ ID

NO:122; VHH of SEQ ID NO:123; VHH of SEQ ID NO:124; VHH of SEQ ID NO:125; VHH of SEQ ID NO:126; and sequences having 85% or greater identity thereto.

[0021] Antibody drug conjugates (ADCs), pharmaceutical compositions, and fusion proteins are described, comprising the described antibodies. Nucleic acid molecules
5 encoding the described antibodies, and vectors or host cells comprising same are also provided.

[0022] Methods of in vitro and in vivo detecting of AXL are described. Methods of transporting molecules of interest into cells expressing AXL are described. Methods and
10 uses of the described antibodies for treating cancer, employing antibody drug conjugates are provided.

[0023] Other aspects and features of the present disclosure will become apparent to those ordinarily skilled in the art upon review of the following description of specific embodiments in conjunction with the accompanying figures.

15 BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Embodiments of the present disclosure will now be described, by way of example only, with reference to the attached Figures.

[0025] **Figure 1** (Prior Art) is a schematic depiction of AXL domain structure.

[0026] **Figure 2** shows results of the effect of anti-AXL mAbs on A549 cell survival.

20 [0027] **Figure 3** shows results of flow cytometry experiments determining the binding properties of anti-AXL mAb to cell surface-expressed AXL on H292 cells.

[0028] **Figure 4** shows results of the activity of anti-AXL-DM1 conjugates on cell viability in a H292 tumor cell line, a A549 cell line, human breast adenocarcinoma MDA-MB-231 tumor cell line, and immortalized HaCaT keratinocyte cells.

25 [0029] **Figure 5** is a schematic representation of the human AXL protein ectodomain (hAXL-ECD) and transmembrane domain. Domain binding is shown for ten peptide fragments of the hAXL-ECD.

[0030] **Figure 6** is a schematic representation of the strategy for isolation of anti-rhAXL ECD single domain antibodies (sdAbs).

30 [0031] **Figure 7** shows amino acid sequences of the mAb V_H/V_L domains with CDRs using IMGT definitions, shown in bold underlined font.

[0032] **Figure 8** shows amino acid sequences of the VHHs with CDRs, using IMGT definitions, represented in bold underlined font.

[0033] Figure 9 shows antibody binding capacity in lung (H292), breast (MDA-MB-231), ovarian (SKOV3) and glioblastoma (U87) cell lines.

[0034] Figure 10 shows the results of flow cytometry experiments determining the binding properties of anti-AXL hlgG and sdAbs in lung (NCI-H292), breast (MDA-MB-231), and ovarian (SKOV3) cell lines.

[0035] Figure 11 shows growth inhibition binding curves of hlgG Abs and sdAbs in three tumor cell lines

[0036] Figure 12 shows growth inhibition binding curves of hlgG-F11-5E9-DM1 and hlgG-F107-10G1-DM1 in the presence and absence of Gas6 in H292 cell line.

[0037] Figure 13 shows growth inhibition binding curves of anti-AXL biparatopic construct ADCs compared to monoparatopic parental ADCs in SKOV3 cell line.

[0038] Figure 14 shows tumor growth inhibition in SKOV3 tumor-bearing mice by selected test antibodies.

[0039] Figure 15 shows tumor growth inhibition in SKOV3 tumor-bearing mice by selected test antibodies.

[0040] Figure 16 shows the study design and time points for tumor growth measurements in MDA-MB-231-Luc tumor-bearing mice in Example 17.

[0041] Figure 17 shows the effect of hF111-5E9-DM1 on body weight up to 37 days post-treatment initiation in Example 17.

[0042] Figure 18 shows the effect of hF111-5E9-DM1 on tumor growth up to 28 days post-treatment initiation in Example 17.

[0043] Figure 19 shows the Papp values of FC5Hfc1X0 (positive control), TWIN200-hlgG1 (negative control) and seven anti-AXL sdAbs evaluated in Example 18.

DETAILED DESCRIPTION

[0044] Generally, the present disclosure provides anti-AXL monoclonal and single domain antibodies for use in treatment, identification/diagnosis, and staging/prognosis of diseases, such as cancer.

[0045] An isolated or purified antibody is described, which specifically binds to an epitope in an immunoglobulin-like (IgL) domain of human AXL ectodomain (ECD). The AXL ECD may, for example, have the amino acid sequence of SEQ ID NO:71. The epitope of the antibody may be located in IgL1 or IgL2 of AXL. The antibody may be capable of internalization into AXL expressing tumor cells.

[0046] The antibody can be a single domain antibody, a monoclonal antibody, a humanized antibody, a chimeric antibody, a biparatopic antibody, a bispecific antibody, or may be an AXL-binding antibody fragment thereof. If the antibody is a chimeric antibody, it may comprise framework regions from human IgG1, and may comprise framework regions
5 from human kappa light chain and human IgG heavy chain. The antibody may comprise framework regions from IgA, IgD, IgE, IgG, or IgM.

[0047] The antibody may be a full-length IgG, Fv, scFv, Fab, or F(ab')₂, VHH, VL, or VH. The antibody may have an equilibrium dissociation constant (KD) of < 50 nM to human AXL ectodomain. Optionally, the binding to the epitope in an IgL domain of AXL ECD may be
10 one that does not compete with the binding of Growth arrest-specific 6 (Gas6) ligand to AXL.

[0048] The antibody or fragment may have variable light chain (VL) CDR1, CDR2, and CDR3 sequences selected from the group consisting of: SEQ ID NO:98, 99, and 100; SEQ ID NO:92, 93 and 94; SEQ ID NO:110, 111, and 112; SEQ ID NO:86, 87, and 88; SEQ ID NO:80, 81, and 82; SEQ ID NO:104, 105, and 106; SEQ ID NO:116, 117, and 118; and
15 sequences substantially identical thereto.

[0049] The antibody or fragment thereof may be one comprising a variable light chain (VL) sequence selected from the group consisting of: SEQ ID NO:38; 40; 42; 44; 46; 74; 76; and sequences substantially identical thereto. The antibody or fragment may comprise variable heavy chain (VH) CDR1, CDR2, and CDR3 sequences selected from the group
20 consisting of: SEQ ID NO:95-97; 89-91; 107-109; 83-85; 77-79; 101-103; 113-115; and sequences substantially identical thereto.

[0050] An antibody or fragment thereof is described, which comprises a variable heavy chain (VH) sequence selected from the group consisting of: SEQ ID NO:39; 41; 43; 45; 47; 73; 75; and sequences substantially identical thereto.

[0051] The antibody or fragment thereof may be one comprising variable heavy chain (VL) CDR1, CDR2, and CDR3 sequences; and (VH) CDR1, CDR2, and CDR3 sequences selected from the group consisting of: VL, SEQ ID NO:98-100 and VH, SEQ ID NO:95-97; VL, SEQ ID NO:92-94 and VH, SEQ ID NO:89-91; VL, SEQ ID NO:110-112 and VH, SEQ ID NO:107-109; VL, SEQ ID NO:86-88 and VH, SEQ ID NO:83-85; VL, SEQ ID NO:80-82 and
25 VH, SEQ ID NO:77-79; VL, SEQ ID NO:104-106 and VH, SEQ ID NO:101-103; VL, SEQ ID NO:116-118 and VH, SEQ ID NO:113-115; and sequences substantially identical thereto.

[0052] The antibody described may comprise single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences selected from the group

consisting of: SEQ ID NO:127-129; SEQ ID NO:130-132; SEQ ID NO:133-135; SEQ ID NO:136-138; SEQ ID NO:139-141; SEQ ID NO:142-144; SEQ ID NO:145-147; SEQ ID NO:148-150; and sequences substantially identical thereto.

[0053] An antibody or fragment thereof is described which has a single domain (sd)

5 heavy chain variable (VHH) domain sequence selected from the group consisting of any one of: SEQ ID NOs:119 to 126; and sequences substantially identical thereto.

[0054] An antibody or fragment thereof is described, which is selected from the group consisting of: VH of SEQ ID NO:39, and VL of SEQ ID NO:38; VH of SEQ ID NO:41, and VL of SEQ ID NO:40; VH of SEQ ID NO:43, and VL of SEQ ID NO:42; VH of SEQ ID NO:45, and VL of SEQ ID NO:44; VH of SEQ ID NO:47, and VL of SEQ ID NO:46; VH of SEQ ID NO:73, and VL of SEQ ID NO:74; VH of SEQ ID NO:75, and VL of SEQ ID NO:76; and the VHH sequences from 119-126, as well as sequences having 85% or greater identity thereto.

[0055] For example, the antibody or fragment thereof may have a sequence identity of >90%, >95%, or > 98% as compared with specifically exemplified sequences listed herein.

15 **[0056]** The described antibody may comprise light chain CDR1, CDR2 and CDR3 sequences of SEQ ID NO:1, 2, and 11; and heavy chain CDR1, CDR2 and CDR3 sequence of SEQ ID NO:3, 4, and 5; wherein the antibody binds an epitope in the IgL1 domain and not in the IgL2 domain of AXL. The antibody may comprise light chain CDR1, CDR2 and CDR3 sequences of SEQ ID NO:1, 6, and 11; and heavy chain CDR1, CDR2 and CDR3 sequence of SEQ ID NO:7, 8, and 5; wherein the antibody binds an epitope in the IgL1 domain and not in the IgL2 domain of AXL.

[0057] The antibody may comprise light chain CDR1, CDR2 and CDR3 sequences of SEQ ID NO:9, 10, and 11; and heavy chain CDR1, CDR2 and CDR3 sequence of SEQ ID NO:12, 13, and 14.

25 **[0058]** Alternatively, the antibody may comprise light chain CDR1, CDR2 and CDR3 sequences of SEQ ID NO:15, 16, and 11; and heavy chain CDR1, CDR2 and CDR3 sequence of SEQ ID NO:17, 18, and 19.

[0059] The antibody may be one which does not compete with Gas6 for AXL binding.

[0060] The antibody may be one that is biparatopic. Such a biparatopic antibody
30 may be, for example: sdAb001/005 or sdAb001/006.

[0061] An antibody drug conjugate (ADC) is described which comprises the antibody described herein, which is conjugated to a drug moiety. The drug moiety may be, for example, an anti-cancer drug. An exemplary drug moiety is DM1.

[0062] A pharmaceutical composition is described comprising an ADC and a pharmaceutically acceptable excipient.

[0063] A fusion protein is described, comprising the antibody described herein and human IgG1 Fc.

5 **[0064]** A nucleic acid molecule is described, which encodes the antibody. A vector comprising a nucleic acid molecule is described. The viral vector may be adenoviral, lentiviral or retroviral.

[0065] A host cell is described, which may comprise the vector noted above. The host cell may be a recombinant microbial host cell or may be a mammalian cell, and said cell
10 may produce the antibody described herein.

[0066] A hybridoma or an organism that produces the antibody is also provided.

[0067] In some instances, the antibody may be immobilized onto a surface. Further, the antibody may be linked to a cargo molecule. Optionally, the cargo molecule may be a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a
15 cytokine, a receptor trap, an antibody thereof, a chemical compound, a carbohydrate moiety, a DNA-based molecule, a cytotoxic agent, or a viral vector.

[0068] When the antibody has a cargo molecule, an exemplary size of the cargo molecule may be from about 1 to about 500 kDa. The cargo molecule can be loaded in a liposome or nanocarrier. Such a nanocarrier may comprise one or more nanoparticle,
20 nanowire, nanotube, or quantum dots. The cargo molecule may be a cytotoxic agent.

[0069] The DNA-based molecule may comprise an anti-sense oligonucleotide, microRNA, siRNA, or a plasmid.

[0070] An in vitro method of detecting AXL is described, comprising: a) contacting a sample with one, or more than one, isolated or purified antibody linked to a detectable agent;
25 and b) detecting the detectable agent linked to the antibody thereof bound to AXL in the sample.

[0071] A method is described for detecting AXL in circulating cells where the sample may be a serum sample.

[0072] The step of detecting, in such a method, may be performed using optical
30 imaging, immunohistochemistry, molecular diagnostic imaging, or ELISA.

[0073] There is described herein an in vivo method of detecting AXL expression in a subject, comprising: a) administering to the subject one or more antibody, as described

herein, linked to a detectable agent; and b) detecting the detectable agent linked to the antibody thereof bound to AXL.

[0074] Optionally, the step of detecting may be performed using PET, SPECT, or fluorescence imaging.

5 **[0075]** A method of transporting a molecule of interest into cells expressing AXL is described, comprising administering to a subject one or more antibody linked to the molecule of interest, wherein the antibody delivers the molecule of interest to the subject's cells expressing AXL. There may a molecule of interest, such as a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a cytokine, a receptor trap, an antibody
10 thereof, a chemical compound, a carbohydrate moiety, a DNA-based molecule, a cytotoxic agent, or a viral vector. In such a method, the molecule of interest may be loaded on or in one or more liposome or nanocarrier. The nanocarrier may comprise one or more nanoparticle, nanowire, nanotube, or quantum dots. Further, the molecule of interest may comprise a cytotoxic agent, such as a cytotoxic agent used for treating cancer.

15 **[0076]** A method of treating cancer is described, comprising administering an anti-cancer antibody drug conjugate (comprising the antibody described herein) to a subject in need thereof. Further, there is described herein a method of treating cancer comprising administering a cellular therapy, a chimeric antigen receptor (CAR-T cell) therapy, or an oncolytic virus.

20 **[0077]** Uses for antibody described herein may be for preparation of a medicament comprising an anti-cancer antibody drug conjugate. The antibody is useful in treating cancer in a subject in need thereof. A further use is described in which an anti-cancer antibody drug conjugate is used for treating cancer in a subject in need thereof.

[0078] The use of such an antibody is described, and the antibody may be used for
25 preparation of a cellular therapy, a chimeric antigen receptor (CAR-T cell) therapy, or an oncolytic virus.

[0079] Anti-AXL antibody drug conjugates are described herein for use as cancer therapeutics. It is desirable to provide immunotherapeutics and immunoconjugates that possess potent and selective anti-cancer activity. Antibody-drug conjugates (ADCs) for the
30 treatment of cancer based on AXL as a cancer target require development of antibodies that can function in a conjugate. AXL is in the TAM family of receptor tyrosine kinases, the overexpression of which has been detected in a wide array of human cancers. Antibodies are described herein that are suited for AXL ADC, determined in part on the basis of surrogate

ADC screening of over 250 monoclonal antibodies (mAbs) in several cell line cancer models and cross-reactivity with AXL protein from cynomolgus monkey for toxicity testing. Anti-AXL antibodies for ADC development are described, and Gas6 competition and single domain antibody (sdAb) characterization data is provided herein.

5 **[0080]** Gas6 competition. In anti-AXL ADCs, the ability of Gas6 (an AXL ligand) to block antibody binding to AXL is an important feature for AXL ADC selection. This ability is referred to herein as Gas6 competition. High concentrations of the Gas6 ligand present systemically or in the tumor microenvironment (but not found in tumor cell lines grown in culture) may account for discrepancies in antibody binding activity seen in vivo versus in
10 vitro. Thus, selection of antibodies to have activities unaffected or weakly affected by Gas6 would ensure that antibody binding is not thwarted by Gas6 in vivo.

[0081] Monoclonal Antibodies (mAbs). AXL ADCs were screened from within an anti-AXL mouse mAb library to identify potent ADC internalizers ($IC_{50} < 1 \text{ nM}$) having activities unaffected by the presence of Gas6.

15 **[0082]** Single Domain Antibodies (sdAbs). AXL ADC candidates are identified from isolated anti-AXL single domain antibodies (sdAbs, heavy chain variable domains VHH only) by panning from phage display libraries. Techniques used for assessment of sdAbs, herein, include internalization assays and competitive binding assays. Gas6 competition and sdAb characterization data was obtained. In general, single domain antibodies may be up to about
20 110 amino acids in length, comprising one variable domain (VH) of a heavy-chain antibody. sdAbs lack an Fc region, and thus sdAb-Fc fusions may be prepared.

[0083] Epitopes. Epitopes and/or binding domains are described herein.

[0084] The term “antibody”, also referred to in the art as “immunoglobulin” (Ig), as used herein refers to a protein constructed from paired heavy and light polypeptide chains;
25 various Ig isotypes exist, including IgA, IgD, IgE, IgG, and IgM. Single domain sdAb as well as monoclonal antibodies mAbs are described herein. When an antibody is correctly folded, each chain folds into a number of distinct globular domains joined by more linear polypeptide sequences. For example, the immunoglobulin light chain folds into a variable (VL) and a constant (CL) domain, while the heavy chain folds into a variable (VH) and three constant
30 (CH, CH2, CH3) domains. Interaction of the heavy and light chain variable domains (VH and VL) results in the formation of an antigen binding region (Fv). Each domain has a well-established structure familiar to those of skill in the art.

[0085] The term “antibody” may also be used to refer to a fragment of an immunoglobulin molecule, or a derivative of the molecule, which retains the ability to specifically bind to the antigen for a typical comparable time period as observed for the immunoglobulin. The binding region or binding domain of the antibody may comprise variable regions of both the heavy and light chains of the immunoglobulin molecule, or may be a single domain (VHH) molecule. The term “antibody” is used to encompass such fragments of an antibody that retain antigen-binding (which may be referred to herein as an “antibody fragment” or simply “fragment”). Fragments that retain binding ability may include Fab’ or Fab fragments, a monovalent fragment which may have VL, VH, CL and CH1 domains, F(ab’)₂ fragments, biparatopic fragments, bivalent fragments, Fd fragments having VH and CH1 domains, camelid antibodies, nanobodies, isolated complementarity determining region (CDRs), multiple fragments joined by linkers, and other such molecules.

[0086] The term “antibody” encompasses polyclonal antibodies, monoclonal antibodies, single domain antibodies, antibody-like polypeptides, humanized antibodies, chimeric antibody molecules, bispecific or biparatopic antibodies, and any fragments not mentioned here specifically which are capable of antigen-binding. The antibody fragment may be a naturally-occurring antibody fragment, may be obtained by manipulation of a naturally-occurring antibody or by using recombinant methods, and may be a non-naturally occurring antibody or fragment. For example, an antibody fragment may include, but is not limited to a Fv, single-chain Fv (scFv; a molecule consisting of VL and VH connected with a peptide linker), Fab, F(ab’)₂, and multivalent presentations of any of these. Antibody fragments such as those just described may require linker sequences, disulfide bonds, or other type of covalent bond to link different portions of the fragments; those of skill in the art will be familiar with various approaches. Single domain antibody fragments as well as monoclonal antibody fragments are encompassed by the term “antibody”.

[0087] In a non-limiting example, the antibody fragment may be an sdAb derived from naturally-occurring sources. Heavy chain antibodies of camelid origin lack light chains and thus their antigen binding sites consist of one domain, termed V_HH. sdAb have also been observed in shark and are termed V_{NAR}. Other sdAb may be engineered based on human Ig heavy and light chain sequences. As used herein, the term “sdAb” includes those sdAb directly isolated from V_H, V_HH, V_L, or V_{NAR} reservoir of any origin through phage display or other technologies, sdAb derived from the aforementioned sdAb, recombinantly produced sdAb, as well as those sdAb generated through further modification of such sdAb by

humanization, affinity maturation, stabilization, solubilization, camelization, or other methods of antibody engineering. Also encompassed are homologues, derivatives, or fragments that retain the antigen-binding function and specificity of the sdAb.

[0088] SdAb possess desirable properties for antibody molecules, such as high
5 thermostability, high detergent resistance, relatively high resistance to proteases and high production yield. They can also be engineered to have very high affinity by isolation from an immune library or by in vitro affinity maturation. Further modifications to increase stability, such as the introduction of non-canonical disulfide bonds may also be brought to the sdAb.

[0089] The structure of a single-domain antibody is established and well known. An
10 sdAb comprises a single immunoglobulin domain that retains the immunoglobulin fold; most notably, only three CDR/hypervariable loops form the antigen-binding site. However, not all CDRs may be required for binding the antigen. For example, and without being limiting, one, two, or three of the CDRs may contribute to binding and recognition of the antigen by the sdAbs described herein. The CDRs of the sdAb or variable domain are referred to herein as
15 CDR1, CDR2, and CDR3.

[0090] The described antibodies (including fragments thereof) are specific for AXL, part of the receptor tyrosine kinase subfamily. AXL (Entrez Gene: 558) comprises 894 amino acids organized into an extracellular domain that includes 2 immunoglobulin-like domains and 2 fibronectin type III repeats; a transmembrane domain; and an intracellular domain that
20 includes the tyrosine kinase domain (see Prior Art **Figure 1**). The AXL protein binds the ligand Gas6, a vitamin K-dependent protein. AXL is normally expressed across many cell types, though expression levels are increased in a number of disease states.

[0091] The antibody should exhibit a high degree of internalization. By the term “high degree of internalization”, it is meant that the antibody shows sub nM (IC₅₀) potency in a
25 saporin- or DM1-conjugated secondary antibody cytotoxicity screening assay in more than one tumor cell line known to express AXL. The antibodies presently described bind to the extracellular domain of the cell surface AXL receptor. The antibodies may then be internalized by the cell and delivered into subcellular organelles, including endosomes and lysosomes. The antibody may also comprise a neutralization function, i.e., it may block
30 signalling through the AXL receptor.

[0092] The light and heavy chain variable regions are responsible for binding the target antigen and can therefore show significant sequence diversity between antibodies. The constant regions show less sequence diversity, and are responsible for binding a

number of natural proteins to elicit important biochemical events. The variable region of an antibody contains the antigen-binding determinants of the molecule, and thus determines the specificity of an antibody for its target antigen. The majority of sequence variability occurs in six hypervariable regions, three each per variable heavy (VH) and light (VL) domain; the hypervariable regions combine to form the antigen-binding site, and contribute to binding and recognition of an antigenic determinant. The specificity and affinity of an antibody for its antigen is determined by the structure of the hypervariable regions, as well as their size, shape, and chemistry of the surface they present to the antigen. Various schemes exist for identification of the regions of hypervariability, one of which (Kabat Scheme) defines the “complementarity-determining regions” (CDR) based on sequence variability at the antigen-binding regions of the VH and VL domains. Another (Chothia Scheme) defines the “hypervariable loops” (H or L) based on the location of the structural loop regions in the VH and VL domains. As these individual schemes define CDR and hypervariable loop regions that are adjacent or overlapping, those of skill in the antibody art often utilize the terms “CDR” and “hypervariable loop” interchangeably, and they may be so used herein. The International Immunogenetics Information System (IMGT) is used herein, with Kabat numbering also shown. The IMGT numbering system was developed to facilitate comparison of variable domains. In this system, conserved amino acids (such as Cys23, Trp41, Cys104, Phe/Trp118, and a hydrophobic residue at position 89) always have the same position. Additionally, a standardized delimitation of the framework regions (FR1: positions 1 to 26; FR2: 39 to 55; FR3: 66 to 104; and FR4: 118 to 129) and of the CDR (CDR1: 27 to 38, CDR2: 56 to 65; and CDR3: 105 to 117) is provided.

[0093] Herein, the CDR sequences noted as IMGT are identified by the descriptive names followed by –A, whereas the descriptive names of Kabat sequences are followed with –B.

[0094] The CDR/loops are referred to herein according to the Chothia scheme for CDR H1, and the Kabat scheme for all other CDR. The CDR of the antibodies described herein are referred to as CDR L1, L2, L3 for CDR in the light chain, and CDR H1, H2, H3 for CDR in the heavy chain.

[0095] SEQ ID NO:72 provides a known AXL full length His6 sequence, and is provided for information only.

[0096] In addition to causing the internalization and activation of conjugated or bound cytotoxic agents, the antibody may lead to antibody-dependent cell-mediated cytotoxicity

(ADCC) or complement-dependent cytotoxicity (CDC). As would be known to those of skill in the art, the ADCC and CDC activities are mediated through the constant domains present in various antibody structures.

[0097] The antibody may be from any source, human, mouse, or other; may be any isotype, including IgA, IgD, IgE, IgG, and IgM; and may be any type of fragment, including but not limited to Fv, scFv, Fab, F(ab')₂.

[0098] The antibody may also be chimeric, formed as a combination of protein sequences originating from more than one species. As is known to those of skill in the art, a chimeric antibody is produced by combining genetic material from a nonhuman source (for example but not limited to a mouse) with genetic material from a human. For example, human constant domains can be fused to mouse VH and VL sequences.

[0099] The antibody may be "humanized" using any suitable method known in the art, for example, but not limited to CDR grafting and veneering. Humanization of an antibody comprises replacing an amino acid in the sequence with its human counterpart, as found in the human consensus sequence, without loss of antigen-binding ability or specificity; this approach reduces immunogenicity of the antibody when introduced into human subjects. In the process of CDR grafting, one or more than one of the heavy chain CDR defined herein may be fused or grafted to a human variable region (VH or VL), or to other human antibody (IgA, IgD, IgE, IgG, and IgM) or fragment framework regions (Fv, scFv, Fab) or proteins of similar size and nature onto which CDR can be grafted (for example, see Nicaise et al, 2004). In such a case, the conformation of said one or more than one hypervariable loop is likely preserved, and the affinity and specificity of the antibody for its target (i.e., AXL) is likely minimally affected. CDR grafting is known in the art. In one non-limiting example of such a humanized antibody, a CDR as described above from a mouse source may be grafted onto human light chain and heavy chain framework regions.

[00100] Veneering, also referred to in the art as "variable region resurfacing", involves humanizing solvent-exposed positions of the antibody. Thus, buried non-humanized residues, which may be important for CDR conformation, are preserved while the potential for immunological reaction against solvent-exposed regions is minimized. Veneering is known in the art and essentially involves replacing exposed residues in the framework region of the native antibody or fragment thereof with the amino acid residues in their human counterpart (Padlan, 1991; Gonzales et al 2005).

[00101] Persons of skill in the art would also be amply familiar with methods of preparing such humanized antibody fragments and humanizing amino acid positions. See, for example, Vincke et al., 2009. As known by those of skill in the art, it may be necessary to incorporate certain native amino acid residues into the human framework in order to retain binding and specificity. Humanization by CDR grafting is known in the art (for example, see Tsurushita et al, 2005; Jones et al, 1986; Tempest et al, 1991; Riechmann et al, 1988; Queen et al, 1989; Gonzales et al, 2005), and thus persons of skill would be amply familiar with methods of preparing such humanized antibody or fragments thereof.

[00102] An isolated or purified antibody specific for AXL that may be a chimeric antibody comprising the VH and VL as defined above and a human IgG1 framework. For example, the human IgG1 framework may comprise a human kappa light chain and a human IgG1 heavy chain.

[00103] Monoclonal Antibodies

[00104] High-affinity monoclonal antibodies (mAb) directed against AXL are described. The mAbs were generated by genetic immunization (i.e., by injecting DNA that encodes rhAXL-ECD). The mAb were screened using a functional assay designed to identify antibodies that exhibit a high degree of internalization.

[00105] Five anti-hAXL mAb identified were functionally characterized for potential as an antibody-drug conjugate (ADC). These antibodies are designated herein as: F107-7H5, F107-8D12, F111-5E9, F111-3C8, and F107-10G1. These anti-human AXL antibodies were selected because they showed sub nM (IC₅₀) potency in a saporin-conjugated secondary antibody-based cytotoxicity screening assay in at least two of the following cell lines: U87 glioblastoma, A549 alveolar adenocarcinoma, and MDA-MB-231 breast adenocarcinoma. They also showed toxicity on H292 lung carcinoma cells, A549 cells and MDA-MB-231 cells, when conjugated to DM1. Epitope binning analyses indicated that the selection of these antibodies, which was based on potency in the conjugated secondary antibody screening assay, resulted in all five mAb binding to the following epitopes: two located in the Ig-like domain 1 (F107-7H5 and F107-8D12) and one in Ig-like domain 2 (F111-3C8 and F111-5E9) of AXL. It is noted that binding of F107-10G1 is also in the Ig-like domain 1, but at a site distinct from that of F107-7H5 and F107-8D12.

[00106] Sequence Identity and Characteristics

[00107] A substantially identical sequence may comprise one or more conservative amino acid mutations. It is known in the art that one or more conservative amino acid mutations to a reference sequence may yield a mutant peptide with no substantial change in physiological, chemical, physico-chemical or functional properties compared to the reference sequence; in such a case, the reference and mutant sequences would be considered “substantially identical” polypeptides. A conservative amino acid substitution is defined herein as the substitution of an amino acid residue for another amino acid residue with similar chemical properties (e.g. size, charge, or polarity). These conservative amino acid mutations may be made to the framework regions of the antibody while maintaining the CDR sequences listed above and the overall structure of the antibody; thus the specificity and binding of the antibody are maintained.

[00108] In a non-limiting example, a conservative mutation may be an amino acid substitution. Such a conservative amino acid substitution may substitute a basic, neutral, hydrophobic, or acidic amino acid for another of the same group. By the term “basic amino acid” it is meant hydrophilic amino acids having a side chain pK value of greater than 7, which are typically positively charged at physiological pH. Basic amino acids include histidine (His or H), arginine (Arg or R), and lysine (Lys or K). By the term “neutral amino acid” (also “polar amino acid”), it is meant hydrophilic amino acids having a side chain that is uncharged at physiological pH, but which has at least one bond in which the pair of electrons shared in common by two atoms is held more closely by one of the atoms. Polar amino acids include serine (Ser or S), threonine (Thr or T), cysteine (Cys or C), tyrosine (Tyr or Y), asparagine (Asn or N), and glutamine (Gln or Q). The term “hydrophobic amino acid” (also “non-polar amino acid”) is meant to include amino acids exhibiting a hydrophobicity of greater than zero according to a normalized consensus hydrophobicity scale. Hydrophobic amino acids include proline (Pro or P), isoleucine (Ile or I), phenylalanine (Phe or F), valine (Val or V), leucine (Leu or L), tryptophan (Trp or W), methionine (Met or M), alanine (Ala or A), and glycine (Gly or G). “Acidic amino acid” refers to hydrophilic amino acids having a side chain pK value of less than 7, which are typically negatively charged at physiological pH. Acidic amino acids include glutamate (Glu or E), and aspartate (Asp or D).

[00109] Sequence identity is used to evaluate the similarity of two sequences; it is determined by calculating the percent of residues that are the same when the two sequences are aligned for maximum correspondence between residue positions. Any known method may be used to calculate sequence identity; for example, computer software is available to

calculate sequence identity. Sequence identity can, for example, be calculated by software such as NCBI BLAST2 service maintained by the Swiss Institute of Bioinformatics, BLAST-P, Blast-N, or FASTA-N, or any other appropriate software that is known in the art.

[00110] Substantially identical sequences, as described herein, may be at least 90% identical; in another example, the substantially identical sequences may be at least 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identical, or any percentage there between, at the amino acid level to sequences described herein. Importantly, the substantially identical sequences retain the activity and specificity of the reference sequence. In a non-limiting embodiment, the difference in sequence identity may be due to conservative amino acid mutation(s). In a non-limiting example, an antibody comprising a sequence at least 85%, 90%, 95%, 98% or 99% identical to the specific sequence described herein is encompassed.

[00111] When sequences are compared, the present antibodies noted as mAbs 3C7, 8D12 and 7H5 are related to one another with about 90% similarity or greater (or $\geq 90\%$ identity). Variations in a sequence that nevertheless result in substantially identical activity would be considered substantially identical sequences.

[00112] Changes in the sequences due to alterations, such as humanization, may alter the sequences in a manner that is predictable, and understood to those of skill in the art. Thus, such altered sequences may have a greater number of altered residues so that identity is below the 90% value mentioned above. Variation in the sequence may be present in regions outside of the CDRs, or may be within the CDRs, provided that substantially identical binding is achieved.

[00113] The antibody may also comprise additional sequences to aid in expression, detection or purification of a recombinant antibody. Any such sequences or tags known to those of skill in the art may be used. For example, the antibody may comprise a targeting or signal sequence (for example, but not limited to ompA), a detection/purification tag (for example, but not limited to c-Myc, His₅, His₆, or His₈G), or a combination thereof. In another example, the signal peptide may be MVLQTQVFISLLLWISGAYG (SEQ ID NO:59) or MDWTWRILFLVAAATGTHA (SEQ ID NO:60). In a further example, the additional sequence may be a biotin recognition site. As is also known to those of skill in the art, linker sequences may be used in conjunction with the additional sequences or tags, or may serve as a detection/purification tag.

[00114] Multimerization, Linking, and Cargo

[00115] The antibody may be in a multivalent display format, also referred to herein as multivalent presentation. Multimerization may be achieved by any suitable method of known in the art. For example, and without being limiting in any manner, multimerization may be achieved using self-assembly molecules, and/or by producing pentabodies by expressing a fusion protein comprising the antibody and the pentamerization domain of the B-subunit of an AB₅ toxin family. The pentamerization domain then assembles into a pentamer. A multimer may also be formed using the multimerization domains, which may be referred to as a “combody” form to describe a fusion of the antibody, with a coiled-coil peptide resulting in a multimeric molecule. Other forms of multivalent display are also encompassed herein. For example, and without being limiting, the antibody may be presented as a dimer, a trimer, or any other suitable oligomer. This may be achieved by methods known in the art, for example with direct linking connection, with a c-jun/Fos interaction, using a knob into holes interaction.

[00116] Each subunit of the multimers described above may comprise the same or different antibodies, which may have the same or different specificity. Additionally, the multimerization domains may be linked to the antibody using a linker, as required; such a linker should be of sufficient length and appropriate composition to provide flexible attachment of the two molecules, but should not hamper the antigen-binding properties of the antibody. For example, and without being limiting in any manner, the antibody may be linked to a cargo molecule. The cargo molecule may be any suitable molecule. For example, the cargo molecule may be a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a cytokine, a receptor trap, an antibody or fragment thereof (e.g., IgG, scFv, Fab, V_HH, V_H, V_L, etc.) a chemical compound, a carbohydrate moiety, DNA-based molecules (anti-sense oligonucleotide, microRNA, siRNA, plasmid), a cytotoxic agent, viral vector (adeno-, lenti-, retro-), one or more liposomes or nanocarriers loaded with any of the previously recited types of cargo molecules, or one or more nanoparticle, nanowire, nanotube, or quantum dots. The antibody may be linked to the cargo molecule using any method known in the art (recombinant technology, chemical conjugation, etc.).

[00117] In one non-limiting example, the cargo molecule may be a detectable label, a radioisotope, a paramagnetic label such as gadolinium or iron oxide, a fluorophore, a fluorescent agent, Near Infra-Red (NIR) fluorochrome or dye such as Cy5.5, an echogenic microbubble, an affinity label (for example biotin, avidin, etc), a detectable protein-based molecule, nucleotide, quantum dot, nanoparticle, nanowire, or nanotube or any other suitable agent that may be detected by imaging methods. In a specific, non-limiting example, the anti-

AXL antibody may be linked to a near infrared fluorescence (NIRF) imaging dye, for example Cy5.5, Alexa680, Dylight680, or Dylight800.

[00118] The antibodies described herein may be used in cellular therapies, in chimeric antigen receptor (CAR-T cell) therapy, in oncolytic viruses, and for preparation of these and in other therapeutic compositions.

[00119] In another specific, non-limiting embodiment, the antibody is linked to a drug, thus providing an antibody-drug conjugate (ADC). The drug may be any type of drug, for example but not limited to a cytotoxic or chemotherapeutic agent. The cytotoxic or chemotherapeutic agent may include, but is not limited to microtubule inhibiting agents (such as maytansines and auristatins), DNA damaging agents (such as calicheamicin and duocarmyidin), RNA polymerase inhibitors (such as alpha-amantin), anti-metabolites, agents that react with DNA (such as alkylating agents, coordination compounds, platinum complexes, DNA cross-linking compounds, etc.), inhibitors of transcription enzymes, tyrosine kinase inhibitors, topoisomerase inhibitors, DNA minor-groove binding compounds, antimitotic agents (such as vinca alkyloids and taxanes), antitumor antibiotics, hormones, enzymes, and other potent drugs. As is known to those of skill in the art, the antibody-drug conjugate allows for targeted delivery of a drug, thus limiting systemic exposure. In this construct, the antibody binds to the extracellular domain of the cell surface AXL receptor; the drug linked to the antibody is thus internalized and delivered intracellularly. The drug is then either active in the cell directly or is first activated via the endosome and can then act on the cell.

[00120] The cargo molecule as described herein may be linked, also referred to herein as “conjugated”, to the antibody by any suitable method known in the art. For example, and without being limiting, the cargo molecule may be linked to the peptide by a covalent bond or ionic interaction. The linkage may be achieved through a chemical cross-linking reaction, or through fusion using recombinant DNA methodology combined with any peptide expression system, such as bacteria, yeast or mammalian cell-based systems. When conjugating the cargo molecule to the antibody, a suitable linker may be used. Methods for linking an antibody to a cargo molecule such as a therapeutic or detectable agent would be well-known to a person of skill in the art.

[00121] Nucleic acid sequences encoding the molecules as described herein are also encompassed. Given the degeneracy of the genetic code, a number of nucleotide sequences would have the effect of encoding the desired polypeptide, as would be readily understood

by a skilled artisan. The nucleic acid sequence may be codon-optimized for expression in various micro-organisms. Vectors comprising the nucleic acids as just described are also encompassed. Furthermore, cells comprising the nucleic acid and/or vector as described are encompassed.

5 **[00122]** Isolated or purified antibodies may be immobilized onto a surface using various methodologies; for example, the antibody may be linked or coupled to the surface via His-tag coupling, biotin binding, covalent binding, adsorption, or by other routes.

Immobilization of the antibody may be useful in various applications for capturing, purifying or isolating proteins. The solid surface may be any suitable surface, for example, but not limited
10 to the well surface of a microtiter plate, channels of surface plasmon resonance (SPR) sensorchips, membranes, beads (such as magnetic-based or sepharose-based beads or other chromatography resin), glass, plastic, stainless steel, a film, or any other useful surface such as nanoparticles, nanowires and cantilever surfaces. A purified antibody immobilized onto a surface may be used in a variety of methods, including diagnostic methods.

15 **[00123]** An in vitro method of detecting AXL is described, comprising contacting a sample with one or more than one isolated or purified antibody linked to a detectable agent. The AXL-antibody complex can then be detected using detection and/or imaging technologies known in the art. The sample in the method as just described may be any suitable tissue sample, for example but not limited to a serum sample, a vascular tissue
20 sample, or a tumour tissue sample; the sample may be from a human or animal subject. The step of contacting is done under suitable conditions, known to those skilled in the art, for formation of a complex between the antibody and AXL. The step of detecting may be accomplished by any suitable method known in the art, for example, but not limited to optical imaging, immunohistochemistry, molecular diagnostic imaging, ELISA, or other suitable
25 method. For example, the isolated or purified antibody linked to a detectable agent may be used in immunoassays (IA) including, but not limited to enzyme IA (EIA), ELISA, "rapid antigen capture", "rapid chromatographic IA", and "rapid EIA". In a specific, non-limiting embodiment, the in vitro method is for detection of AXL in circulating cells and the sample is a serum sample.

30

[00124] AXL Detection and Disease Diagnosis

[00125] An in vivo method of detecting AXL expression in a subject is described. The method comprises administering one or more than one isolated or purified antibody linked to

a detectable agent to the subject, then detecting the labelled antibody bound to AXL. The step of detecting may comprise any suitable method known in the art, for example, but not limited to PET, SPECT, or fluorescence imaging, or any other suitable method. The method as just described may be useful in detecting the expression of AXL in tissues, for example but not limited to tumor tissues.

[00126] The in vivo detection step in the methods described above may be whole body imaging for diagnostic purposes or local imaging at specific sites, such as but not limited to sites of solid tumor growth, in a quantitative manner to assess the progression of disease or host response to a treatment regimen. The detection step in the methods as described above may be immunohistochemistry, or a non-invasive (molecular) diagnostic imaging technology including, but not limited to: optical imaging; positron emission tomography (PET), wherein the detectable agent is an isotopes such as ^{11}C , ^{13}N , ^{15}O , ^{18}F , ^{64}Cu , ^{62}Cu , ^{124}I , ^{76}Br , ^{82}Rb and ^{68}Ga , with ^{18}F being the most clinically utilized; single photon emission computed tomography (SPECT), wherein the detectable agent is a radiotracer such as $^{99\text{m}}\text{Tc}$, ^{111}In , ^{123}I , ^{201}Tl , ^{133}Xe , depending on the specific application; magnetic resonance imaging (MRI), wherein the detectable agent may be, for example and not limited to gadolinium, iron oxide nanoparticles and carbon-coated iron-cobalt nanoparticles thereby increasing the sensitivity of MRI for the detection of plaques; or Contrast-Enhanced Ultrasonography (CEUS) or ultrasound, wherein the detectable agent is at least one acoustically active and gas-filled microbubble. Ultrasound is a widespread technology for the screening and early detection of human diseases, and is less expensive than MRI or scintigraphy and safer than molecular imaging modalities such as radionuclide imaging because it does not involve radiation.

[00127] Delivery of Molecules to Cells Expressing AXL

[00128] A method is described for transporting a molecule of interest into cells expressing AXL. The method comprises administering the molecule linked to an antibody to a subject. The molecule may be any desired molecule, including the cargo molecules, as previously described; the molecule may be "linked" to the antibody using any suitable method, including, but not limited to conjugation or expression as a fusion protein. The administration may be by any suitable method, for example parenteral administration, including but not limited to intravenous (iv), subcutaneous (sc), and intramuscular (im)

administration. In this method, the antibody delivers the desired molecule to cells in a targeted fashion.

[00129] Compositions

5 **[00130]** Compositions are provided, which comprise one or more than one isolated or purified antibody. The composition may comprise a single antibody as described above, or may be a mixture of antibodies. Furthermore, in a composition comprising a mixture of antibodies, the antibodies may have the same specificity, or may differ in their specificities; for example, the composition may comprise antibodies specific to AXL (same or different
10 epitope). The antibody may be biparatopic. The composition may also comprise one or more antibody linked to one or more than one cargo molecule. For example, the composition may comprise one or more than one ADC as described herein.

[00131] An antibody-drug conjugate (ADC) approach, in which an anti-AXL antibody is used to deliver a potent cytotoxic drug, increases the patient population that is likely to
15 respond to the drug. Without being bound by theory, this may be due largely to the requirement for increased expression of AXL rather than a requirement for ongoing AXL activity. Another advantage is that an ADC can frequently be given in the case of acquired resistance to antibodies.

[00132] A composition in which the antibody is provided may also comprise a
20 pharmaceutically acceptable diluent, excipient, or carrier. The diluent, excipient, or carrier may be any suitable diluent, excipient, or carrier known in the art, and must be compatible with other ingredients in the composition, with the method of delivery of the composition, and is not deleterious to the recipient of the composition. The composition may be in any suitable form; for example, the composition may be provided in suspension form, powder form (for
25 example, but limited to lyophilised or encapsulated), capsule or tablet form. For example, when the composition is provided in suspension form, the carrier may comprise water, saline, a suitable buffer, or additives to improve solubility and/or stability; reconstitution to produce the suspension is effected in a buffer at a suitable pH to ensure the viability of the antibody. Dry powders may also include additives to improve stability and/or carriers to increase
30 bulk/volume; for example the dry powder composition may comprise sucrose or trehalose. In a specific, non-limiting example, the composition may be so formulated as to deliver the antibody to the gastrointestinal tract of the subject. Thus, the composition may comprise encapsulation, time-release, or other suitable technologies for delivery of the antibody. It

would be within the competency of a person of skill in the art to prepare suitable compositions comprising the present compounds.

[00133] The antibody may be used in the treatment of diseases or conditions where AXL expression is dysregulated, or may be used in the preparation of a medicament for such use. The antibody may be linked to a cargo molecule, or may be included in a composition. Diseases and conditions that may be treated using the antibody described herein include, but are not limited to solid cancer tumors, including, but not limited to, breast, renal, endometrial, ovarian, thyroid, and non-small cell lung carcinoma, melanoma, prostate carcinoma, sarcoma, gastric cancer and uveal melanoma; liquid tumors, including but not limited to, leukemias (particularly myeloid leukemias) and lymphomas; endometriosis, vascular disease/injury (including but not limited to restenosis, atherosclerosis and thrombosis), psoriasis; visual impairment due to macular degeneration; diabetic retinopathy and retinopathy of prematurity; kidney disease (including but not limited to glomerulonephritis, diabetic nephropathy and renal transplant rejection), rheumatoid arthritis; osteoarthritis, osteoporosis and cataracts. Diseases and conditions that may be treated also include those affected by the following biological processes: Invasion, migration, metastasis, or drug resistance as manifested in cancer; stem cell biology as manifested in cancer; invasion, migration, adhesion, or angiogenesis; vascular remodeling; bone homeostasis; viral infection; or differentiation as manifested in obesity. The compounds of formula (I) may also be used to modulate inflammatory processes by treating sepsis, acting as vaccine adjuvants, and/or potentiating the immune response in immuno-compromised patients. In one non-limiting example, the disease or condition may be metastatic cancer.

[00134] The present invention will be further illustrated in the following examples. However, it is to be understood that these examples are for illustrative purposes only and should not be used to limit the scope of the present invention in any manner.

[00135] EXAMPLES

[00136] Example 1: Production of anti-AXL antibodies

[00137] Monoclonal antibodies (mAb) against AXL were generated by immunizing mice with the extracellular domain of a recombinant human AXL protein (rhAXL-ECD; SEQ ID NO:58) as well as by genetic immunization.

[00138] Production of rhAXL-ECD: A pTT5 construct containing a synthetic recombinant fragment of C-terminally His8-tagged recombinant human (rh)AXL extracellular

domain (ECD; SEQ ID NO:71) was expressed in CHO cells, purified by Ni-agarose and verified by SDS-PAGE (data not shown).

[00139] Immunizations: Mouse mAb were generated by genetic immunization. For genetic immunizations, mice were bled (pre-immune serum) and immunized on day 0 and day 42 by hydrodynamic tail vein delivery technique (HTV) using 100 µg of a pTT40 expression plasmid for expression of soluble rhAXL-ECD. Mice were bled 7-10 days later and the serum titer was measured by ELISA. Four to five months later, a booster injection using 100 µg of pTT40-AXL-H8G was done by HTV 3 to 4 days prior to fusion experiment.

[00140] Fusion experiment and hybridoma selection. All manipulations were done under sterile conditions. Spleen cells were harvested in IMDM (Hy-Clone) and fused to NSO myeloma cell line using PEG fusion protocol. To this end, spleen cells and myeloma cells were washed in IMDM, counted in RBC lysing buffer (Sigma) and mixed together at a 5:1 ratio. Pelleted cells were fused together by adding 1 ml of a 50% solution of PEG 4000 (EMD-Millipore) in PBS drop-wise over one minute, and incubated at 37°C for an additional 90 sec. The reaction was stopped by addition of 30 ml of IMDM at 22°C over 2 min. After a 10 min incubation, freshly fused cells were spun at 233 xg for 10 min. Cells were washed once in IMDM supplemented with 10% heat inactivated FBS (Sigma), and suspended at a concentration of 2×10^5 input myeloma cells per ml in HAT selection medium (IMDM) containing 20% heat inactivated FBS, penicillin-streptomycin (Sigma), 1 ng/ml mouse IL-6 (Biosource), HAT media supplement (Sigma) and L-glutamine and incubated at 37°C, 5% CO₂. The next day, hybridoma cells were washed and suspended at a concentration of 2×10^5 input myeloma cells per ml in semi-solid medium D (StemCell) supplemented with 5% heat inactivated FBS, 1 ng/ml mouse IL-6 and 10 µg/ml FITC- Fab'2 Goat anti-mouse IgG (H+L) (Jackson). The cell mixture was plated in Petri dish (Genetix) and further incubated for 6-7 days at 37°C, 5% CO₂. Secreter clones were then transferred using a mammalian cell clone picker (CLONEPIX™ FL, Molecular Devices) into sterile 96-w plates (Costar) containing 200 µl of IMDM supplemented with 20% heat inactivated FBS, penicillin-streptomycin (Sigma), 1 ng/ml mouse IL-6 (Biosource), HT media supplement (Sigma) and L-glutamine and incubated for 2-3 days at 37°C, 5% CO₂.

[00141] Hybridoma supernatant was collected and evaluated for binding to the rhAXL-ECD protein by ELISA. To this end, 96-w half-area plates (Costar) were coated with 25 µl of rhAXL-ECD at 5 µg/ml in PBS and incubated overnight at 4°C. Microplates were washed 3 times with PBS, blocked with PBS-BSA 1%, and 25 µl of hybridoma supernatant were added

and incubated at 37°C, 5% CO₂ for 2 hours. Plates were washed 4 times with PBS-TWEEN™ 20 0,05% and incubated for one hour at 37°C, 5% CO₂ with 25 µl of secondary antibody alkaline phosphatase conjugated F(ab)'2 goat anti-mouse IgG (H+L) (Jackson ImmunoResearch) diluted 1/3000 in blocking buffer. After 4 washes with PBS-TWEEN™ 20 0,05%, 25 µl of a 1 mg/ml pNPP substrate solution was added and further incubated for one hour at 37°C. OD405nm measurements were done using a microplate reader (Spectramax 340 PC, Molecular Devices).

[00142] ELISA positive antibodies were selected (including clones F107-7H5, F107-8D12, F111-5E9, F111-3C8, and F107-10G1) and small scale purifications done on 10 ml volumes of hybridoma supernatant. mAb were purified via adsorption onto Protein G Mag SEPHAROSE™ Extra (GE Healthcare Life Sciences #28-9670-70), washes in PBS, elution twice with 200 µl of glycine 200 mM pH 2.5 and neutralization in TRIS-HCl 1M pH 9.0. Purified antibodies were desalted using Zeba Spin resin (Pierce) pre-equilibrated in PBS and filter sterilized through 0.22 µm membrane (Millipore). The final concentration of the antibody solutions was determined by nano-drop (#ND-1000).

[00143] Example 2: Cross-Reactivity Assessment of Anti-AXL mAbs

[00144] The cross-reactivity binding properties of the anti-hAXL monoclonal antibodies purified in Example 1 for recombinant AXL ectodomain were evaluated.

[00145] To evaluate the cross-reactivity of the mAb to other members of the TAM receptor tyrosine kinase subfamily, binding of the mAb to rhAXL-ECD, rhMER-ECD, rhTyro-3 and BSA (negative control) was measured by ELISA. 96-w half-area plates (Costar) were coated with 25 µl of rhAXL-ECD, rhMER-ECD, rhTyro-3 or BSA at 5 µg/ml in PBS and incubated overnight at 4°C. Microplates were washed 3 times with PBS, blocked with PBS-BSA 1%, and 25 µl of a 10 µg/ml diluted in PBS-BSA 1% were added and incubated at 37°C, 5% CO₂ for 2 hours. Plates were washed 4 times with PBS-TWEEN™ 20 0.05% and incubated for one hour at 37°C, 5% CO₂ with 25 µl of secondary antibody alkaline phosphatase conjugated F(ab)'2 goat anti-mouse IgG (H+L) (Jackson ImmunoResearch) diluted 1/5000 in blocking buffer. After 4 washes with PBS-Tween 20 0.05%, 25 µl of a 1 mg/ml pNPP substrate solution was added and further incubated for one hour at 37°C. OD405nm measurements were done using a microplate reader (Spectramax 340 PC, Molecular Devices).

[00146] Table 1 provides the results of cross-reactivity assessment of anti-AXL monoclonal antibodies on other targets from the same protein family. Measurements at OD405nm are shown.

Table 1				
Cross-Reactivity Assessment of Anti-AXL mAbs				
Purified Antibody	Coating antigen			
	AXL	MER	Tyro3	BSA
F107-7H5-2	2,403	0,000	0,000	0,000
F107-8D12-2	2,592	0,000	0,000	0,000
F107-10G1-3	1,758	0,000	0,000	0,000
F111-3C8-2	2,301	0,000	0,000	0,000
F111-5E9-2	1,364	0,000	0,000	0,000

5

[00147] The results of the cross-reactivity assessment indicate that the five mAb specifically bind the extracellular domain of AXL, i.e. they do not cross-react with BSA or the extracellular domains of other members of the TAM family, namely Tyro3 and MER.

10 **[00148]** Example 3: Functional characterization for ADC potential

[00149] The anti-hAXL-ECD monoclonal antibodies purified in Example 1 were evaluated for their internalizing ability and antibody-drug conjugate (ADC) potential in an ADC surrogate screening assay. Non-small cell lung cancer (NSCLC) A549 cells, breast cancer MDA-MB 231 cells, or glioblastoma U87MG cells were used, all of which express moderate to high levels of AXL. mAbs were selected that show low or sub nM (IC₅₀) potency in secondary conjugate saporin-based cytotoxicity screening in more than one tumor cell line known to express the tumor target of interest (i.e., AXL).

[00150] Cell culture. The following cell lines were obtained from ATCC and cultured according to supplier's recommendations: A549 human non-small lung cancer (NSCLC), MDA-MB-231 triple negative (ER-/PR-/HER2low) breast cancer (TNBC), and U87MG glioblastoma cell lines. Generally, cells were passaged once or twice a week and used within 4-6 weeks for all experiments.

20 **[00151]** Evaluation of antibody-mediated cytotoxicity as antibody-drug conjugates. Primary mouse antibodies (typically 1nM in concentration) were incubated with an equimolar

concentration of anti-mouse secondary antibody chemically conjugated with saporin toxin (Advanced Targeting Systems, San Diego, CA), a ribosome inactivating enzyme that needs to be internalized to cause cell death. The antibody complex was then added to the cell types indicated (plated in triplicate) and their effects on cell viability measured after 3 days of incubation at 37°C. Incubation with no primary antibody (secondary antibody alone) or an irrelevant primary antibody (control human IgG) were used to assess non-target-directed cytotoxicity. Cell viability & metabolism were measured using conventional methods such as the use of a resurizin dye like Alamar Blue or a reagent to measure protein content, such as sulforhodamine B.

[00152] A549 NSCLC cells were seeded in RPMI-5% FBS in 96-well plates at a density of 2000 cells/well in 100 µl RPMI-5% FBS. The next day, 20 nM of anti-AXL mAb was mixed with 20nM of the Mab-Zap secondary antibody (Advanced Targeting Systems, San Diego, CA), and was incubated for 30 min. at room temperature; 11µl of this mixture was added to the cells in triplicate such that a 1 nM final concentration of the 1:1 complex of anti-AXL mAb (F107-7H5, F107-8D12, F111-5E9, F111-3C8, or F107-10G1) and the saporin-conjugated anti-mouse secondary antibody (Mab-Zap) was incubated with the cells. After 72hr of incubation (37°C, 5% CO₂, humidified incubator) cell viability was determined using Alamar Blue according to manufacturer's direction (BIOSOURCE). Subsequently, antibodies that caused more than a 50% decrease in A549 cell viability at 1nM concentration were also tested for cytotoxic effects on breast cancer MDA-MB 231 cells, or glioblastoma U87MG cell lines using the method described above.

[00153] **Figure 2** shows results of the ADC surrogate screening assay for anti-hAXL-ECD mAb. The antibodies in the assay caused a marked reduction in A549 cell survival compared to cells treated with secondary conjugate alone (Mab-Zap). Displayed are the average values+SEM of a triplicate experiment.

[00154] In **Figure 2**, where the survival of the cells is expressed relative to that of the mAb-ZAP secondary conjugate alone (shown as 100%). The selected mAb clones (F107-7H5, F107-8D12, F107-10G1, F111-3C8, and F111-5E9) caused more than 50% reduction in viability in A549 cells and in at least one more cell line and were selected for direct conjugation to the highly cytotoxic drug DM1. The high degree of cytotoxicity associated with these 5 antibodies (IC₅₀<1nM), from among more than 250 primary mouse mAb tested, demonstrates that they exhibit a high degree of internalization and appropriate intracellular routing to achieve activation of the saporin toxin, making them ideal for ADC development.

Not surprisingly, antibodies identified in this screen all exhibited appreciable binding to AXL on the surface of tumor cells (**Figure 3**).

[00155] **Figure 3** shows results of flow cytometry experiments determining the binding properties of anti-AXL mAb to cell surface-expressed AXL. Panel A shows the linear scale dose-response curve, while Panel B shows the log scale dose response curve.

[00156] Example 4: DM1 conjugation and ADC testing

[00157] The anti-hAXL-ECD monoclonal antibodies purified in Example 1 were conjugated via lysine residues to succinimidyl trans-4-[maleimidylmethyl] cyclohexane-1-carboxylate (SMCC) linked to N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)-maytansine (DM1). Product purity and drug:antibody ratio were determined by UPLC based size-exclusion chromatography (SEC).

[00158] Conjugation: Purified anti-hAXL-ECD mAb (F107-7H5, F107-8D12, F111-5E9, F111-3C8, or F107-10G1; Example 1) were buffer-exchanged into Conjugation buffer (100 mM Sodium phosphate, 20 mM NaCl, 2 mM EDTA pH 7.2) using pre-equilibrated spin desalting columns. The concentration of each mAb was adjusted to 2 mg/mL with conjugation buffer and 200 µg total of each was used for conjugation. A stock solution of SMCC-DM1 was prepared in dimethylacetamide (DMA). SMCC-DM1 from the DMA stock solution was added to each mAb to achieve a molar SMCC-DM1:mAb ratio of 10.0. The solution was mixed thoroughly and incubated at 37°C for 3 hours. The reaction was stopped by passing the reaction mixture through two spin desalting columns equilibrated in Conjugation buffer with 0.02% w/v Polysorbate-20 added.

[00159] Drug-antibody ratio (DAR) was determined by integrating the monomeric peak from the UPLC-SEC chromatogram at both 280 nm and 252 nm and comparing these to the ratios of extinction coefficients for the unconjugated antibody and free drug at the same wavelengths. Percent monomer was determined from the total integrated areas of monomer, high-molecular weight species and low-molecular weight species observed in the chromatogram. Results are shown in **Table 2**, which provides drug-antibody ratio and percent aggregate determined by UPLC-SEC for F107-7H5, F107-8D12, F111-5E9, F111-3C8, and F107-10G1 conjugated to DM1.

Table 2 Drug-antibody Ratio and Percent Aggregate

mAb	DAR	Percent Aggregate
F111-3C8	7.21	27.0
F107-7H5	5.46	1.1
F107-8D12	4.90	1.8
F107-10G1	5.21	0.7
F111-5E9	5.22	1.1

[00160] Cell culture. The NCI-H292 and A549 human non-small cell lung cancer (NSCLC) and the MDA-MB-231 triple negative (ER-/PR-/HER2low) breast cancer (TNBC) cell lines were obtained from ATCC and cultured according to supplier's recommendations.

5 HaCaT cells are a spontaneously immortalized, human keratinocyte line that has been widely used for studies of skin biology and differentiation. This cell line was obtained from Cell Line Services, DKFZ, Heidelberg, Germany and cultured according to supplier's recommendations. Generally, cells were passaged once or twice a week and used within 4-6 weeks for all experiments.

10 **[00161]** ADC testing: Anti-AXL ADC prepared above were tested for their effects on viability of various cultured cell lines known to express AXL, including the non-small cell lung cancer (NSCLC) NCI-H292 and A549 cell lines, human breast adenocarcinoma MDA-MB-231 tumor cell line, as well as immortalized keratinocytes. Following 5 days of exposure, cell growth/viability was assessed using sulforhodamine B reagent and dose-response curves
15 were generated to measure their potency (IC_{50}) and efficacy (% maximal inhibition) using the log(inhibitor) vs. response -- Variable slope (four parameters) model from GraphPad Prism v6.0 software.

[00162] **Figure 4** shows results of the activity of anti-AXL-DM1 conjugates on cell viability in H292 tumor cell lines, A549 cell lines, human breast adenocarcinoma MDA-MB-
20 231 tumor cell line, as well as immortalized HaCaT keratinocyte cells, which express AXL.

[00163] The DM1-conjugated anti-AXL antibodies demonstrated good efficacy (from 55-74% maximal growth inhibition) and strong potency ($IC_{50} < 0.1$ nM) in the tumor cell lines analyzed compared to the non-targeted irrelevant human IgG-DM1 conjugate negative control. In contrast, very little toxicity was seen on non-tumorigenic, immortalized HaCaT
25 keratinocyte cells, which express AXL.

[00164] Table 3 provides the results of ADC testing of anti-AXL antibodies. % max inh = percent maximal inhibition.

Table 3 Results of ADC Testing of Anti-AXL Antibodies.							
cell line		F107-7H5-DM1	F107-8D12-DM1	F107-10G1-DM1	F111-3C8-DM1	F111-5E9-DM1	hIgG-DM1
H292	IC ₅₀ (nM)	0.113	0.176	0.044	0.419	0.159	>10
NSCLC	% max inh	74.0	72.8	57.7	70.3	64.6	incomplete
A549	IC ₅₀ (nM)	0.120	0.218	0.058	0.313	0.169	>10
NSCLC	% max inh	58.5	61.8	55.7	71.2	70.0	incomplete
231	IC ₅₀ (nM)	0.037	0.056	~ 0.029	0.147	0.067	>10
TNBC	% max inh	71.4	73.4	64.9	77	69.2	incomplete
	IC ₅₀ (nM)	no effect	no effect	no effect	no effect	no effect	no effect
HaCaT	% max inh	incomplete	incomplete	incomplete	incomplete	incomplete	incomplete

5 **[00165]** Example 5: Cell surface binding by flow cytometry

[00166] The binding properties of the anti-hAXL-ECD monoclonal antibodies purified in Example 1 to AXL on cell surfaces were evaluated by flow cytometry using the H292 cell line, due to its strong inhibition by the anti-hAXL-ECD mAb (see Example 2).

[00167] Detection of antibody binding to surface AXL by flow cytometry. Prior to analysis, cells were plated such that they were not more than 80% confluent on the day of analysis. Cells were washed in PBS and harvested by the addition of cell dissociation buffer (Sigma). A cell suspension containing 2.5×10^5 cells (in 500 μ l corresponding cell culture media) was incubated with various concentrations (0.01-10nM) of anti-AXL antibodies for 2 h at 4°C (to prevent internalization). Following one wash with cell culture media, primary antibody was incubated with 2 μ g DYLIGHT™ 488 conjugated AffiniPure goat anti-mouse IgG Alexa 488 secondary antibody (Jackson ImmunoResearch) in 100 μ l of media for 1h at 4°C. Prior to analysis, cell pellets were re-suspended in 300-500 μ l media and filtered through a 50 μ m nylon mesh filter to remove cell aggregates. Flow cytometry analyses were performed on 10,000 viable cells gated on forward scattering, side scattering parameters and propidium iodide dye exclusion using a BD LSRII flow Cytometer (Becton-Dickinson Biosciences, CA, USA) and a standard filter set using BD FACSDiva™ acquisition software, according to manufacturer's instructions.

[00168] Specific detection of antibody binding was calculated as the mean fluorescent intensity of binding to each primary antibody after background level subtraction of the mean fluorescent intensity of binding in the absence of primary antibody (but containing detection antibody). Binding parameters B_{max}, (maximum fluorescence signal at receptor saturation), and K_D (apparent cell binding constant) were then determined using a One site - Specific binding with Hill slope model in GraphPad Prism v 6.0.

[00169] Results are shown in **Figure 3** and **Table 4**. These results showed that mAb 7H5, 8D12, and 5E9 bound strongly (with an apparent binding constant, K_D ≤ 1nM) compared to the negative-binding control (300 nM mouse IgG).

[00170] **Table 4** provides binding properties of anti-AXL mAb to cell-based AXL on H292 cells.

Table 4 Binding Properties of Anti-AXL mAb to Cell-based AXL on H292 Cells					
Best-fit values	F107-8D12	F107-7H5	F111-3C8	F107-10G1	F111-5E9
B _{max} (MFI)	1309	1431	1477	937	1149
K _d (nM)	0.08802	0.1038	1.227	0.1005	0.1767

[00171] Example 6: Epitope binning experiments

[00172] To evaluate whether anti-hAXL-ECD mAb of Example 1 bind to the same epitope region, surface plasmon resonance epitope binning experiments were carried out.

[00173] Antibodies were directly immobilized on the chips surface ('mAb1'), after which the rhAXL ECD was flowed, followed by flowing the same or a different antibody ('mAb2'). All experiments were performed on a ProteOn XPR36 biosensor at 25°C using PBST as running buffer (PBS with 0.05% v/v Tween20). GLM sensor chips and coupling reagents (10mM sodium acetate, pH 4.5, sulfo-N-hydroxysuccinimide (SNHS), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), and ethanolamine) were purchased from BioRad, Inc (Hercules, CA).

[00174] Creating the antibody array. Antibodies (Mab1) were immobilized on the GLM chip surface at 30 µl/min. The activation reagents (at stock concentration of 0.4M EDC and 0.1M SNHS in water) were diluted 20-fold each in water. The top (A1) horizontal channel (the Analyte channel in the Proteon control software) was activated for 3 min with the diluted

activation reagents. Next, the mAb (Example 1) were each diluted to 20 µg/ml in 10 mM acetate pH4.5 and injected for 3 min in separate vertical (L1 to L6) channels (the Ligand channel in the Proteon control software), followed with a 3 min injection of ethanolamine to block the reactive spots. The multi-channel module (MCM) was then rotated and another 3 min injection of ethanolamine was done on the activated horizontal channel (A1). This four-step 'activation-binding-and-2x deactivation' procedure was then repeated on each of the horizontal channels (A2 to A6).

[00175] Sandwich epitope binning. The two-step sandwich epitope binning was done at 30 µl/min in the analyte orientation. 100nM rhAXL-ECD protein was injected for 3 min, immediately followed by a 3 min injection of mAb2 at 100nM. Immobilized mAb (mAb1) surfaces were regenerated by a 18s injection of 0.85% phosphoric acid at 100 µl/min. This two-step injection (rhAXL-ECD -mAb2) was repeated for each individual mAb2. Each mAb was also tested simultaneously as mAb1 (immobilized on the chip) and mAb2 (in solution). To monitor the AXL dissociation from the immobilized mAb1 PBST was injected instead of mAb2.

[00176] **Table 5** provides epitope binning results for mAb. Cells labelled "nc" indicate no competition, "sc" cells indicate self-competition, and "C" cells indicate competition.

Table 5					
Epitope Binning Results for mAb					
	F107-8d12	F107-7H5	F111-3C8	F111-5E9	F107-10G1
F107-8D12	sc	C	nc	nc	nc
F107-7H5	C	sc	nc	nc	nc
F111-3C8	nc	nc	sc	C	nc
F111-5E9	nc	nc	C	sc	nc
F107-10G1	nc	nc	nc	nc	sc

[00177] From the results shown in **Table 5**, it can be seen that four of the mAb fall into 2 epitope bins, that is, they compete with each other for binding to AXL; 7H5 competes with 8D12 and vice versa, and 3C8 competes with 5E9 and vice versa. 10G1 does not compete with the other mAbs. In contrast, anti-AXL mAb that were not selected based on their ability to internalize in the ADC surrogate assay tended to distribute themselves into a larger

number of bins (data not shown). This data suggests that functional selection for internalizing antibodies results in a subset that bind selectively to 3 major sites on AXL extracellular domain. Moreover, additional SPR binding studies using similar methods demonstrated that these sites correspond to regions within Immunoglobulin-like domain-1 and -2. Epitope binning experiments (same methods as above) performed with the individual AXL extracellular subdomain (Immunoglobulin-like domain or Fibronectin type III-like domain) showed that mAb F107-10G1 binds a sequence in Immunoglobulin-like domain-1, and that it failed to compete with all other IgL1-binding antibodies tested, consistent with the results utilizing the full AXL ectodomain. These results indicate that there are at least 2 separate epitopes within Immunoglobulin-like domain-1 with which ADC mAbs interact.

[00178] Example 7: Epitope mapping by yeast surface display

[00179] Yeast surface display was used to map the sequences to which the anti-hAXL-ECD mAb of Example 1 bind.

[00180] Epitope mapping. The hAXL ectodomain (ECD) and its fragments were expressed and covalently displayed on the surface of yeast cells using the yeast surface display method (Feldhaus et al., 2003). The YSD vector (pPNL6) was from The Pacific Northwest National Laboratory, USA. The hAXL fragments covering the entire hAXL-ECD (**Figure 5**) were expressed as fusion proteins (Aga2-HA-(hAXL)-MYC on the yeast cell surface. The displayed hAXL fragments were used to map the domains of hAXL to which the anti-hAXL-ECD mAb of Example 1 bind. The binding of the mAb to yeast cells was performed using a whole yeast cell ELISA. The relative amount of the displayed fusion protein was measured by probing with an anti-MYC antibody, followed by an HRP-conjugated secondary antibody, and used to normalize the binding signal for the mAb of Example 1.

[00181] **Figure 5** is a schematic representation of the human AXL ectodomain and transmembrane domain. Ten peptides covering the entire hAXL-ECD were expressed on the yeast membrane to map the binding epitope of the anti-AXL mAb. The peptides are represented and numbered 1 to 10.

[00182] **Table 6** shows the results of anti-AXL mAb binding to various regions in the AXL extracellular domain on displayed yeast cells. The mAb of Example 1 binds two regions: either the Immunoglobulin-like domain 1 (IgL1, corresponding to aa 26-138) or the

Immunoglobulin-like domain 2 (IgL2, corresponding to aa 135-226). These results are consistent with the results obtained in Example 6.

Table 6 Results of anti-AXL mAb Binding to Various Regions in the AXL Extracellular Domain		
mAb	Epitope location	Epitope conformation
F107-7H5	IgL1	Conformational
F107-8D12	IgL1	Conformational
F111-5E9	IgL2	Conformational
F111-3C8	IgL2	Conformational
F107-10G1	IgL1	Conformational

[00183] The nature of the epitopes. To determine if the epitopes for the mAb are continuous (linear) or non-continuous (conformational), various fragments of either IgL1 or IgL2 domains were expressed and displayed on yeast cells and assayed for binding to the mAb using cell ELISA as described above. None of the sub-fragments of either IgL domains binds significantly to any mAb of Example 1, although these sub-fragments displayed well on the surface of the yeast cell as judged by the signal of the anti-MYC signal. These results strongly suggest that the epitopes for the mAb of Example 1 require the entire sequence information of the IgL domain, and therefore are largely conformational.

[00184] Example 8: Antibody sequencing

[00185] The VH and VL domains of the anti-hAXL mAb of Example 1 were sequenced and analyzed.

[00186] Sequences of the VH and VL domains as well as the CDR regions are shown in **Table 7**. Analysis of the sequence for a consensus binding sequence of the CDR 1-3 regions of the VH and VL chains was conducted using web-based software. The results of this analysis indicated that the CDR regions of both the VH and VL regions of mAb F107-7H5 is very similar to that of F107-8D12 (2 amino acid change). These antibodies cluster together and recognize a common linear epitope within IgL1. In contrast, the remainder of the antibodies tend to form smaller differentiated clusters, the majority of which bind IgL2, with the exception of F107-10G1 (which bind a site on IgL1 which is distinct from the majority of the IgL1 binding antibodies selected).

Table 7 CDR Sequences for mAb F107-7H5, F107-8D12, F111-5E9, F111-3C8, and F107-10G1					
mAb	Light Chain CDR			Heavy Chain CDR	
F107-7H5	V _L	SEQ ID NO:38		V _H	SEQ ID NO:39
	L1	KSSQSLLNSRTRKIYLA (SEQ ID NO:9)		H1	GYTFTSYWIN (SEQ ID NO:12)
	L2	WASTRQS (SEQ ID NO:10)		H2	NIYPDSSSTNYNEKFKS (SEQ ID NO:13)
	L3	KQSYNLWT (SEQ ID NO:11)		H3	DTYGGSPDY (SEQ ID NO:14)
F107-8D12	V _L	SEQ ID NO:40		V _H	SEQ ID NO:41
	L1	KSSQSLLNTRTRKNYLA (SEQ ID NO:15)		H1	GYTFISFWIN (SEQ ID NO:17)
	L2	WASTRES (SEQ ID NO:16)		H2	NIFPGSSSTNYNEKFKS (SEQ ID NO:18)
	L3	KQSYNLWT (SEQ ID NO:11)		H3	DYYGGSPDY (SEQ ID NO:19)
F111-3C8	V _L	SEQ ID NO:42		V _H	SEQ ID NO:43
	L1	SASSSVSYMY (SEQ ID NO:26)		H1	GYTFTSYWMH (SEQ ID NO:29)
	L2	RTSNLAS (SEQ ID NO:27)		H2	NINPNSTSADYNEKFKR (SEQ ID NO:30)
	L3	QQYHNYPPT (SEQ ID NO:28)		H3	PLMGPYWYFDV (SEQ ID NO:31)
F111-5E9	V _L	SEQ ID NO:44		V _H	SEQ ID NO:45
	L1	RASQDINNYLN (SEQ ID NO:20)		H1	KYGMN (SEQ ID NO:23)
	L2	YISRLHS (SEQ ID NO:21)		H2	WINTYTGEPTYADDFKG (SEQ ID NO:24)
	L3	QQGNTLPFT (SEQ ID NO:22)		H3	GGYYSNPIYPMDY (SEQ ID NO:25)
F107-10G1	V _L	SEQ ID NO:46		V _H	SEQ ID NO:46
	L1	KASQDVTTAVA (SEQ ID NO:32)		H1	NYGMS (SEQ ID NO:35)
	L2	WASTRHT (SEQ ID NO:33)		H2	SISGGGGRTYYLDNVKG (SEQ ID NO:36)
	L3	QQHFTTPLT (SEQ ID NO:34)		H3	GARASYFAMDY (SEQ ID NO:37)

[00187] Example 9: Recombinant antibody production and purification

[00188] To facilitate large scale mAb productions and consistency between productions, the anti-hAXL-ECD mAb identified in Example 8 were produced recombinantly in CHO cells.

[00189] The VH and VL regions (see Example 8) were cloned as fusions with human IgG1 constant regions (human IgG1 heavy chain and human kappa light chain, respectively) into the pTT5 vector, thereby generating chimeric mAb.

[00190] Table 8 provides sequences for each of the chimeric mAbs: hFC-F107-7H5, hFC-F107-8D12, hFC-F111-5E9, hFC-F111-3C8, and hFC-F107-10G1. In addition, all light chain sequences comprised a signal sequence MVLQTQVFISLLLWISGAYG (SEQ ID NO:59) at the N-terminus, while heavy chain sequences comprised the signal sequence MDWTWRILFLVAAATGTHA (SEQ ID NO:60) at the N-terminus.

Table 8 Sequences of Chimerized mAb	
mAb	Sequence
hFC-F107-7H5 light chain	SEQ ID NO:48
hFC-F107-7H5 heavy chain	SEQ ID NO:49
hFC-F107-8D12 light chain	SEQ ID NO:50
hFC-F107-8D12 heavy chain	SEQ ID NO:51
hFC-F111-3C8 light chain	SEQ ID NO:52
hFC-F111-3C8 heavy chain	SEQ ID NO:53
hFC-F111-5E9 light chain	SEQ ID NO:54
hFC-F111-5E9 heavy chain	SEQ ID NO:55
hFC-F107-10G1 light chain	SEQ ID NO:56
hFC-F107-10G1 heavy chain	SEQ ID NO:57

[00191] Chimeric mAb expression was validated via a 2 mL expression scout: CHO cells were transiently transfected with VL and VH containing constructs (1:1 ratio); conditioned medium (CM) was harvested on day 7, and mAb expression levels were evaluated by SDS-PAGE (data not shown). The chimeric mAb expressed well and a small-scale production (50 mL) was initiated by transiently transfecting CHO cells with the same construct ratio. Conditioned medium (CM) was harvested on day 7, chimeric mAb were purified (ProtA), quantitated, and evaluated by SDS-PAGE. The data showed that all five chimeric mAb were well expressed by the transiently transfected CHO cells.

[00192] To confirm that the recombinantly expressed chimeric mAb behave similarly to the hybridoma-expressed mAb, SPR binding experiments are performed to ensure that AXL binding is not compromised.

[00193] Example 10: Isolation of anti-AXL mAbs and sdAbs

[00194] In this example, isolation of llama sdAbs directed against human AXL ectodomain is conducted.

[00195] Materials and Methods

[00196] Production of recombinant human AXL ectodomain (rhAXL ECD). A mammalian expression vector (pTT5) containing a synthetic recombinant fragment of C-terminally His₈-tagged recombinant human AXL extracellular domain was transiently
5 transfected in CHO cells, purified by Ni-agarose and verified by SDS-PAGE (data not shown).

[00197] Llama Single Domain Antibody (sdAb) generation. Llama sdAbs (VHHs) were generated by immunizing a llama with rhAXL ECD protein followed by panning of a phage-displayed VHH library constructed from peripheral blood lymphocytes. Briefly, one llama
10 (Lama glama) was immunized with 100 µg of rhAXL on days 0, 21, 28, 35 and 42. The antigen was mixed with Freund's complete adjuvant on day 0 and Freund's incomplete adjuvant on days 21, 28, 35 and 42. Serum was drawn before the first immunization of day 0 (pre-immune) as well as on days 35 and 49 then fractionated into separate conventional IgG (cIgG) from heavy-chain IgG (hclgG) using protein A and protein G affinity chromatography.
15 ELISA against rhAXL on unfractionated and fractionated sera was performed. Briefly, 1 µg of rhAXL diluted in PBS was coated overnight (100 µl/well, 18 h, 4°C) in 96 well MAXISORP™ plates (Nalge Nunc International, Rochester, NY). Plates were blocked with 5% skim milk/PBST, washed with PBS-T (PBS + 0.05% (v/v) Tween 20), and serial dilutions of pre-immune total (unfractionated) serum, post-immune total serum (Day 35 and 49) and
20 fractionated serum (Day 49; 100 µg/ml starting concentration) was applied. After incubation at room temperature for 1.5 h and washing with PBS-T, goat anti-llama IgG-HRP (1:10,000 in PBS) was added for 1 h at 37°C. A final PBS-T wash preceded the addition of 100 µl/well TMB substrate (KPL, Gaithersburg, MD) for 10 min. The reaction was stopped with 100 µl/well 1 M H₃PO₄ and read on a BioRad plate reader (Hercules, CA) at 450 nm.

[00198] For phage-displayed VHH library construction, RNA was isolated from approximately 5 x 10⁷ lymphocytes collected on Day 35 and 49 post-immunization using the QIAamp RNA Blood Mini Kit (QIAGEN, Mississauga, ON, Canada). Approximately 10 µg of total RNA was used as template for first strand cDNA synthesis with oligo dT primers using the First-Strand cDNA Synthesis Kit (GE Healthcare, Baie-d'Urfé, QC, Canada). The cDNA
30 was PCR-amplified by an equimolar mix of three variable region-specific sense primers (MJ1, MJ2, and MJ3) and two antisense CH2-specific primers (CH2 and CH2b3). Briefly, the PCR reaction mixture was set up in a total volume of 50 µl with the following components: 5 µl cDNA, 5 pmol of MJ1-3 primer mixture, 5 pmol of either CH2 or CH2b3 primers, 5 µl of 10x

reaction buffer, 1 µl of 10 mM dNTP and 2.5 units of PLATINUM® Taq DNA polymerase (Invitrogen/Life Technologies, Burlington, ON, Canada). The PCR protocol consisted of an initial step at 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 57°C for 45 s, 72°C for 1 min and a final extension step at 72°C for 10 min. The amplified PCR products were run in a 2% agarose gel and two major bands were observed: a band of about 850 bp, corresponding to conventional IgG, and a second band of around 600 bp, corresponding to heavy-chain antibodies (hclgGs). The smaller bands were cut and purified using the QIAquick Gel Extraction Kit (QIAGEN) and re-amplified in a second PCR in a total volume of 50 µl using 1 µl of DNA template, 5 pmol of each of MJ7 primer and MJ8 primer, 5 µl of 10x reaction buffer, 1 µl of 10 mM dNTP and 2.5 units of Platinum® Taq DNA polymerase. The PCR protocol consisted of an initial step at 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 57°C for 30 s and 72°C for 1 min and a final extension step at 72°C for 7 min. The amplified PCR products, ranging between 340 bp and 420 bp and corresponding to VHH fragments of hclgGs, were purified using the QIAquick PCR Purification Kit (QIAGEN), digested with SfiI restriction enzyme (New England BioLabs, Pickering, ON, Canada) and re-purified using the same kit. Fifty micrograms of pMED1 phagemid was digested with SfiI overnight at 50°C.

[00199] To minimize self-ligation, 20 units of XhoI and PstI restriction enzymes were added and the digestion reaction was incubated for an additional 2 h at 37°C. Twenty micrograms of digested phagemid DNA was ligated with 6 µg of digested VHH fragments for 3 h at room temperature using T4 DNA ligase (Invitrogen) and its protocol. The ligated materials were purified using the QIAquick PCR Purification Kit in a final volume of 100 µl and electroporated in 5 µl portions into commercial electrocompetent TG1 E. coli cells (Stratagene, La Jolla, CA) as described. The size of the library was determined as described. The library was grown for 2 h at 37°C, 250 rpm in the presence of 2% (w/v) glucose. The bacterial cells were pelleted, re-suspended in 2xYT/Amp/Glu (2 x YT medium with 100 µg/ml ampicillin and 2% (w/v) glucose) with 35% (v/v) glycerol and stored at -80°C in small aliquots.

[00200] The phage-displayed library was rescued using M13K07 helper phage. AXL-specific VHHs were selected from the library using two strategies. In the first strategy, the library was panned simultaneously against immobilized rhAXL ECD and against A549 tumor cells as described in Zhou et al., 2010. In the second strategy, the library was panned against rhAXL ECD and VHH-displaying phage were eluted competitively with AXL-specific murine mAbs (5E9 and 10G1) as described in Henry et al., 2016. The library phage and the

phage eluted from pannings were interrogated using Illumina MiSeq next-generation DNA sequencing as described in Henry et al., 2016.

[00201] Results

5 **[00202]** VHHs directed against rhAXL ECD were identified by constructing a phage-displayed VHH library from an immunized llama and performing various selections of the library as described in methods section, above. The input (library) VHH-displaying phage and the output (eluted) VHH-displaying phage were sequenced, and individual VHH sequences selectively enriched in particular selections were identified.

10 **[00203]** **Figure 6** illustrates the strategy for isolation of anti-rhAXL ECD sdAbs. In panels A and C, the phage-displayed VHH library constructed from a llama immunized with rhAXL-ECD was panned simultaneously on immobilized rhAXL and on AXL+ A549 tumour cells. VHHs simultaneously enriched for binding to rhAXL ECD and internalization into A549 cells (NRC-sdAb001, SEQ ID NO:119; NRC-sdAb002, SEQ ID NO:120) as well as VHHs
15 enriched only for internalization into A549 cells (NRC-sdAb003, SEQ ID NO:121; NRC-sdAb004, SEQ ID NO:122) were identified. In panels B and D, the phage-displayed VHH library constructed from a llama immunized with rhAXL-ECD was panned against rhAXL ECD and VHHs competitively eluted using AXL-specific mAbs 5E9 or 8D12. VHHs enriched using this selection strategy were identified using next-generation DNA sequencing.

20

[00204] Example 11: Determination of DNA and Amino Acid sequences of anti-AXL mAbs and sdAbs

[00205] In this example the DNA and amino acid sequences of anti-AXL mAbs and sdAbs (isolated according to Example 10) are described.

25 **[00206]** Materials and Methods

[00207] For murine mAb sequencing, total RNA was extracted from hybridoma clones (Qiagen, RNEasy) and reverse transcribed into cDNA (SuperScript™, ThermoFisher Scientific, Waltham, MA, USA). DNA encoding VH and VL domains was PCR amplified (Platinum Taq or equivalent) using mixtures of degenerate forward primers annealing in FR1
30 and a single reverse primer annealing in CH1 (Novagen/EMD Millipore cat. no 69831-3). The resulting amplicons were subcloned (TOPO-TA) and sequenced using the Sanger method on an ABI 3730xl instrument.

[00208] For determination of llama VHH DNA sequences, a phage-displayed VHH library was subjected to a variety of in vitro selections (described in Example 10) and sequenced using 2×250 bp reads on an Illumina MiSeq instrument. VHH sequences of interest were identified by increased frequency post-selection compared to prior. DNA constructs encoding VHHs were synthesized commercially (Geneart, Life Technologies) and subcloned into a bacterial expression vector, then confirmed again using the Sanger method on an ABI 3730xl instrument.

[00209] Results

[00210] The DNA sequences of the VH and VL domains of seven murine mAbs were determined by Sanger sequencing of amplicons derived from hybridoma cells. DNA constructs encoding the mAb VH/VL domains were synthesized commercially in mammalian expression vectors containing fragment crystallizable regions (Fc regions) and verified by Sanger sequencing.

[00211] **Figure 7** shows amino acid sequences of the mAb VH/VL domains with CDRs using International ImMunoGeneTics (IMGT) information system definitions represented in bold underlined font. IMGT definitions are used when referencing CDR sequences herein. Kabat definitions of CDRs are shown as shaded font in **Figure 7**, as an alternative definition to IMGT, primarily for interest only. IMGT definitions of CDRs may include slightly more N-terminal sequence, while Kabat definitions show CDRs as starting later and extending further C-terminally.

[00212] The DNA sequences of eight llama VHHs were determined using 2×250 bp reads on an Illumina MiSeq instrument (sequences were determined as part of the isolation of these VHHs, as described in Example 10). DNA constructs encoding the VHHs were synthesized commercially (GeneArt, Life Technologies) in a bacterial expression vector and their sequences were confirmed using Sanger sequencing.

[00213] **Figure 8** shows amino acid sequences of the VHHs with CDRs, using IMGT definitions, represented in bold underlined font. IMGT definitions are used when referencing CDR sequences herein. Kabat definitions of CDRs are shown in shaded font in **Figure 8**, as an alternative to IMGT. Sequences referenced as NRC-sdAb001 to NRC-sdAb008 are shown.

[00214] Example 12: Binding of mAbs and sdAbs to Recombinant AXL Extracellular Domain (ECD)

[00215] The purpose of this Example is to characterize binding of mAbs/sdAbs to recombinant AXL extracellular domain (ECD), evaluating affinity and kinetics, species cross-reactivity, and domain and epitope mapping.

[00216] Materials and Methods

5 [00217] Production of murine mAbs. Murine mAbs were either purified directly from hybridoma supernatant or produced recombinantly (with human IgG1 Fc regions) by transient transfection of CHO cells. For small-scale purification from 10 mL hybridoma supernatant, mAbs were purified via adsorption onto Protein G Mag Sepharose™ Extra (GE Healthcare Life Sciences #28-9670-70), washed in PBS, eluted twice with 200 µl of
10 glycine 200 mM pH 2.5 and neutralized in TRIS-HCl 1M pH 9.0. Purified mAbs were desalted using Zeba Spin resin (Pierce) pre-equilibrated in PBS and filter sterilized through 0.22 µm membrane (Millipore). The final concentration of the mAbs was determined by nano-drop (#ND-1000). For recombinant production, DNA constructs were transiently transfected in CHO cells and purified as described in Raymond et al. (2015).

15 [00218] Production of VHHs and VHH-Fcs. VHHs were produced as 6xHis- and Myc-tagged monomers in E. coli as described previously by Baral et al. (2013). VHH-Fcs were produced by transient transfection of HEK293 cells with DNA encoding VHHs fused N-terminally to human IgG1 Fc as described in Zhang et al., 2009.

[00219] Surface plasmon resonance. For murine mAbs, binding to human and
20 cynomolgus AXL ECD was assessed using a Biacore T200 instrument. Briefly, either anti-mouse or anti-human Fc antibody was immobilized on a research-grade CM5 series S sensor chip using amine coupling in acetate buffer. Murine or recombinant chimeric murine-human mAbs (mouse VH/VL; human constant regions) were captured, then recombinant human or cyno AXL ECD was flowed over the surface at different concentration ranges (0.1-
25 500 nM) depending on the antibody. The running buffer was PBS/Tween-20 and the flow rate 100 µL/min. All binding studies were performed at 25 °C. Surfaces were regenerated using 10 mM glycine, pH 1.5.

[00220] For VHHs and VHH-Fcs, binding to human and cynomolgus AXL ECD was assessed using either a Biacore T200 instrument or a Biacore 3000 instrument. Either
30 human AXL ECD or anti-human Fc antibody was immobilized on a research-grade CM5 series S sensor chip (T200, rhAXL ECD) or a C1 sensor chip (3000, anti-human Fc) using amine coupling in acetate buffer. VHH-Fcs were captured on anti-human Fc surfaces. Either monomeric VHHs (for rhAXL ECD surfaces) or human and cyno AXL ECD (for VHH-Fc

surfaces) were flowed over the surfaces at different concentration ranges. Running buffers were HBS-EP (3000) and HBS-EP+ (T200). Flow rates were 10-40 ul/min. All binding studies were performed at 25 °C. Surfaces were regenerated using 10 mM glycine, pH 1.5.

[00221] For epitope binning experiments, two test articles (VHH or mAb) were first injected separately (50 nM mAb; 25-fold K_D for VHH monomer) and allowed to dissociate. The mAb (50 nM) was then injected again and at the peak of the response, a mixture of VHH (25-fold K_D) and mAb (50 nM) was injected to observe any additional binding.

[00222] ELISA. ELISAs against human, cyno and mouse AXL ECDs were conducted by immobilizing 100 ng of each protein in wells of Nunc MaxiSorp microplates overnight. Human AXL (aa 1-451) and cyno AXL (aa 1-449) were produced recombinantly as described in Example 10. Mouse AXL was purchased from Sino (cat. 51026-M08H). The next day, plates were blocked with 5% (w/v) dried milk in PBS. Murine mAbs (either hybridoma- or recombinantly-produced) or VHH-Fcs were diluted in PBS containing 1% BSA and 0.1% Tween-20 and added to wells for 2 hours at room temperature. Wells were washed 5x with PBS containing 0.1% Tween-20, then HRP-labeled protein A (diluted 1:1000 in PBS containing 1% BSA and 0.1% TweenTM-20) was added to wells for 1 hour at room temperature. Wells were washed again 5x with PBS/0.1% Tween-20 and developed using TMB substrate. Absorbance was measured at 450 nm. As a positive control for mouse AXL binding, a commercial anti-mouse AXL mAb (clone 175128, R&D Systems MAB8541, rat IgG2A) was used and detected with donkey anti-mouse:HRP secondary antibody.

[00223] Yeast surface display. Yeast surface display of human AXL, Tyro3 and Mer extracellular domains was conducted. Briefly, yeast were transformed with DNA constructs encoding myc-tagged TAM-family RTK ectodomains fused to Aga2p protein. Constructs were designed to encode complete AXL ECD (aa 1-449) or individual subdomains (Ig1, Ig2, Fn1, Fn2 from N- to C-terminal). For fine epitope mapping, individual amino acids of AXL ECD were substituted with Ala in the constructs. The resulting yeast cells expressing partial or complete AXL, Tyro3 and Mer extracellular domains were adsorbed in microwell plates and binding of mAbs/VHH-Fcs were assessed by ELISA and detected using goat anti-human F(ab')₂-HRP. Anti-AXL mAb/VHH-Fc binding were normalized to binding levels of an anti-myc antibody to control for expression level.

[00224] Results

[00225] Binding of murine mAbs to human and cyno AXL ECD was assessed in SPR using chimeric murine-human mAbs produced recombinantly. Binding of llama VHHs to

human and cyno AXL ECD was assessed in SPR using both monomeric VHHs and chimeric recombinant VHH-hlgG1 Fc fusions.

[00226] Affinity determination by SPR. Affinities and kinetics of murine mAbs for recombinant human and cynomolgus AXL ECD are shown in **Table 9**.

5

Table 9						
Affinities and kinetics of mAbs for recombinant human and cynomolgus AXL ECD						
mAb	Human AXL			Cyno AXL		
	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (nM)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (nM)
mAb F107-10G1	1.1×10^6 ^a	5.9×10^{-4} ^b	0.5 ^a	8.2×10^5 ^a	4.3×10^{-4} ^a	0.5 ^a
	1.2×10^6 ^b	5.6×10^{-4} ^b	0.4 ^b	1.8×10^6 ^b	2.3×10^{-4} ^b	0.2 ^b
mAb F111- 5E9	7.8×10^5 ^a	1.8×10^{-3} ^a	2 ^a	6.9×10^5 ^a	2.7×10^{-3} ^a	4 ^a
mAb F107-8D12	2.7×10^6 ^a	1.3×10^{-2} ^a	5 ^a	-	-	1835 ^c
mAb F107-7H5	1.4×10^6	1.5×10^{-3}	1 ^a	-	-	925 ^c
mlgG2 F155-3C7	5.6×10^5 ^b	1.8×10^{-3} ^b	3 ^b	2.9×10^5 ^b	7.3×10^{-3}	25 ^b
mAb F111-3C8	8.6×10^4 ^a	1.0×10^{-3} ^a	12 ^a	6.0×10^4	1.1×10^{-3}	18 ^a
mlgG2 F149-4G4	7.5×10^5 ^b	1.2×10^{-3} ^b	1 ^b	-	-	>160 ^b
^a Measured using single-cycle kinetics ^b Measured using multi-cycle kinetics ^c Measured using steady-state analysis						

[00227] The data in **Table 10** indicate affinities and kinetics of llama VHHs for recombinant human and cynomolgus AXL extracellular domain (ECD).

Table 10						
Affinities and kinetics of llama V _H Hs for recombinant human and cynomolgus AXL ECD						
sdAb	Human AXL			Cyno AXL		
	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (nM)	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (nM)
NRC-sdAb001	8.5×10^6 ^a	3.7×10^{-2} ^a	4 ^a			

	1.3×10^6 ^c	7.2×10^{-3} ^c	6 ^c	9.2×10^5 ^c	7.2×10^{-3} ^c	8 ^c
NRC-sdAb002	-	-	96 ^e	nd	nd	nd
NRC-sdAb003	8.5×10^3 ^a	5.4×10^{-3} ^a	955 ^a	nd	nd	nd
NRC-sdAb005	1.7×10^5 ^b 1.2×10^5 ^c	9.7×10^{-3} ^b 4.1×10^{-3} ^c	58 ^b 34 ^c	1.3×10^5 ^c	4.6×10^{-3} ^c	35 ^c
NRC-sdAb006	2.4×10^5 ^b 8.9×10^4 ^c	4.5×10^{-3} ^b 2.1×10^{-3} ^c	19 ^b 24 ^c	1.0×10^5 ^c	2.6×10^{-3} ^c	26 ^c
NRC-sdAb007	-	-	>100 ^c	nd	nd	nd
NRC-sdAb008	3.3×10^6 ^d 5.3×10^6 ^c	3.3×10^{-3} ^d 8.2×10^{-3} ^c	1 ^d 2 ^c	-	-	>200 ^c
^a Measured using single-cycle kinetics (immobilize AXL ECD, flow VHH monomer) ^b Measured using multi-cycle kinetics (immobilize AXL ECD, flow VHH monomer) ^c Estimated using single-concentration kinetics (capture VHH-Fc, flow AXL ECD) ^d Measured using single-cycle kinetics (capture VHH-Fc, flow AXL ECD) ^e Measured using steady-state analysis						

[00228] The cross-reactivity of cynomolgus and murine AXL was determined, and results shown below in **Table 11**. These data show half-maximal effective concentrations EC₅₀ for mAb/sdAb binding to recombinant human and mouse AXL ECD in ELISA.

5

Table 11		
Half-maximal Effective Concentration for Binding ELISA		
mAb/sdAb	Human AXL (nM)	Mouse AXL (nM)
MAB8541 (R&D)	61	1
mAb F107-10G1	2	n.b.
mAb F111- 5E9	2	n.b.
mAb F107- 8D12	1	n.b.
mAb F107- 7H5	6	n.b.
mIgG2 F155- 3C7	14	n.b.
mAb F111- 3C8	ND	ND
mIgG2 F149-4G4	15	n.b.
NRC-sdAb001	1	n.b.
NRC-sdAb002	ND	ND

NRC-sdAb003	ND	ND
NRC-sdAb005	1	n.b.
NRC-sdAb006	1	n.b.
NRC-sdAb007	ND	ND
NRC-sdAb008	1	n.b.
n.b., no binding; ND, not determined		

[00229] The determination of cross-reactivity to TAM family RTKs (Mer, Tyro3) using Yeast Surface Display was determined, and results are shown in **Table 12**.

Table 12 Cross-Reactivity to TAM using Yeast Surface Display						
	Goat anti-human F(ab') ₂ -HRP at ~1:5000					Anti-Myc-HRP
	hFC-107-10G1	NRC-sdAb0001-Fc	NRC-sdAb0002-Fc	NRC-sdAb0003-Fc	hFC-anti-RSV	myc
huAXL-ECD	1.392	1.026	0.882	0.659	0.087	1.000
cyAXL-ECD	1.141	0.824	0.760	0.668	-0.004	1.000
huMER-ECD	-0.020	-0.010	0.010	-0.009	-0.001	1.000
huTyro3-ECD	-0.023	0.017	-0.004	-0.005	0.059	1.000
pNL6 vector	-0.039	-0.025	0.020	-0.025	-0.036	1.000
Non-denatured yeast: normalized to myc expression level						

5

[00230] Epitope binning was assessed, and data are summarized in **Table 13**. Epitope bins targeted by anti-AXL mAbs/sdAbs are shown. Domain mapping using yeast surface display was evaluated.

Table 13 Epitope bins targeted by Anti-AXL mAbs/sdAbs		
mAb/sdAb	Domain	Epitope Bin
mAb F107-10G1	Ig1	1A
mAb F111- 5E9	Ig2	2
mAb F107- 8D12	Ig1	1B
mAb F107- 7H5	Ig1	1B

mIgG2 F155- 3C7	ND	ND
mAb F111- 3C8	Ig2	ND
mIgG2 F149-4G4	ND	ND
NRC-sdAb001	ND (Ig1 based on binning) similar epitope to that of 10G1 based on binding competition	1A
NRC-sdAb002	ND	ND
NRC-sdAb003	ND (Ig1 based on binning)	1A
NRC-sdAb005	ND (Ig2 based on binning)	2
NRC-sdAb006	ND (Ig2 based on binning)	2
NRC-sdAb007	ND (Ig1 based on binning)	1B
NRC-sdAb008	ND (Ig1 based on binning)	1B

[00231] Example 13: Characterization of binding to tumor cell lines

[00232] The purpose of this Example is to characterize binding of mAbs/sdAbs to tumor cell lines expressing moderate to high levels of AXL in terms of K_d, and maximum antibody binding (B_{max})

[00233] Materials and Methods

[00234] Cell culture. The NCI-H292 human non-small cell lung cancer (NSCLC) and the SKOV3 (human ovarian adenocarcinoma) cell lines were obtained from ATCC and cultured according to supplier's recommendations. The MDA-MB-231 triple negative (ER-/PR-/HER2low) breast cancer (TNBC) was purchased from Cedarlane Labs, Burlington NC, USA. Stable transfectants with the firefly luciferase gene were generated. This cell line was cultured in RPMI-1640 + 5%FBS. U87 glioblastoma cells were used, as described above in Example 3. Cells were passaged twice a week and used within 4-6 weeks for all cell lines.

[00235] FACS binding assay. Adherent cells were dissociated with non-enzymatic Sigma Cell Dissociation Solution (Cat. No. C5789). Cells suspensions were added to polypropylene, V-bottom 96-well plates and incubated for 2 hours on ice with primary antibodies at concentrations ranging from 100 nM to 0.001 nM or at a single dose of 100 nM (for the non-specific control). After incubation and washes, cells were incubated with fluorescently labeled secondary antibody (Alexa Fluor® 488 anti-human IgG from Jackson Cat. No 709-546-098) for 1 hour on ice, then washed. Cell viability was determined using Fixable viability dye 450 (20 min on ice). Stained cells were fixed in 1% formaldehyde at 4°C and stored at 4°C until acquisition which was performed the next day.

[00236] Data acquisition was performed in the LSR-Fortessa Flow cytometer (Beckton Dickinson) equipped with FACS Diva Software and HTS unit (automated sampling in 96-well plates). FACS data was exported into Excel data files and MFI (Median Fluorescence intensity) of the AF488-stained, alive (single cells) peak was taken for calculations.

- 5 Background subtraction was calculated for all wells using the MFI values of the cells incubated in the absence of secondary antibody.

[00237] Background subtracted data was analyzed in GraphPad™ 6.0 using the One-site specific binding with Hill slope non-linear regression curve fit model to determine apparent Bmax and Kd for each of the test articles. The model used was according to

10 Formula I.

[00238]
$$\text{Model Y} = \frac{B_{\text{max}} \times X^h}{K_d^h + X^h} \quad (\text{Formula I})$$

[00239] Bmax is the maximum specific binding, in the same unit as Y.

[00240] Kd is the ligand concentration needed to achieve half maximum binding at equilibrium, expressed in the same unit as X.

- 15 **[00241]** The variable “h” is the hill slope.

[00242] AXL receptor density measurements. 10µl of Human AXL Alexa Fluor 488 MAb (Clone 108724) was used to label Quantum™ Simply Cellular Beads (Catalogue No. 814 from Bangs Laboratories, Fishers, IN, USA) for 30 min at 4 °C, or AXL expressing cell lines (100 000 cells/test) for 2h at 4 °C.

- 20 **[00243]** Data acquisition for beads and cells were performed under identical FACS settings and analysed using FlowJo™ software. The GeoMean was used to generate the standard curve and interpolate the values for each cell type. The blank beads value is considered as the detection threshold and the values of the unstained cells were subtracted from GeoMean values in each case. The resulting antibody binding capacity was used as a
25 measure of receptor density for each of the cell lines tested.

[00244] Results

[00245] Binding of recombinant chimeric Abs to three cell lines was assessed by FACS as described above. The apparent Bmax and Kd determination is shown below in
30 **Table 14.**

Table 14

Apparent Kd of human Fc containing mAbs in three AXL expressing cell lines			
Primary Ab	SKOV3 Kd (nM)	NCI-H292 Kd (nM)	MDA-MB-231 luciferase Kd (nM)
NRC-sdAb001	0.1122	0.1053	0.2327
NRC-sdAb005	2.0330	1.4740	1.4720
NRC-sdAb006	13.4300	2.1170	0.8317
NRC-sdAb008	0.0549	0.0414	0.1216
hIgG-F107-10G1	0.0608	0.0708	0.1012
hIgG-F107-7H5	0.0700	0.0509	0.1696
hIgG-F111-5E9	ND*	ND*	0.3659
*incomplete curve			

[00246] **Figure 9** illustrates Antibody Binding Capacity of AXL, based on receptor density (receptors/cell) in the following cell lines: H292, MDA-MB-231, SKOV, and U87 glioblastoma.

- 5 **[00247]** **Figure 10** illustrates FACS binding curves of human (h) IgG Abs and sdAbs in three cell lines for lung, breast and ovarian cancers. Non-specific binding (x), in all instances is shown are negligible while hIgG curve (F107-10G1, F107-7H5, F111-5E9) shows slightly higher mean of fluorescence intensity (MFI) than sdAbs (sdAB001, sdAb008, sdAb006) in some cases.

10

[00248] Example 14: In Vitro Cytotoxicity Assays

[00249] In this Example, anti-AXL monoclonal antibodies were evaluated for internalizing ability and antibody-drug conjugate (ADC) potential in a growth inhibition assay in AXL-expressing cells, in terms of potency (IC₅₀) and maximum percentage growth inhibition (efficacy).

15

[00250] Materials and Methods

[00251] Cell Culture. The NCI-H292 human non-small lung cancer (NSCLC) and the SKOV3 (human ovarian adenocarcinoma) cell lines were obtained from ATCC and cultured according to supplier's recommendations. The MDA-MB-231 triple negative (ER-/PR-/HER2low) breast cancer (TNBC) line was purchased from Cedarlane Labs. Stable transfectants with the firefly luciferase gene were generated by Perkin Elmer. This cell line was cultured in RPMI-1640+5%FBS. Cells were passaged twice a week and used within 4-6 weeks for all cell lines.

20

[00252] Growth Inhibition Assay. Anti-AXL ADCs were tested for their effects on viability on various cultured cell lines known to express AXL, including NCI-H292 (non-small cell lung cancer), MDA-MB-231 (human breast adenocarcinoma) and SKOV3 (ovarian carcinoma). Cells were seeded at 125, 200 and 200 cells/well for the three cell lines respectively in 384-well plates (Corning® 384 Well White Flat Bottom Polystyrene TC-Treated Microplates, Cat. # 3570). Cells were allowed to grow for five days in the presence of serial dilutions of the test articles or benchmark controls ranging from 100 nM to 0.0017 nM and in the presence or absence of added Gas6 ligand (2 µg/mL, R&D CF885). After five days (37°C, 5% CO₂, humidified incubator), the number of viable cells in culture was determined using CellTiterGlo™ (Promega, Madison), based on quantitation of the ATP present in each well, which signals the presence of metabolically active cells.

[00253] Signal output was measured on a luminescence plate reader (Envision, Perkin Elmer) set at an integration time of 0.1 sec. Integration time is adjusted to minimize signal saturation at high ATP concentration.

[00254] Data analysis. Each concentration point (S) is normalized to the negative control wells (NC) and expressed as % survival, calculated according to Formula II.

$$\% \text{ survival} = \frac{NC - S}{NC} \times 100 \quad (\text{Formula II})$$

[00255]

[00256] Dose-response curves of % survival vs. log concentration were fit using GraphPad™ Prism 6.0 with a four parameter logistic model to estimate IC₅₀ and maximum efficacy. Model Y was used to calculations according to Formula III.

$$\text{Model Y} = \frac{\text{Bottom} + (\text{Top} - \text{Bottom})}{1 + 10^{(\log(\text{IC}_{50}) - \log(\text{Conc})) \times \text{HillSlope}}} \quad (\text{Formula III})$$

[00258] IC₅₀ is the concentration of agonist that gives a response half way between Bottom and Top.

[00259] HillSlope describes the steepness of the curves.

[00260] Top and Bottom are plateaus in the units of the Y axis.

[00261] Results

[00262] Growth inhibiting activity of recombinant chimeric Abs conjugated to DM1 or MMAE in three cell lines was assessed as described above.

[00263] The cytotoxicity as direct drug conjugates was evaluated, as shown in **Table 15**. Potency (IC₅₀) and efficacy (% maximum inhibition) of anti AXL ADCs in three AXL expressing cell lines. Results are expressed as: average +/- stdev (N).

Table 15 Potency and efficacy of anti AXL ADCs in AXL expressing cell lines.						
	SKOV3		MDA-MB-231-luc		NCI-H292	
	IC50 (nM)	% max inh	IC50 (nM)	% max inh	IC50 (nM)	% max inh
hFc-F111-5E9-VC-MMAE	0.021 +/- 0.006 (2)	75 +/- 3 (2)	0.942 (1)	105 (1)	~1 (1)	incomplete curve (1)
hFc-F111-5E9-DM1	0.038 +/- 0.043 (3)	73 +/- 7 (3)	0.452 +/- 0.279 (6)	82 +/- 20 (6)	0.084 +/- 0.060 (9)	78 +/- 9 (9)
hFc-sdAb005-DM1	0.631 +/- 0.202 (2)	78 +/- 6 (2)	ND	ND	6.664 +/- 1.718 (6)	83 +/- 4 (6)
hFc-sdAb006-DM1	0.162 +/- 0.03 (2)	76 +/- 6 (2)	0.869 +/- 0.001 (2)	83 +/- 2 (2)	2.714 +/- 0.871 (2)	87 +/- 13 (6)
hFc-F107-10G1-DM1	0.022 (1)	66 (1)	0.029 +/- 0.037 (2)	62 +/- 9 (2)	0.013 +/- 0.014 (4)	71 +/- 5 (4)
hFc-sdAb001-DM1	0.055 +/- 0.006 (2)	81 +/- 0 (2)	ND	ND	0.887 +/- 0.218 (6)	81 +/- 3 (6)
hFc-F107-7H5-DM1	0.014 +/- 0.019 (2)	91 +/- 31 (2)	0.076 +/- 0.079 (2)	65 +/- 13 (2)	0.014 +/- 0.026 (9)	97 +/- 50 (9)
hFc-F107-8D12-DM1	0.001 (1)	103 (1)	0.033 (1)	72. (1)	0.011 +/- 0.006 (3)	89 +/- 1 (3)
hFc-sdAb008-DM1	~0.001	~80	ND	ND	0.034 +/- 0.012 (6)	83 +/- 8 (6)
mlgG-F155-3C7-DM1	0.092 (1)	89 (1)	0.630 (1)	74 (1)	0.327 +/- 0.098 (8)	82 +/- 5 (8)
mlgG-F149-4G4-DM1	0.005 (1)	90 (1)	0.051 (1)	64 (1)	0.015 +/- 0.009 (8)	79 +/- 4 (8)

[00264] Assessment of Gas6 sensitivity. The potency (IC₅₀) of anti-AXL ADCs in NCI-H292 in the presence or absence of Gas6 ligand (2 µg/mL) was evaluated. Results are expressed in **Table 16** as: average +/- stdev (N).

5

Table 16 Assessment of Gas6 Sensitivity		
	-Gas6 IC50 (nM)	+Gas6 IC50 (nM)
hFc-F111-5E9-VC-MMAE	~1 (1)	ND
hFc-F111-5E9-DM1	0.084 +/- 0.060 (9)	0.074 +/- 0.050 (2)
hFc-sdAb006-DM1	2.714 +/- 0.871 (2)	2.2.6 (1)
hFc-F107-10G1-DM1	0.013 +/- 0.014 (4)	0.460 (1)
hFc-sdAb001-DM1	0.887 +/- 0.218 (6)	not convergent (1)
hFc-F107-7H5-DM1	0.014 +/- 0.026 (9)	0.098 +/- 0.035 (2)
hFc-F107-8D12-DM1	0.011 +/- 0.006 (3)	0.296 (1)
hFc-sdAb008-DM1	0.034 +/- 0.012 (6)	0.240 (1)

mlgG-F155-3C7-DM1	0.327 +/- 0.098 (8)	3.900 +/- 0.780 (2)
mlgG-F149-4G4-DM1	0.015 +/- 0.009 (8)	0.013 +/- 0.002 (2)
hFc-sdAb005-DM1	6.664 +/- 1.718 (6)	3.777 (1)

[00265] Growth inhibition was assessed, and growth inhibition binding curves of hlgG Abs and sdAbs in three tumor cell lines are shown in **Figure 11**. Lung, breast and ovarian cell lines (H292, MDA-MB-231 luciferase, and SKOV3, respectively) are shown. Percent surviving fraction (5d) is evaluated.

[00266] In Class 1, hlgG-F107-10G1-DM1 versus non-specific binding are shown for all cells, with sdAb001 also evaluated for H292.

[00267] In Class 2, hlgG-F107-7H5-DM1 and hlgG-F107-8D12-DM1 versus non-specific binding curves are shown for MDA-MB-231 luciferase and SKOV3 cells. sdAb008 and hlgG-F107-7H5-DM1 were evaluated versus non-specific binding for H292.

[00268] In Class 3, hlgG-F111-5E9-DM1 and hlgG-F111-5E9-MMAE versus non-specific binding are shown for all cells, with sdAb006 also evaluated for H292.

[00269] **Figure 12** illustrates growth inhibition binding curves of hlgG-F11-5E9-DM1 and hlgG-F107-10G1-DM1 in the presence and absence of Gas6 in H292 cell line, as compared with non-specific binding.

[00270] Example 15: Assessment of activity of biparatopic/monovalent anti-AXL antibodies.

[00271] In this example, biparatopic/monovalent anti-AXL antibodies were prepared and assessed for potency and efficacy.

[00272] Biparatopic antibodies targeting two non-overlapping epitopes can induce target clustering, which in turn promotes robust internalization, lysosomal trafficking, of receptor targets, including HER2 (Li et al. 2016). This example evaluates the ADC potency of biparatopic (2 epitopes on same target) combinations of single domain anti-AXL antibodies. Human Fc containing biparatopic antibodies were constructed according to the method published by Strop et al. (2012) in which complementary point mutations (RRR, EEE) are engineered in human IgG1 Fc regions to facilitate half monomer exchange and stabilize the bispecific antibody. The two antibodies of interest can be expressed and purified separately, and mixed together under appropriate redox conditions, resulting in a formation of a stable

bispecific antibody. In all cases, the activity of biparatopic ADCs was compared to the potency of homobivalent monoparatopic sdAb-based ADCs from which they were derived .

[00273] **Table 17** shows the potency (IC₅₀) and efficacy (% maximum inhibition) of anti AXL biparatopic construct ADCs compared to monoparatopic (bivalent) parental ADCs in SKOV3 cell line. Where available, results are expressed as: average +/- stdev (N).

Table 17 Potency (IC ₅₀) and efficacy (% maximum inhibition) of anti-AXL biparatopic and monoparatopic (bivalent) ADCs in SKOV Cell Line					
Sample name	Antibody design	MW	DAR	IC50 (nM)	% max inh
sdAb001-DM1	Monoparatopic	77200	3.13	0.055 +/- 0.006 (2)	81 +/- 0 (2)
sdAb005-DM1	Monoparatopic	77200	3.05	0.631 +/- 0.202 (2)	78 +/- 6 (2)
sdAb006-DM1	Monoparatopic	77800	2.77	0.162 +/- 0.03 (2)	76 +/- 6 (2)
sdAb008-DM1	Monoparatopic	78200	3.08	<0.001	~80
sdAb 001R/005E-DM1	Biparatopic	77200	3.6	0.008	89
sdAb 001R/006E-DM1	Biparatopic	77500	3.39	0.0004	73
sdAb 008R/005E-DM1	Biparatopic	77700	2.61	0.014	78
sdAb 008R/006E-DM1	Biparatopic	78000	2.69	0.003	70

[00274] Preliminary results indicate that both ADCs comprising biparatopic sdAbs directed epitopes found in Domain 1A/domain 2 (sdAb 001R/005E-DM1, sdAb 001R/006E-DM1) appear much more potent (7-100x) than ADCs derived from the corresponding bivalent Fc containing sdAbs. Other biparatopic combinations tested in this example did not exhibit increased potency.

[00275] **Figure 13** shows growth inhibition binding curves of anti AXL biparatopic/monovalent constructs ADCs compared to monoparatopic/bivalent parental ADCs in SKOV3 cell line. Percent surviving fraction after 5 days is plotted as a log concentration (nM). Panel A provides the chart of SKOV3 combination 008R/005E, sdAb008-DM1 (squares), sdAb005-DM1 (triangles), and biparatopic 008/005 (stars) are compared. Panel B provides the illustrated SKOV3 combination 008R/006E chart: sdAb006-

DM1 (diamonds), sdAb008-DM1 (squares), and biparatopic 008/006 (hollow circles) are compared. Panel C provides the chart of hFc/sdAb001R/005E, sdAb005-DM1 (triangles), sdAb001-DM1 (solid circles), and the biparatopic 001/005 construct (shown as "X") are compared. Panel D provides the chart of SKOV3 combination 001R/006E, sdAb001-DM1 (solid circles), sdAb006-DM1 (diamonds) and the biparatopic 001/006 construct (large asterisks) are compared.

[00276] Example 16: In Vivo Tumor Growth Inhibition by ADCs in SKOV3 tumor Xenograft Mouse Models

[00277] In this Example, in vitro cytotoxicity is evaluated using inhibition of tumor growth inhibition in SKOV3 cells. Anti-hAXL antibody-drug conjugates ADCs were evaluated for ability to cause tumor growth inhibition in xenograft models expressing AXL.

[00278] Materials and Methods

[00279] Protocol. The protocol and procedures involving the care and use of animals in this study were reviewed and approved by Ottawa-NRC Animal Care Committee (Protocol # 2014.02). Six week old, 18-20 grams female CD1 Albino mice (CrI:CD1-Foxn1nu), were ordered from Charles River Canada (St-Constant, Quebec, Canada). Animals were cared for and used in accordance with the guidelines of the Canadian Council on Animal Care (CCAC).

[00280] The anti-hAXL-ECD monoclonal antibodies were purified as outlined in Example 1 and were conjugated via lysine residues to succinimidyl trans-4-[maleimidylmethyl] cyclohexane-1-carboxylate (SMCC) linked to N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)-maytansine (DM1) as in Example 4.

[00281] Cell culture and tumor growth measurements. SKOV3 (human ovarian adenocarcinoma) cells were obtained from ATCC and cultured according to supplier's recommendations. The MDA-MB-231 triple negative (ER-/PR-/HER2low) breast cancer (TNBC) was purchased from ATCC (Cedarlane Labs). Stable transfectants with the firefly luciferase gene were generated by Perkin Elmer. This cell line was cultured in RPMI-1640+5%FBS.

[00282] Cells were passaged twice a week and used within 4-6 weeks for all cell lines. Cells were subcutaneously inoculated (5×10^6 cells in a volume of 0.1 mL PBS per injection site) in the left flank of isoflurane anesthetized nude mice (n=8) under sterile condition. Tumors were allowed to grow to a size between 80 and 100 mm³ in volume, after which

animals were randomized one day prior to the dosing day to ensure that each cohort contains animals with variable tumor sizes. The appropriate volume of test/control articles for each animal was prepared on the day of dosing and injected intravenously (i.v.). Mice were injected with the test articles at 5 mg/kg on day 0 and 4 (96 h interval) via tail vein. A control group was treated with saline.

[00283] Tumor growth was monitored every three days by caliper measurement for 29 days post treatment or until they were euthanized for ethical reasons (humane endpoints).

[00284] Tumor volumes were calculated according to Formula IV:

[00285] Estimated tumor volume [mm³] =

[00286] $\pi/6(\text{length [mm]} \times (\text{width [mm]} \times (\text{height [mm]})))$ (Formula IV)

[00287] Tumor volumes in each group are shown as mean $\pm$ SEM and plotted as a function of measurement time after SKOV3 cell inoculation. Group comparisons for the tumor volume data were conducted using two-way ANOVA with Tukey's multiple comparisons test using GraphPad Prism version 7.0. Differences between treatment and control groups were statistically significant at P < 0.05.

[00288] Results

[00289] Compared to vehicle (PBS control) treated animals, AXL ADCs administered at 5 mg/kg (mpk) were shown to cause significant tumor growth inhibition in SKOV3 tumor xenograft mouse models (n=2). A high reproducibility in tumor growth was observed in both control and treated groups in two separate studies (referenced herein as "Study 15A" and "Study 15b"). The data from these two separate studies are represented in **Figure 14** and **Figure 15**.

[00290] **Figure 14** shows tumor growth inhibition in Study 15a for SKOV3 tumor-bearing mice treated twice (day 0 and 4) with selected ADCs at 5 mg/kg. The ADCs used were: hF107-10G1-DM1 (2a), hF111-5E9-DM1 (3a); hF107-7H5-DM1 (4a); hF107-8D12-DM1 (5a); mF149-4G4-DM1 (6a); and mF155-3C7-DM1 (7a), for comparison with saline (1a) Tumor volumes (mm³) were recorded every three days. Each data point represents mean $\pm$ SEM, (n=8).

[00291] **Figure 15** shows tumor growth inhibition in Study 15b for SKOV3 tumor-bearing mice treated twice (day 0 and 4) with selected ADCs at 5 mg/kg. The ADCs used were: hF111-5E9-DM1 (2b); hF107-7H5-DM1 (3b); hF107-10G1-DM1 (4b); sdAb001-DM1 (6b); sdAb006-DM1 (7b); sdAb008-DM1 (8b); and mF149-4G4-DM1 (9b) for comparison with

saline control (1b). Tumor volumes (mm³) were recorded every three days. Each data point represents mean $\pm$ SEM, (n=8).

[00292] A statistically significant ($p < 0.05$) effect on tumor growth between ADC-treated and saline-treated groups was observed from days 13-18 to day 28 with a tumor volume reduction of from 37.0% to 66.9% at day 28.

[00293] Table 18 shows the percentage of tumor volume reduction compared to the control groups (saline) at day 28 post-treatment initiation, for the data of Study 15a and 15b (see Figure 14 and Figure 15, respectively). Percentage of tumor volume (TV) reduction compared to control was calculated as: $\% \text{ reduction} = (TV_{\text{control}} - TV_{\text{treated}}) / TV_{\text{control}} * 100$ (Formula V).

Table 18 Percentage of Tumor Volume Reduction by ADCs in SKOV3 Xenograft Mouse Model		
Study Number	Test ADC	% of tumor volume reduction compared to control
Study 15A Figure 14	hF107-10G1-DM1	57.9 $\pm$ 2. 9
	hF111-5E9-DM1	62.5 $\pm$ 5.0
	hF107-7H5-DM1	48.9 $\pm$ 3.2
	hF107-8D12-DM1	55.4 $\pm$ 4.4
	mF149-4G4-DM1	54.4 $\pm$ 9.0
	mF155-3C7-DM1	38.4 $\pm$ 10. 7
Study 15B Figure 15	hF111-5E9-DM1	61.0 $\pm$ 5.1
	hF107-7H5-DM1	66.9 $\pm$ 3.1
	hF107-10G1-DM1	63.7 $\pm$ 7.3
	sdAb001-DM1	47.6 $\pm$ 6.8
	sdAb006-DM1	47.7 $\pm$ 9.48
	sdAb008-DM1	37.0 $\pm$ 16.0
	mF149-4G4-DM1	54.2 $\pm$ 10.3

[00294] Example 17: Evaluation of efficacy of Anti-AXL ADC hits in MDA-MB-231-Luc xenograft mice following repeated intravenous administrations

[00295] AXL is expressed in various types of cancer and associated with invasiveness, metastasis as well as angiogenesis. Anti-AXL ADCs generated have anti-AXL antibody conjugated to microtubule inhibitor, mertansine (DM1) via SMCC linker. These ADCs show promising in vitro efficacy in various cell lines and have been selected based on potency and Gas6 sensitivity. In this Example several Anti-AXL ADC hits are screened and the the role of Gas6 sensitivity on in vivo efficacy of anti-AXL ADCs is evaluated.

[00296] Efficacy and pharmacokinetics are evaluated for several Anti-AXL ADCs following two intravenous bolus administration to female NU/NU nude mice. This Example further investigates the role of Gas6 sensitivity on efficacy of Anti-AXL ADCs.

[00297] Methods

[00298] Cell Line. MDA-MB-231 is a triple negative (ER⁻, PR⁻, HER2⁻) human mammary gland adenocarcinoma cell line, which are epithelial adherent cells originally derived from a 51 years Caucasian female patient. These cells are tumorigenic in nude mice forming moderately well differentiated adenocarcinoma consistent with primary ovarian tumors. MDA-MB-231 cells are tumorigenic cells that express epidermal growth factor (EGF) and transforming growth factor alpha (TGF α). These cells form poorly differentiated adenocarcinoma (grade III) in ALS treated BALB/c mice and form poorly differentiated adenocarcinoma (grade III) in nude mice. The cell line was originally obtained from the American Tissue Culture Collection (ATCC, Manassas, Virginia, USA).

[00299] Cells were grown in ATCC-formulated RPMI 1640 medium (ATCC catalog #: 30-2001) with 10% fetal bovine serum (FBS, Hyclone SH30070.03) to a maximum of 80% confluence. Cells were maintained in a humidified 5% CO₂ atmosphere at 37°C. They were harvested by trypsinization (0.25% trypsin/EDTA, Gibco/BRL 15090-046) followed by washing in cold phosphate-buffered saline solution (PBS) and assessed for viability by capacity to exclude trypan blue dye. All cell populations for growth as xenografts in mice were, at a minimum, 98% viable.

[00300] Animal Management: Receipt, Acclimation, Housing and Handling. The protocol and procedures involving the care and use of animals in this study were reviewed and approved by Ottawa-NRC Animal Care Committee (Protocol # 2014.02). The care and use of animals were in accordance with the guidelines of the Canadian Council on Animal Care (CCAC). Six week old, 18-20 grams female CD1 Albino mice (CrI:CD1-Foxn1nu), were ordered from Charles River Canada (18 LaSalle, St-Constant, Quebec, Canada). Upon arrival, the mice were subjected to a general physical examination by the staff members to

ensure normal health status. No obvious abnormalities were detected in the animals received. The mice were acclimated for a period of at least 5 days to allow them to become accustomed to the housing environment. Mice were housed 4 per cage in polycarbonate cages on corn cob bedding, equipped with a filter top to avoid contamination. Each cage was
5 clearly labelled with a color-coded cage card indicating protocol number, principal investigator's name, animal number and gender. Each mouse was uniquely identified using ear punching.

[00301] The mice were housed in a positively-pressured Tecniplast Green Line Individually Ventilated Cages (IVC) set for 75 air changes per hour under the following
10 conditions: Temperature: 23-24 °C; Humidity: 50%; Light cycle: 12 hours light and 12 hours dark.

[00302] Aseptic techniques were used at all times when handling the mice. All equipment used in this study was sterilized with 70% ethanol prior to using. Animals were handled under the Biological Safety Cabinet (BSC) which was also disinfected with 70%
15 ethanol each time before use. All individuals handling the mice worn a steam sterilized gown, mask, hair bonnet and sterile gloves. The autoclaved IVC cages filled with 1/8" irradiated corncob bedding (Envigo, Madison, WI) and a small volume of Enviro-dri paper (Shepherds Specialty Papers) were changed at least once weekly in a BSC.

[00303] Diet. All animals had free access to food and water. Water bottles were filled
20 with ultra-filtered water and placed with caps separately in old mouse shoebox cages and steam autoclaved. Once autoclaved the caps were installed while working in the confines of the BSC. The water bottles were changed once a week or more frequently when the water levels were low. Animals were fed ad libitum, gamma-irradiated maintenance rodent diet (2914 Teklad Global 14% Protein Maintenance Diet, Envigo) during the acclimation and
25 experimental periods.

[00304] Subcutaneous xenograft tumor model. Cells were subcutaneously inoculated (5x10⁶ cells in a volume of 0.1 mL PBS per injection site) in the left flank of isoflurane anesthetized nude mice (n=8) under sterile condition. Tumors were allowed to grow to a size
30 between 80 and 100 mm³ in volume, after which animals were randomized one day prior to the dosing day to ensure that each cohort contains animals with variable tumor sizes.

[00305] Tumor monitoring. Tumor growth was monitored every three days by caliper measurement for 29 days post treatment or until they were euthanized for ethical reasons (humane endpoints): 1) more than 10% body weight loss without weight recovery within 48

hours; 2) tumor volume over 2500 mm³; 3) tumor ulcerations; and 4) clear signs of distress such as immobility and reduced grooming etc.

[00306] Tumor volumes were calculated according to Formula IV:

[00307] Estimated tumor volume [mm³] =

5 **[00308]** $\pi/6(\text{length [mm]} \times (\text{width [mm]} \times (\text{height [mm]})))$ (Formula IV)

[00309] Test Articles and Vehicle. All test articles were shipped on ice from NRC Montreal to Ottawa and stored at 4°C before administration. The ADC, hF111-5E9-DM1 was prepared as batch number: CC31MAY 2016; Drug to Antibody Ratio (DAR): 2.62; Concentration: 1.31mg/mL; Aliquot Volume: 3.7mL; Storage Conditions: 4°C; Endotoxin
10 Levels: <0.15; prepared at the NRC facility HHT Montreal, in Montreal, Quebec, Canada.

[00310] The vehicle for treatment was an ADC buffer with the following characteristics: Sterility: Sterile-filtered; Storage Conditions: 20-25°C; Supplier: NRC- HHT Montreal.

[00311] Dose Formulation Preparation. Each formulation was prepared on the day of dosing. To obtain a dose level of 5 mg/kg, the injection volume of test/control article was
15 calculated based on their stock concentrations and body weight of the animals. Preparation of the final dosing solutions was done under a clean HEPA/UV lamp biological hood by adding saline to obtain the desired volumes. The test/control articles were kept at 4°C during the preparation period. The appropriate volume of test/control articles for each animal was injected intravenously.

20 **[00312]** Clinical Observations and Body Weight. All animals were observed once daily for mortality and signs of ill health. Individual body weight and tumor size of animals was measured and recorded on the day of treatment and every three days afterwards.

[00313] Treatment. Mice were injected with the test articles at 5 mg/kg on day 0 and 4 (96 h interval) via tail vein. A control group was treated with saline. The animal weight on the
25 day of injection and the injected volume were recorded.

[00314] Sample Collection, Processing and Storage. Blood samples were collected via the mandibular vein at 1, 2, 4, 6, 24, 48, 96, 120, 168, 216, 264 and 336 hours post second dose from three animals per time-point.

[00315] **Figure 16** shows the study design, including where blood samples were taken
30 at: pre-2nd dose, 1h, 2h, 4h, 6h, 24h, 48h, 96h, 120h, 168h, 216h, 264h, and 336h. Tumor volume measurements were taken, and dosing was at days 1 and 4, while tumor volume measurements were taken at intervals indicated. Also shown in **Figure 16**, below the study design, are the animal numbers and time points for the study. Approximately 80-100 µL of

the obtained blood was allowed to clot at room temperature for 15-30 minutes and then centrifuged at 1500 g for 10 minutes at room temperature. Following centrifugation, the liquid component (serum) was immediately, transferred into the pre-labelled tubes, snap-frozen immediately on dry ice and stored at -80°C.

5 **[00316]** At the end of the study, tumors were collected. Half portion of each tumor was fixed in 4% paraformaldehyde at room temperature overnight, washed with PBS and paraffin embedded. Subsequently, the paraffin embedded tumors will be processed for AXL expression as per discretion of project lead. The other half of the tumor was rapidly frozen using dry ice and stored in -80°C for further analysis.

10 **[00317]** Statistical Analysis. Tumor volumes in each group are shown as mean $\pm$ SEM and plotted as a function of measurement time after MDA-MB-231-Luc cell inoculation. Group comparisons for the tumor volume data were conducted using two-way ANOVA with Tukey's multiple comparisons test using GraphPad™ Prism version 7.0. Differences between treatment and control groups were statistically significant at $P < 0.05$. Grubbs' test was used
15 to determine outliers within the control/treatment groups ($\alpha=0.05$).

[00318] Results & Discussion

[00319] **Figure 17** shows the effect of anti-AXL ADCs on body weight, as a percentage of initial body weight (BW) in MDA-MB-231-Luc tumor-bearing mice after being treated twice (day 0 and 4) with hF111-5E9-DM1 at 5 mg/kg. The ADC buffer (circles) and
20 hF111-5E9-DM1 (triangles) groups are illustrated.

[00320] Body weight was measured as an indicator of off-target potential toxicity. No significant difference was observed between the control animals (ADC buffer group) and hF111-5E9-DM1 treated animals. The mice in both groups gained about 10% body weight during the experimental period.

25 **[00321]** **Figure 18** shows the effect of anti-AXL ADCs on tumor growth, assessed as tumor volume. Tumor growth curves in MDA-MB-231-Luc tumor-bearing mice treated twice (day 0 and 4) with either ADC buffer (CTR) or 5 mg/kg hF111-5E9-DM1 are shown, with tumor volumes (mm^3) being recorded every three days. Each data point represents mean $\pm$ SEM, for ADC buffer (circles) and hF111-5E9-DM1 (triangles) groups.

30 **[00322]** A statistically significant ($p<0.05$) effect on tumor growth was observed between hF111-5E9-DM1 -treated and ADC buffer-treated groups from days 8 to day 28. One animal from the hF111-5E9-DM1 group showed significantly higher tumor growth rate

compared to the rest in the same group. This animal was further identified as an outlier using the Grubbs' test ($\alpha=0.05$).

[00323] The average tumor volume reduction in the hF111-5E9-DM1 -treated group was from about 90 to 60 mm³, compared to the control group, which experienced tumor growth from about 90 to 360 mm³ at day 28. This striking difference in reduced tumor volume shows that anti-AXL ADCx can reduce tumor volume in a breast cancer tumor model.

[00324] Example 18: Evaluation of anti-AXL sdAbs ability to cross the Blood Brain Barrier in vitro

[00325] The ability of anti-AXL antibodies to cross the blood brain barrier is evaluated in this example. Due to the expression of AXL in the central nervous system (CNS), antibodies targeting AXL and linked to a toxin (such as DM1, MMAE, etc) could exhibit very potent on-target toxicity in the brain if they are able to cross the blood brain barrier (BBB). Thus, the capacity of certain anti-AXL sdAbs to cross the BBB is evaluated using a human in vitro BBB model.

[00326] MATERIAL AND METHODS

[00327] Human Blood Brain Barrier Model. A stem cell based human BBB model is used. The model utilizes brain endothelial cells (BECs) generated from human amniotic fluid-derived induced pluripotent stem cells. This in vitro human blood-brain barrier (BBB) model enables pre-clinical screening of prospective BBB-permeant compounds and CNS-targeting antibodies. These BECs exhibit substantial barrier properties such as well-organized continuous tight junctions, high transendothelial electrical resistance (TEER) and polarized expression of efflux and receptor-mediated transcytosis (RMT) transporters.

[00328] Transwell-based in vitro BBB model. The Transwell BBB assay is composed of BECs seeded onto gelatin-coated permeable Transwell membrane inserts that are placed into 12-well companion plates. BEC were seeded at 500,000 cells/membrane on rat-tail collagen coated 0.83 cm² Falcon™ cell inserts, 1 μ m pore size in 1 mL of BEC feeding medium without phenol red. The wells of a 12-well tissue culture plate (i.e., bottom chamber for transport) contained 2 mL of BEC medium without phenol red. The TEER values were measured for each insert and only inserts with a TEER of $> 300\Omega\text{cm}^2$ were used for transcytosis studies. Transport experiments were performed in the transport buffer (10 mM HEPES, 5 mM MgCl₂, and 0.01% BSA in phosphate buffered saline, pH 7.4; 1 mL upper chamber and 2 mL bottom chamber) in "multiplexed" fashion by adding a mixture of test

antibody (**Table 19**) and negative control (A20.1) at 1.25 μ M of each to the top chamber and by collecting 100 μ L aliquot from the bottom chamber at 15, 30, 60 and 90 min (followed by the replacement of 100 μ L of transport buffer into the bottom chamber after each aliquot collection) for simultaneous quantification of all test antibodies by nanoLC-MS/MS (multiple reaction monitoring – MRM).

[00329] Specific peptide signatures for the test articles and for A20.1 were determined for MRM analysis. For test antibodies, Fc peptide (TTPPVLDSDGSFFLYSK; SEQ ID NO:187) and for negative control A20.1 peptide (EFVAAGSSTGR; SEQ ID NO:188) were detected by MRM. The integrity of the BBB during the period of the assay was monitored by incorporation of a BBB non-crossing sdAb (A20.1) in each test sample. FC5Hfc1X0 was used as a positive control and TWIN200-hlgG1 as a negative control. The sdAbs and control test articles evaluated in this study are outlined in **Table 19**. The experiment was performed in triplicates.

Table 19 Test Articles (Anti-AXL sdAbs fused to Fc domain of hlgG1) and Control Molecules used in in vitro BBB Assay*										
Code	# of inserts	Time (min)	Test Antibody				Negative Control			
			Name	Detail	MW	Top μ M	Name	Details	MW	Top μ M
AOL	3	90	FC5hFc1X0	AP16 0408	78334	1.25	A20.1	140915	15670	1.25
AOM	3	90	NRC-sdAb001-hlgG1	-	76853	1.25	A20.1	140915	15670	1.25
AON	2	90	NRC-sdAb002-hlgG1	-	79514	1.25	A20.1	140915	15670	1.25
AOO	1	90	NRC-sdAb003-hlgG1	-	77267	1.25	A20.1	140915	15670	1.25
AOP	2	90	NRC-sdAb004-hlgG1	-	78810	1.25	A20.1	140915	15670	1.25
AOQ	3	90	NRC-sdAb005-hlgG1	-	76915	1.25	A20.1	140915	15670	1.25
AOR	3	90	NRC-sdAb006-hlgG1	-	77496	1.25	A20.1	140915	15670	1.25
AOS	3	90	NRC-sdAb008-hlgG1	-	77838	1.25	A20.1	140915	15670	1.25
AOT	1	90	TWIN200-hlgG1		79009	1.25	A20.1	140915	15670	1.25

*For test antibodies, Fc peptide (SEQ ID NO:187) and for negative control A20.1 peptide (SEQ ID NO:188) were monitored (MRM).

[00330] RESULTS

[00331] A comparison of in vitro Papp values of FC5Hfc1X0 (positive control), TWIN200-hlgG1 (negative control) and seven anti-AXL sdAbs is shown in **Figure 19**. Control molecules of various molecular weights, 15.6 kDa (A20.1), and 79.0 kDa (TWIN200-hlgG1), showed similar low Papp values, ranging between about $9.0\text{--}15.0 \times 10^{-6}$ cm/min; in contrast, Papp values for FC5Hfc1X0 were about 175×10^{-6} cm/min (>10-fold higher than A20.1). All the anti-AXL sdAbs exhibited Papp values similar to the negative controls, indicating no significant penetrability of the in vitro BBB, except for NRC-sdAb004 and NRC-sdAb005 antibodies which show low Papp values (of about 2-fold) relative to their respective controls. The grey line at the bottom of the chart indicates Lower Level of Quantification (LLOQ). The asterisk shown in the chart for NRC-sdAb006-hlgG1 indicates leaky insert AOR1 data was removed.

[00332] **Table 20** provides the Papp data, and shows the statistical analysis of the Papp values for the test articles compared to A20.1 (negative control).

Table 20 Papp Values and Statistical Significance			
Test Ab	Papp of Test Ab	Papp of A20.1	p-value (Test vs. A20.1)
FC5hFc	175.8 ± 26.3	11.9 ± 0.8	$p < 0.001$
NRC-sdAb001-hlgG1	9.56 ± 1.52	10.7 ± 2.6	ns
NRC-sdAb001-hlgG1	7.02 ± 1.04	14.1 ± 2.2	ns
NRC-sdAb002-hlgG1	5.22 ± 0.0	9.15 ± 0.0	ns
NRC-sdAb003-hlgG1	9.17 ± 0.21	12.5 ± 0.3	ns
NRC-sdAb004-hlgG1	17.1 ± 8.3	9.75 ± 1.25	$p = 0.01$
NRC-sdAb005-hlgG1	24.3 ± 10.2	10.9 ± 0.2	$p = 0.01$
NRC-sdAb006-hlgG1	11.4 ± 3.3	11.8 ± 2.9	ns
NRC-sdAb008-hlgG1	13.6 ± 0.0	12.5 ± 0.0	ns

[00333] In the preceding description, for purposes of explanation, numerous details are set forth in order to provide a thorough understanding of the embodiments. However, it will be apparent to one skilled in the art that these specific details are not required.

[00334] The above-described embodiments are intended to be examples only.

- 5 Alterations, modifications and variations can be effected to the particular embodiments by those of skill in the art. The scope of the claims should not be limited by the particular embodiments set forth herein, but should be construed in a manner consistent with the specification as a whole.

10 **[00335]** Sequences

[00336] A consolidated list of sequences referenced here is found in **Table 21**.

Table 21 Consolidate List of Sequences		
SEQ ID NO:	Sequence	Description
1	KSSQSLNX ₁ RTRKX ₂ YLA, X ₁ =S or T; X ₂ =I or N	CDR L1-B consensus
2	WASTRX ₁ X ₂ , where X ₁ =E, H or Q and X ₂ =S or T	CDR L2-B consensus
3	GX ₁ TFX ₂ X ₃ X ₄ X ₅ X ₆ X ₇ , X ₁ =F or Y; X ₂ =I, S or T; X ₃ =S, N or K; X ₄ =F or Y; X ₅ =G or W; X ₆ =I or M; X ₇ =N, S or H	CDR H1-B consensus
4	NIX ₁ PX ₂ SX ₃ SX ₄ X ₅ YNEKFKX ₆ , X ₁ =F, N, or Y; X ₂ =G, N, or D; X ₃ =S or T; X ₄ =A or T; X ₅ =N or D; X ₆ =S or R	CDR H2-B consensus
5	DX ₁ YGGSPDY, X ₁ =T or Y	CDR H3-B consensus
6	WASTRX ₁ S, X ₁ =E or Q	CDR L2-B consensus (2)
7	GYTFTSX ₁ WIN, X ₁ =F or Y	CDR H1-B consensus (2)
8	NIX ₁ PX ₂ SSSTNYNEKFKS, X ₁ =F or Y and X ₂ =G or D	CDR H2-B consensus (2)
9	KSSQSLLSRTRKIYLA	F107-7H5 CDR L1-B
10	WASTRQS	F107-7H5 CDR L2-B
11	KQSYNLWT	F107-7H5/F107 & 8D12 CDR L3-B
12	GYTFTSYWIN	F107-7H5 CDR H1-B
13	NIYPDSSSTNYNEKFKS	F107-7H5 CDR H2-B
14	DTYGGSPDY	F107-7H5 CDR H3-B
15	KSSQSLLNTRTRKNYLA	F107-8D12 CDR L1-B
16	WASTRES	F107-8D12 CDR L2-B
17	GYTFISFWIN	F107-8D12 CDR H1-B
18	NIFPGSSSTNYNEKFKS	F107-8D12 CDR H2-B
19	DYYGGSPDY	F107-8D12 CDR H3-B
20	RASQDINNYLN	F111-5E9 CDR L1-B

21	YISRLHS	F111-5E9 CDR L2-B
22	QQGNTLPFT	F111-5E9 CDR L3-B
23	KYGMN	F111-5E9 CDR H1-B
24	WINTYTGEPTYADDFKG	F111-5E9 CDR H2-B
25	GGYYSNPIYPMDY	F111-5E9 CDR H3-B
26	SASSSVSYMY	F111-3C8 CDR L1-B
27	RTSNLAS	F111-3C8 CDR L2-B
28	QQYHNPPT	F111-3C8 CDR L3-B
29	GYTFTSYWMH	F111-3C8 CDR H1-B
30	NINPNSTSADYNEKFKR	F111-3C8 CDR H2-B
31	PLMGPYWYFDV	F111-3C8 CDR H3-B
32	KASQDVTTAVA	F107-10G1 CDR L1-B
33	WASTRHT	F107-10G1 CDR L2-B
34	QQHFTTPLT	F107-10G1 CDR L3-B
35	NYGMS	F107-10G1 CDR H1-B
36	SISGGGGRTYYLDNVKG	F107-10G1 CDR H2-B
37	GARASYFAMDY	F107-10G1 CDR H3-B
38	DIVMSQSPSSLAVSAGEKVTMSCKSSQSLNSRTRKIYLAWYQ QKPGQSPKLLIYWASTRQSGVPDRFTGSGSGTDFTLTISVQA EDLAVYYCKQSYNLWTFGGGKLEIK	F107-7H5 VL
39	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWINWVKQRPG QGLEWIGNIYPDSSSTNYNEKFKSKATLTVDKSSSTAYIQFSSLT SEDSAVYYCTRDYGGSPDYWGQGTTTLTVSS	F107-7H5 VH
40	DIVMSQSPSSLAVSAGERVTMSCKSSQSLNTRTRKNYLAWYQ QKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQA EDLAVYYCKQSYNLWTFGGGKLEIK	F107-8D12 VL
41	QVQLQQPGAELVKPGASVQLSCKASGYTFISFWINWVKQRPG QGLEWMGNIFPGSSSTNYNEKFKSKATLTVDKSSSTAYMQLSS LTSEDSAVYFCARDYYGGSPDYWGQGTTTLTVSS	F107-8D12 VH
42	QIVLTQSPAIMASAPGEKVTISCSASSSVSYMYWYQQKPGSSP KPWIYRTSNLASGVPARFSGSGSGTSYSLTISMEADAATYY CQQYHNPPTFGGGKLEIK	F111-3C8 VL
43	QVQLQQPGAELGKPGTSVKLSCKASGYTFTSYWMHWKRV GQGLEWIGNINPNSTSADYNEKFKRKATLTVDKSSSTAYMQLS TLTSEDSAVYYCTRPLMGPYWYFDVWGTGTTTVTVSS	F111-3C8 VH
44	DIQMTQTTSSLSASLGDRVTISCRASQDINNYLNWYQQKPDGT VKLLIYYISRLHSGVPSRFSGSGSGTDYSLTISNLEEDVATYFC QQGNTLPFTFGSGTKLEIK	F111-5E9 VL
45	QIQLVQSGPELKKPGETVKISCKASGYFTKYGMNWWKQAPGK GLKWMGWINTYTGEPTYADDFKGRFAFSLETSASTAYLQINNL TTEDMVTYFCAKGGYYSNPIYPMDYWGQGTSVTVSS	F111-5E9 VH
46	VIVMTQSHKFMSTSVGDRVSITCKASQDVTTAVAWYQQKPGQ SPKLLIYWASTRHTGVPDRFTGSGSGTDYSLTISNVQTEDLAFY YCQQHFTTPLTFGAGTKLELK	F107-10G1 VL
47	EVNLVESGGGVVKPGASLKLSCASGFTFSNYGMSWWRQTSD KRLEWVASISGGGGRTYYLDNVKGRFIISRENAKNTLYLQMSSL KSEDTALFYCARGARASYFAMDYWGQGSSVTVSS	F107-10G1 VH
48	DIVMSQSPSSLAVSAGEKVTMSCKSSQSLNSRTRKIYLAWYQ	Chimeric F107-7H5 light

	QKPGQSPKLLIYWASTRQSGVPDRFTGSGSGTDFTLTISVQA EDLAVYYCKQSYNLWTFGGGKLEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC	chain
49	QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWINWVKQRPG QGLEWIGNIYPDSSSTNYNEKFKSKATLTVDKSSTTAYIQFSSLT SEDSAVYYCTRDYGGSPDYWGQGTTLTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPG	Chimeric F107-7H5 heavy chain
50	DIVMSQSPSSLAVSAGERVTMSCKSSQSLLNTRTRKNYLAWYQ QKPGQSPKLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQA EDLAVYYCKQSYNLWTFGGGKLEIKRTVAAPSVFIFPPSDEQL KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC	Chimeric F107-8D12 light chain
51	QVQLQQPGAELVKPGASVQLSCKASGYTFISFWINWVKQRPG QGLEWMGNIFPGSSSTNYNEKFKSKATLTVDKSSTAYMQLSS LTSEDSAVYFCARDYYGGSPDYWGQGTTLTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT EVTCTVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPG	Chimeric F107-8D12 heavy chain
52	QIVLTQSPAIMASAPGEKVTISCSASSSVSYMYWYQQKPGSSP KPWIYRTSNLASGVPARFSGSGSGTSYSLTISMEAEADAATYY CQQYHNYPPFTFGGGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	Chimeric F111-3C8 light chain
53	QVQLQQPGAELGKPGTSVKLSCKASGYTFTSYWMHWWKRVP GQGLEWIGNINPNSTADYNEKFKRKATLTVDKSSTAYMQLS TLTSEDSAVYYCTRPLMGPIYWFVWGTGTTTVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPG	Chimeric F111-3C8 heavy chain

54	DIQMTQTTSSLSASLGDRVTISCRASQDINNYLNWYQQKPDGT VKLLIYYISRLHSGVPSRFSGSGSGTDYSLTISNLEEDVATYFC QQGNTLPFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	Chimeric F111-5E9 light chain
55	QIQLVQSGPELKKPGETVKISCKASGYTFTKYGMNWWKQAPGK GLKWMGWINTYTGEPTYADDFKGRFAFSLETSASTAYLQINNL TTEDMVTYFCAKGGYYSNPIYPMDYWGGQTSVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK VDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEALHNHYTQKSLSLSPG	Chimeric F111-5E9 heavy chain
56	VIVMTQSHKFMSTSVGDRVSITCKASQDVTTAVAWYQQKPGQ SPKLLIYWASTRHTGVPDRFTGSGSGTDYSLTISNVQTEDLAFY YCQQHFTTPTLTFGAGTKLELKRVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDST YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	Chimeric F107-10G1 light chain
57	EVNLVESGGGVVKPGASLKLSCEASGFTFSNYGMSWWRQTS KRLEWWASISGGGGRTYYLDNVKGRFIISRENAKNTLYLQMSSL KSEDTALFYCARGARASYFAMDYWGQGSSVTVSSASTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCSV MHEALHNHYTQKSLSLSPG	Chimeric F107-10G1 heavy chain
58	MAWRCPRMGRVPLAWCLALCGWACMAPRGTAEEPSFVGN PGNITGARGLTGTLRCQLQVQGEPEVHWLRDGGQILELADSTQ TQVPLGEDEQDDWIVVSQLRITSLQLSDTGQYQCLVFLGHQTF VSQPGYVGLEGLPYFLEEPEDRTVAANTPFNLSCQAQGPPEPV DLLWLQDAVPLATAPGHGPQRS LHVPGLNKTSSFSCAEHNAK GVTTSTRTATITVLPQQPRNLHLVSRQPTELEVAWTPGLSGIYPL THCTLQAVLSDDGMGIQAGEPDPEEPLTSQASVPPHQLRLGS LHPHTPYHIRVACTSSQGPSSWTHWLPVETPEGVPLGPPENIS ATRNGSQAFVHWQEPRAPLQGTLLGYRLAYQGQDTPEVLMDI GLRQEVTLLELQGDGSVSNLTVCAAYTAAGDGPWWSLPVPLEA WRPGQAQPVHQLVKEPSTPAFSWPWWGSGGGSSTG	rhAXL-ECD
59	MVLQTQVFISLLLWISGAYG	Light chain signal sequence
60	MDWTWRILFLVAAATGTHA	Heavy chain signal sequence
61	gacattgtgatgtcacagtctccatcctccctggctgtgtcagcaggagagaaggtcacta tgagctgcaaataccagtcagagctctgctcaacagtagaaccgaaagatctacttggtt	F107-7H5 VL sequence

	ggtagcagcagaaaccaggtcagctctcctaaactgctgatctattgggcatccactaggc aatctgggggtccctgatcgcttcacaggcagtgatctgggacagattcactctcaccatc agcagtgctcaggctgaagacctggcagtttattactgcaagcaatcttataatctgtggac gttcgggtggaggcaccaagctggaaatcaaacgg	
62	caggtccaactgcagcagcctggggctgagcttgtaagcctggggcttcagtgaact gtcctgcaaggcttctggctacactttcaccagctactggataaactgggtgaagcagag gcctggacaaggccttgagtgattggaaatattatcctgatagtagtagtactaactaca atgagaagttcaagagcaaggccacactgactgtagacaagtctccaccacagccta catacagttcagcagcctgacatctgaggactctgcggtctattattgtacaagagatacct atgggtgtagccctgactactggggccaaggcaccactctcacagctctcctca	F107-7H5 VH sequence
63	gacattgtgatgtcacagtctccatcctcctggctgtgtcagcaggagagagggtcacta tgagctgcaaaccagtcagagctgtctcaacactagaacccgaaagaactacttggtt ggtagcagcagaaaccagggcagctctcctaaactgctgatctactgggcatccactagg gaatctgggggtccctgatcgcttcacaggcagtgatctgggacagattcactctcaccat cagcagtgctcaggctgaagacctggcagtttattactgcaagcaatcttataatctgtgga cggtcgggtggaggcaccaagctggaaatcaaa	F107-8D12 VL sequence
64	caggtccaactgcagcagcctggggctgagcttgtaagcctggggcttcagtgcagct gtcctgcaaggcttctggctacactttcatcagcttctggataaactgggtgaagcagagg cctggacaaggccttgagtgatgggaaatattttcctggtagtagtagtacgaactaca atgagaagttcaagagcaaggccacactgactgtagacaaatcctccagcagcagccta catgcagctcagcagcctgacatctgaggactctgcggtctattttgtgcaagagattact atgggtgtagccctgactactggggccaaggcaccactctcacagtycctca	F107-8D12 VH sequence
65	gatatccagatgacacagactacatcctccctgtctgcctctctgggagacagagtcacc atcagttgcagggcaagtcaggacattaacaattatttaaactggatcagcagaaacca gatggaactgttaaactcctgatctactacatatcaagattacactcaggagtcctcatca gggtcagtggcagtggtctggaacagattattctctcaccattagcaacctggagctaga agatgttgccacttacttttgccaacagggttaatacgttccattcacgttcggctcggggac aaagttggaataaaa	F111-5E9 VL sequence
66	cagatccagttggtgcagctctggacctgagctgaagaagcctggagagacagtcaagat ctcctgcaaggcttctgggtataccttcacaaaatattggaatgaactgggtaaagcaggct ccaggaaaggggttaagtgatgggtggataaacctacactggagagccaacat atgctgatgactcaagggacgggttgccttctcttggaaacctctgccagcactgcctattt gcagatcaacaacctcacaactgaggacatgggtcacatatttctgtgcaaaaggggggt attatagtaaccttatctatctatggactactgggtcaaggaacctcagtcaccgtctcct ca	F111-5E9 VH sequence
67	caaattgttctcaccagctccagcaatcatgtctgcactccaggggagaagggtcacca tatcctgcagtgccagctcaagtgtaagttacatgtattggtagcagaagccaggatc ctcccccacacctggattatcgacatccaacctggcttctggagtcctgctcgttca gtggcagtggtctgggaccttactctctcacaatcagcagcatggaggctgaagatg ctgccacttattactgccagcagtatcataattaccacccacgttcggaggggggacca agctggaaataaaaacgg	F111-3C8 VL sequence
68	caggtccaactgcagcagcctggggctgaactgggcaagcctgggacatcagtgaag ctgtcctgcaaggcttctggctacaccttcaccagctattggatgactgggtgaagcgggt gcctggacaaggccttgagtgattggaaatattaatcctaatagtactagtctgactac aatgagaagttcaagaggaaggccacattgactgtagacaaatcctccagcagcagcct acatgcagctcagcaccctgacatctgaggactctgcggtctactactgtacaagacccc taatgggtccttactgtgacttcgatgtctggggcacagggaaccaggtcaccgtctcctca	F111-3C8 VH sequence
69	gtcattgtgatgaccagcttcacaaattcatgtccacatcagtaggagacagggtcagta tcacctgcaaggccagtcaggatgtgactactgctgtagcctggatcaacaaaaacca	F107-10G1 VL sequence

	gggcaatctcctaaactactgattactgggcatccacccggcacactggagtcctgatc gcttcacaggcagtgatctgggacagattattctctcaccatcagcaatgtgcagactga agacctggcattttattactgtcagcaacattttaccactcctctcacgttcggtgctgggacc aagttggagctgaaa	
70	gaagtgaacctggaggctctgggggaggcgtagtgaagcctggagcgtctctgaaact ctcctgtgaagcctctggattcactttcagtaactatggcatgtcttgggtcgccagacttca gacaagaggctggagtggtcgcatccattagtggtggtggtgtagaacctactatcta gacaatgtaaagggccgattcatcatctccagagagaatgccaagaacaccctgtacct gcaaagtagtagtctgaagctgaggacacggcctgttttactgtgcaagaggagctcg ggcctcttactttgctatggactactgggtcaaggaaggtcagtcaccgtctcctca	F107-10G1 VH sequence
71	MAWRCPRMGRVPLAWCLALCGWACMAPRGTAEEESPFVGN PGNITGARGLTGTLRCQLQVQGEPEVHWLRDGGQILELADSTQ TQVPLGEDEQDDWIVVSQLRITSLQLSDTGQYQCLVFLGHQTF VSQPGYVGLEGLPYFLEEPEDRTVAANTPFNLSCQAQGPPEPV DLLWLQDAVPLATAPGHGPPQRSRLHVPGLNKTSSFSCAHHNAK GVTTSTRATITVLPQQPRNLHLVSRQPTELEVAWTPGLSGIYPL THCTLQAVLSDDGMGIQAGEPDPPEEPLTSQASVPPHQLRLGS LHPHTPYHIRVACTSSQGPSSWTHWLPVETPEGVPLGPPENIS ATRNGSQAFVHWQEPRAPLQGTLLGYRLAYQGQDTPEVLMDI GLRQEVTLLELQGDGSSVSNLTVCAAYTAAGDGPWSLPVPLEA WRPGQAQPVHQLVKEPSTPAFSWPWWGSGGGSSTGHHHHH HHHG	(rh)AXL-ECD His8
72	MAWRCPRMGRVPLAWCLALCGWACMAPRGTAEEESPFVGN PGNITGARGLTGTLRCQLQVQGEPEVHWLRDGGQILELADSTQ TQVPLGEDEQDDWIVVSQLRITSLQLSDTGQYQCLVFLGHQTF VSQPGYVGLEGLPYFLEEPEDRTVAANTPFNLSCQAQGPPEPV DLLWLQDAVPLATAPGHGPPQRSRLHVPGLNKTSSFSCAHHNAK GVTTSTRATITVLPQQPRNLHLVSRQPTELEVAWTPGLSGIYPL THCTLQAVLSDDGMGIQAGEPDPPEEPLTSQASVPPHQLRLGS LHPHTPYHIRVACTSSQGPSSWTHWLPVETPEGVPLGPPENIS ATRNGSQAFVHWQEPRAPLQGTLLGYRLAYQGQDTPEVLMDI GLRQEVTLLELQGDGSSVSNLTVCAAYTAAGDGPWSLPVPLEA WRPGQAQPVHQLVKEPSTPAFSWPWWYVLLGAVVAAACVLIL ALFLVHRRKKETRYGEVFEPTVERGELVVRYRVRKSYSRRTTE ATLNSLGISEELKEKLRDVMVDRHKVALGKTLGEGEFGAVMEG QLNQDDSIKLVAVKTMKIAICTRSELEDFLSEAVCMKEFDHPNV MRLIGVCFQGSERESFPAPVVILPFMKHGDLSFLLYSRLGDQP VYLPTQMLVKFMADIASGMEYLSTKRFIHRDLAARNCMLNENM SVCVADFGLSKKIYNGDYRQGRKAMPVKWIAIESLADRVYTS KSDVWSFGVTMWEIATRGQTPYPGVENSEIYDYLRRGNRLKQ PADCLDGLYALMSRCWELNPQDRPSFTELREDLENTLKALPPA QEPDEILYVNMDEGGGYPEPPGAAGGADPPTQPDPKDSCSCL TAAEVHPAGRYVLCPTTPSPAQPADRGSPAAPGQEDGAYLE CGRYASHHHHHH	AXL – full length His6
73	QVQLQQPGTELVKPGASVKLSCKASGYIFTNFWINWWKQRP QGLEWIGNIFPGSNSSNYNEKFNKATLTVDKSSSTAYMHLSSL TSEDSAVYYCVRDYYGGSPDYWGQGTTTLTVSS	F155-3C7 VH
74	DIVMSQSPSSLAVSAGEKVTMSCKSSQSLNSKTRKNYLAWYQ QKPGQSPKLLIYWASTRESGVPARFTGSGSGTDFTLTISVQA	F155-3C7 VL

	EDLAIYYCKHSYNLWTFGGGKLEIR	
75	QIQLVQSGPELKKPGETVKISCKTSGYTFTYYGINWVKQAPGK GLEWMGWINTYLGEPTYADDFKGRFAFSLETSASTAYLQINNL RDEDMATYFCTRGTMSSYSFDYWGQGTALTSS	F149-4G4 VH
76	QNVLTQSPAIMSASPGEEVTMTCRASSSVSSSYLHWYQQKSG ASPKLWIYSTSKLASGVPARFSGSGSGTSYSLTISSVEAEDAAT YYCHQYSGDPLTFGSGTKLEVK	F149-4G4 VL
77	GFTFSNYG	F107-10G1 CDR H1-A
78	ISGGGGRT	F107-10G1 CDR H2-A
79	ARGARASYFAMDY	F107-10G1 CDR H3-A
80	QDVTTA	F107-10G1 CDR L1-A
81	WAS	F107-10G1 CDR L2-A
82	QQHFTTPLT	F107-10G1 CDR L3-A
83	GYTFTKYG	F111-5E9 CDR H1-A
84	INTYTGEPT	F111-5E9 CDR H2-A
85	AKGGYYSNPIYPMDY	F111-5E9 CDR H3-A
86	QDINNY	F111-5E9 CDR L1-A
87	YIS	F111-5E9 CDR L2-A
88	QQGNTLPFT	F111-5E9 CDR L3-A
89	GYTFISFW	F107-8D12 CDR H1-A
90	IFPGSSST	F107-8D12 CDR H2-A
91	ARDYYGGSPDY	F107-8D12 CDR H3-A
92	QSLNTRTRKNY	F107-8D12 CDR L1-A
93	WAS	F107-8D12 CDR L2-A
94	KQSYNLWT	F107-8D12 CDR L3-A
95	GYTFTSYW	F107-7H5 CDR H1-A
96	IYPDSSST	F107-7H5 CDR H2-A
97	TRDTYGGSPDY	F107-7H5 CDR H3-A
98	QSLNLSRTRKIY	F107-7H5 CDR L1-A
99	WAS	F107-7H5 CDR L2-A
100	KQSYNLWT	F107-7H5 CDR L3-A
101	GYIFTNFW	F155-3C7 CDR H1-A
102	IFPGSNSS	F155-3C7 CDR H2-A
103	VRDYYGGSPDY	F155-3C7 CDR H3-A
104	QSLNLSKTRKNY	F155-3C7 CDR L1-A
105	WAS	F155-3C7 CDR L2-A
106	KHSYNLWT	F155-3C7 CDR L3-A
107	GYTFTSYW	F111-3C8 CDR H1-A
108	INPNSTSA	F111-3C8 CDR H2-A
109	TRPLMGPYWYFDV	F111-3C8 CDR H3-A
110	SSVSY	F111-3C8 CDR L1-A
111	RTS	F111-3C8 CDR L2-A
112	QQYHNPPT	F111-3C8 CDR L3-A
113	GYTFTYYG	F149-4G4 CDR H1-A
114	INTYLGEPT	F149-4G4 CDR H2-A
115	TRGTMSYSFDY	F149-4G4 CDR H3-A
116	SSSVSSSY	F149-4G4 CDR L1-A
117	STS	F149-4G4 CDR L2-A

118	HQYSGDPLT	F149-4G4 CDR L3-A
119	QVKLEESGGGLVQAGGSLRLSCTAS <u>ASISSFDIMGWYRQAPGK</u> QRELVA <u>AITTLDI</u> ANYRDSVKGRFTISRDNANTVYLQMDSLKP EDTARYHCAAFQSDQNYWGQGTQVTVSS	NRC-sdAb001
120	QVQLVDSGGGLVQAGGSLRLSCATSTRTVSSAVMAWFRQAPE KVRDFVGFITNSGNIYDDSVKGRFTISRDNANTVYLQMNSLK PEDTAVYYCAAKWSFSSGYGDLRRAAMYDYWGQGTQVTVSS	NRC-sdAb002
121	QVQLVESGGGLVQAGGSLRLSCAASGVTLDYTAIGWFRQAPG KERELVAITSGGNTDYAESAKGRFRISRDNANTVYLQMNSLK PEDTGVYYCAARRGGARGEYDYWDQGTQVTVSS	NRC-sdAb003
122	QVQLVESGGGVVQAGGSLRLSCAFSGAFDYEIGWFRQAPG KEREVAAVTRNGDSVYADSLKARFTASRNNVNTAYLHMNI LQPEDTATYYCAANWRPLRTSSGADDYADWGQGTQVTVSS	NRC-sdAb004
123	QVKLEESGGGLAQAGGSLRLSCAASGSSISINTIGWFRQAPGK QRELVAASDSGANRNYADSVKGRFTISRDNANTVYLQMNNLK PEDTAIYYCRAWGTGTISTMYWGQGTQVTVSS	NRC-sdAb005
124	QVKLEESGGGLVQAGASLRLSCVASESIFGFNTMGWYRQAPG NERELVASISNSKRTMYADSVKGRFTISRDNANTVNLQMNNL KPEDTAVYYCRAWGIITSATVYWGQGTQVTVSS	NRC-sdAb006
125	QVKLEESGGGLVQAGGSLRLSCATSTRTVSSAVMAWFRQAPE KERDFVGFISNSGSVYYDDSVKGRFTISRDNANTVYLQMNSL KPEDTAVYYCAIIWRTSDLTGRFNTWGQGTQVTVSS	NRC-sdAb007
126	QVKLEESGGGLVQAGGSLRLSCAASGSSGMINTMGWYRQAP GKQRELVARRSTGGTTNYADSVKGRFTISRDDANNTVYLQMN SLKPEDTAVYYCAIIWRTSDLTGRFNTWGQGTQVTVSS	NRC-sdAb008
127	ASISSFDI	NRC-sdAb001 CDR1-A
128	ITTL DIA	NRC-sdAb001 CDR2-A
129	AAFQSDQNY	NRC-sdAb001 CDR3-A
130	TRTVSSAV	NRC-sdAb002 CDR1-A
131	ITNSGNI	NRC-sdAb002 CDR2-A
132	AAKWSFSSGYGDLRRAAMYDY	NRC-sdAb002 CDR3-A
133	GVTLDYTA	NRC-sdAb003 CDR1-A
134	ITSGGNT	NRC-sdAb003 CDR2-A
135	AARRGGARGEYDY	NRC-sdAb003 CDR3-A
136	RGAFDYE	NRC-sdAb004 CDR1-A
137	VTRNGDSV	NRC-sdAb004 CDR2-A
138	AANWRPLRTSSGADDYAD	NRC-sdAb004 CDR3-A
139	GSISSINT	NRC-sdAb005 CDR1-A

140	SDSGANR	NRC-sdAb005 CDR2-A
141	RAWGTGTISTMY	NRC-sdAb005 CDR3-A
142	ESIFGFNT	NRC-sdAb006 CDR1-A
143	ISNSKRT	NRC-sdAb006 CDR2-A
144	RAWGIITSATVY	NRC-sdAb006 CDR3-A
145	TRTVSSAV	NRC-sdAb007 CDR1-A
146	ISNSGSV	NRC-sdAb007 CDR2-A
147	AIIWRTSDLTGRFNT	NRC-sdAb007 CDR3-A
148	GSSGMINT	NRC-sdAb008 CDR1-A
149	RSTGGTT	NRC-sdAb008 CDR2-A
150	AIIWRTSDLTGRFNT	NRC-sdAb008 CDR3-A
151	NFWIN	F155-3C7 CDR H1-B
152	NIFPGSNSSNYNEKFKN	F155-3C7 CDR H2-B
153	DYYGGSPDY	F155-3C7 CDR H3-B
154	KSSQSLLSKTRKNYLA	F155-3C7 CDR L1-B
155	WASTRES	F155-3C7 CDR L2-B
156	KHSYNLWT	F155-3C7 CDR L3-B
157	YYGIN	F149-4G4 CDR H1-B
158	WINTYLGEPTYADDFKG	F149-4G4 CDR H2-B
159	GTMSYSFDY	F149-4G4 CDR H3-B
160	RASSSVSSSYLH	F149-4G4 CDR L1-B
161	STSKLAS	F149-4G4 CDR L2-B
162	HQYSGDPLT	F149-4G4 CDR L3-B
163	FDIMG	NRC-sdAb001 CDR1-B
164	AITTLDIANYRDSVKG	NRC-sdAb001 CDR2-B
165	FQSDQNY	NRC-sdAb001 CDR3-B
166	SAVMA	NRC-sdAb002 CDR1-B
167	FITNSGNILYDDSVKG	NRC-sdAb002 CDR2-B
168	KWSFSSGYGDLRRAAMYDY	NRC-sdAb002 CDR3-B
169	YTAIG	NRC-sdAb003 CDR1-B
170	AITSGGNTDYAESAKG	NRC-sdAb003 CDR2-B
171	RRGGARGEYDY	NRC-sdAb003 CDR3-B
172	TYEIG	NRC-sdAb004 CDR1-B
173	AVTRNGDSVVYADSLKA	NRC-sdAb004 CDR2-B
174	NWRPLRTSSGADDYAD	NRC-sdAb004 CDR3-B
175	ASDSGANRNYADSVKG	NRC-sdAb005 CDR1-B

176	ASDSGANRNYADSVKG	NRC-sdAb005 CDR2-B
177	WGTGTISTMY	NRC-sdAb005 CDR3-B
178	FNTMG	NRC-sdAb006 CDR1-B
179	SISNSKRTMYADSVKG	NRC-sdAb006 CDR2-B
180	WGIITSATVY	NRC-sdAb006 CDR3-B
181	SAVMA	NRC-sdAb007 CDR1-B
182	FISNSGSVYYDDSVKG	NRC-sdAb007 CDR2-B
183	IWRTSDLTGRFNT	NRC-sdAb007 CDR3-B
184	INTMG	NRC-sdAb008 CDR1-B
185	RRSTGGTTNYADSVKG	NRC-sdAb008 CDR2-B
186	IWRTSDLTGRFNT	NRC-sdAb008 CDR3-B
187	TTPPVLSDGSFFLYSK	Fc peptide
188	EFVAAGSSTGR	A20.1 peptide
Note- CDR sequences descriptions followed by "-A" indicates IMGT CDR convention. CDR sequences descriptions followed by "-B" indicates Kabat CDR convention.		

[00337] REFERENCES:

[00338] All publications referred to herein are hereby incorporated by reference.

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WHAT IS CLAIMED IS:

1. An isolated or purified antibody that specifically binds to an epitope in an immunoglobulin-like (IgL) domain of human AXL ectodomain (ECD).
- 5 2. The antibody of claim 1, wherein the AXL ECD has the amino acid sequence of SEQ ID NO:71.
3. The antibody of claim 1, wherein the epitope is located in IgL1 or IgL2 of AXL.
4. The antibody of claim 1, capable of internalization into AXL expressing tumor cells.
5. The antibody of claim 1, which is a single domain antibody, a monoclonal antibody, a
10 humanized antibody, a chimeric antibody, a biparatopic antibody, a bispecific antibody, or an AXL-binding antibody fragment thereof.
6. The antibody of claim 5, wherein the chimeric antibody comprises framework regions from human IgG1.
7. The antibody of claim 6, wherein the chimeric antibody comprises framework regions
15 from human kappa light chain and human IgG heavy chain.
8. The antibody of claim 1, comprising framework regions from IgA, IgD, IgE, IgG, or IgM.
9. The antibody of claim 1, comprising a full-length IgG, Fv, scFv, Fab, or F(ab')₂, VHH, VL, or VH.
- 20 10. The antibody of claim 1, having an equilibrium dissociation constant (K_D) of ≤ 50 nM to human AXL ectodomain.
11. The antibody of claim 1, wherein the binding to the epitope in an IgL domain of AXL ECD does not compete with the binding of Growth arrest-specific 6 (Gas6) ligand to AXL.
12. An antibody or fragment thereof comprising variable light chain (VL) CDR1, CDR2,
25 and CDR3 sequences selected from the group consisting of:
SEQ ID NO:98, 99, and 100;

SEQ ID NO:92, 93 and 94;
SEQ ID NO:110, 111, and 112;
SEQ ID NO:86, 87, and 88;
SEQ ID NO:80, 81, and 82;
5 SEQ ID NO:104, 105, and 106;
SEQ ID NO:116, 117, and 118; and
sequences substantially identical thereto.

13. An antibody or fragment thereof comprising a variable light chain (VL) sequence
10 selected from the group consisting of:

SEQ ID NO:38;
SEQ ID NO:40;
SEQ ID NO:42;
SEQ ID NO:44;
15 SEQ ID NO:46;
SEQ ID NO:74;
SEQ ID NO:76; and
a sequence substantially identical thereto.

20 14. An antibody or fragment thereof comprising variable heavy chain (VH) CDR1, CDR2,
and CDR3 sequences selected from the group consisting of:

SEQ ID NO:95, 96, and 97;
SEQ ID NO:89, 90 and 91;
SEQ ID NO:107, 108, and 109;
25 SEQ ID NO:83, 84, and 85;
SEQ ID NO:77, 78, and 79;
SEQ ID NO:101, 102, and 103;
SEQ ID NO:113, 114, and 115; and
sequences substantially identical thereto.

30

15. An antibody or fragment thereof comprising a variable heavy chain (VH) sequence
selected from the group consisting of:

SEQ ID NO:39;

SEQ ID NO:41;

SEQ ID NO:43;

SEQ ID NO:45;

SEQ ID NO:47;

5 SEQ ID NO:73;

SEQ ID NO:75; and

a sequence substantially identical thereto.

16. The antibody according to any one of claims 1 to 4, or a fragment thereof, comprising
10 variable heavy chain (VL) CDR1, CDR2, and CDR3 sequences; and (VH) CDR1, CDR2, and
CDR3 sequences selected from the group consisting of:

VL, SEQ ID NO:98-100; and VH, SEQ ID NO:95-97;

VL, SEQ ID NO:92-94; and VH, SEQ ID NO:89-91;

VL, SEQ ID NO:110-112; and VH, SEQ ID NO:107-109;

15 VL, SEQ ID NO:86-88; and VH, SEQ ID NO:83-85;

VL, SEQ ID NO:80-82; and VH, SEQ ID NO:77-79;

VL, SEQ ID NO:104-106; and VH, SEQ ID NO:101-103;

VL, SEQ ID NO:116-118; and VH, SEQ ID NO:113-115; and

sequences substantially identical thereto.

20

17. The antibody according to any one of claims 1 to 4, or a fragment thereof comprising
single domain (sd) heavy chain variable (VHH) domain CDR1, CDR2, and CDR3 sequences
selected from the group consisting of:

SEQ ID NO:127, 128, and 129;

25 SEQ ID NO:130, 131, and 132;

SEQ ID NO:133, 134, and 135;

SEQ ID NO:136, 137, and 138;

SEQ ID NO:139, 140, and 141;

SEQ ID NO:142, 143, and 144;

30 SEQ ID NO:145, 146, and 147;

SEQ ID NO:148, 149, and 150; and

sequences substantially identical thereto.

18. An antibody or fragment thereof comprising a single domain (sd) heavy chain variable (VHH) domain sequence selected from the group consisting of:

QVKLEESGGGLVQAGGSLRLSCTASASISSFDIMGWYRQAPGKQRELVAAITTLIDIA
NYRDSVKGRFTISRDNANKNTVYLQMDSLKPEDTARYHCAAFQSDQNYWGQGTQVT
VSS (SEQ ID NO:119);

QVQLVDSGGGLVQAGGSLRLSCATSTRTVSSAVMAWFRQAPEKVRDFVGFITNSG
NILYDDSVKGRFTISRDNANQNTVYLQMNSLKPEDTAVYYCAAKWSFSSGYGDLRRA
AMYDYWGQGTQVTVSS (SEQ ID NO:120);

QVQLVESGGGLVQAGGSLRLSCAASGVTLDTAIGWFRQAPGKERELVAAITSGGN
TDYAESAAGRFRISRDNANKNTIYLQMNSLKPEDTGVYYCAARRGGARGEYDYWDQ
GTQVTVSS (SEQ ID NO:121);

QVQLVESGGGVVQAGGSLRLSCAFSRGAFDTYEIGWFRQAPGKEREFVAAVTRNG
DSVYADSLKARFTASRNNNAVNTAYLHMNILQPEDTATYYCAANWRPLRTSSGADD
YADWGQGTQVTVSS (SEQ ID NO:122);

QVKLEESGGGLAQAGGSLRLSCAASGSISSINTIGWFRQAPGKQRELVAASDSGAN
RNYADSVKGRFTISRDNANKNTVYLQMNNLKPEDTAIYYCRAWGTGTISTMYWGQGT
QVTVSS (SEQ ID NO:123);

QVKLEESGGGLVQAGASLRLSCVASESIFGFNTMGWYRQAPGNERELVASISNSKR
TMYADSVKGRFTISRDNANKNTVNLQMNNLKPEDTAVYYCRAWGIITSATVYWGQGT
QVTVSS (SEQ ID NO:124);

QVKLEESGGGLVQAGGSLRLSCATSTRTVSSAVMAWFRQAPEKERDFVGFISNSG
SVYYDDSVKGRFTISRDNANQNTVYLQMNSLKPEDTAVYYCAIIWRTSDLTGRFNTW
GQGTQVTVSS (SEQ ID NO:125);

QVKLEESGGGLVQAGGSLRLSCAASGSSGMINTMGWYRQAPGKQRELVARRESTG
GTTNYADSVKGRFTISRDDANNTVYLQMNSLKPEDTAVYYCAIIWRTSDLTGRFNTW
GQGTQVTVSS (SEQ ID NO:126); and

a sequence substantially identical thereto.

19. An antibody or fragment thereof selected from the group consisting of:
 - 5 VH of SEQ ID NO:39, and VL of SEQ ID NO:38;
 - VH of SEQ ID NO:41, and VL of SEQ ID NO:40;
 - VH of SEQ ID NO:43, and VL of SEQ ID NO:42;
 - VH of SEQ ID NO:45, and VL of SEQ ID NO:44;
 - VH of SEQ ID NO:47, and VL of SEQ ID NO:46;
 - 10 VH of SEQ ID NO:73, and VL of SEQ ID NO:74;
 - VH of SEQ ID NO:75, and VL of SEQ ID NO:76;
 - VHH of SEQ ID NO:119;
 - VHH of SEQ ID NO:120;
 - VHH of SEQ ID NO:121;
 - 15 VHH of SEQ ID NO:122;
 - VHH of SEQ ID NO:123;
 - VHH of SEQ ID NO:124;
 - VHH of SEQ ID NO:125;
 - VHH of SEQ ID NO:126; and
 - 20 sequences having 85% or greater identity thereto.
20. The antibody or fragment of claim 19, wherein the sequence identity is $\geq 90\%$, $\geq 95\%$, or $\geq 98\%$.
- 25 21. The antibody of claim 1, comprising light chain CDR1, CDR2 and CDR3 sequences of SEQ ID NO:1, 2, and 11; and heavy chain CDR1, CDR2 and CDR3 sequence of SEQ ID NO:3, 4, and 5; wherein the antibody binds an epitope in the IgL1 domain and not in the IgL2 domain of AXL.
- 30 22. The antibody of claim 1, comprising light chain CDR1, CDR2 and CDR3 sequences of SEQ ID NO:1, 6, and 11; and heavy chain CDR1, CDR2 and CDR3 sequence of SEQ ID NO:7, 8, and 5; wherein the antibody binds an epitope in the IgL1 domain and not in the IgL2 domain of AXL.

23. The antibody of claim 1, comprising light chain CDR1, CDR2 and CDR3 sequences of SEQ ID NO:9, 10, and 11; and heavy chain CDR1, CDR2 and CDR3 sequence of SEQ ID NO:12, 13, and 14.

5

24. The antibody of claim 1, comprising light chain CDR1, CDR2 and CDR3 sequences of SEQ ID NO:15, 16, and 11; and heavy chain CDR1, CDR2 and CDR3 sequence of SEQ ID NO:17, 18, and 19.

10 25. The antibody of any one of claims 1 to 24, which does not compete with Gas6 for AXL binding.

26. The antibody of claim 5, wherein the antibody is biparatopic and comprises sdAb001/005 or sd Ab 001/006.

15

27. An antibody drug conjugate (ADC) comprising the antibody of any one of claims 1 to 24, conjugated to a drug moiety.

20 28. The antibody drug conjugate of claim 27, wherein the drug moiety is an anti-cancer drug.

29. The antibody drug conjugate of claim 27, wherein the drug moiety is DM1.

25 30. A pharmaceutical composition comprising the ADC of claim 22 and a pharmaceutically acceptable excipient.

31. A fusion protein comprising the antibody according to claim 1, and human IgG1 Fc.

30 32. A nucleic acid molecule encoding the antibody of any one of claims 1 to 24.

33. A vector comprising the nucleic acid molecule of claim 32.

34. A host cell comprising the vector of claim 33.

35. The host cell of claim 34, which is a recombinant microbial host cell or is a mammalian cell.

5 36. The host cell of claim 32 or 33, wherein said cell produces the antibody of claim 1.

37. A hybridoma or an organism that produces the antibody of claim 1.

10 38. The antibody of any one of claims 1 to 24, wherein the antibody is immobilized onto a surface.

39. The antibody of any one of claims 1 to 24, wherein the antibody is linked to a cargo molecule.

15 40. The antibody of claim 39, wherein the cargo molecule is a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a cytokine, a receptor trap, an antibody thereof, a chemical compound, a carbohydrate moiety, a DNA-based molecule, a cytotoxic agent, or a viral vector.

20 41. The antibody of claim 39, wherein the cargo molecule is from about 1 kDa to about 500 kDa in size.

42. The antibody of claim 40, wherein the cargo molecule is loaded in one or more liposome or nanocarrier.

25

43. The antibody of claim 42, wherein the nanocarrier comprises one or more nanoparticle, nanowire, nanotube, or quantum dots.

44. The antibody of claim 40 wherein the viral vector is adenoviral, lentiviral or retroviral.

30

45. The antibody of claim 40, wherein the DNA-based molecule comprises an anti-sense oligonucleotide, microRNA, siRNA, or a plasmid.

46. The antibody of any one of claims 39 to 42, wherein the cargo molecule is a cytotoxic agent.

47. An *in vitro* method of detecting AXL, comprising:

- a) contacting a sample with one or more than one isolated or purified antibody of any one of claims 1 to 24 linked to a detectable agent; and
- b) detecting the detectable agent linked to the antibody thereof bound to AXL in the sample.

48. The method of claim 47, wherein method detects AXL in circulating cells and the sample is a serum sample.

49. The method of claim 47 or 48, wherein the step of detecting is performed using optical imaging, immunohistochemistry, molecular diagnostic imaging, or ELISA.

50. An *in vivo* method of detecting AXL expression in a subject, comprising:

- a) administering to the subject one or more antibody of any one of claims 1 to 24 linked to a detectable agent; and
- b) detecting the detectable agent linked to the antibody thereof bound to AXL.

51. The method of claim 47, wherein the step of detecting is performed using PET, SPECT, or fluorescence imaging.

52. A method of transporting a molecule of interest into cells expressing AXL, comprising administering to a subject one or more antibody of claim 1 linked to the molecule of interest, wherein the antibody delivers the molecule of interest to the subject's cells expressing AXL.

53. The method of claim 52, wherein the molecule of interest is a detectable agent, a therapeutic agent, a drug, a peptide, an enzyme, a growth factor, a cytokine, a receptor trap, an antibody thereof, a chemical compound, a carbohydrate moiety, a DNA-based molecule, a cytotoxic agent, or a viral vector.

54. The method of claim 52, wherein the molecule of interest is loaded on one or more liposome or nanocarrier.

55. The method of claim 54, wherein the nanocarrier comprises one or more nanoparticle,
5 nanowire, nanotube, or quantum dots.

56. The method of any one of claims 52 to 55, wherein the molecule of interest comprises a cytotoxic agent.

10 57. The method of claim 56, wherein the cytotoxic agent is for use in treating cancer.

58. A method of treating cancer comprising administering an anti-cancer antibody drug conjugate comprising the antibody of any one of claims 1 to 24 to a subject in need thereof.

15 59. A method of treating cancer comprising administering a a cellular therapy, a chimeric antigen receptor (CAR-T cell) therapy, or an oncolytic virus comprising the antibody of any one of claims 1 to 24.

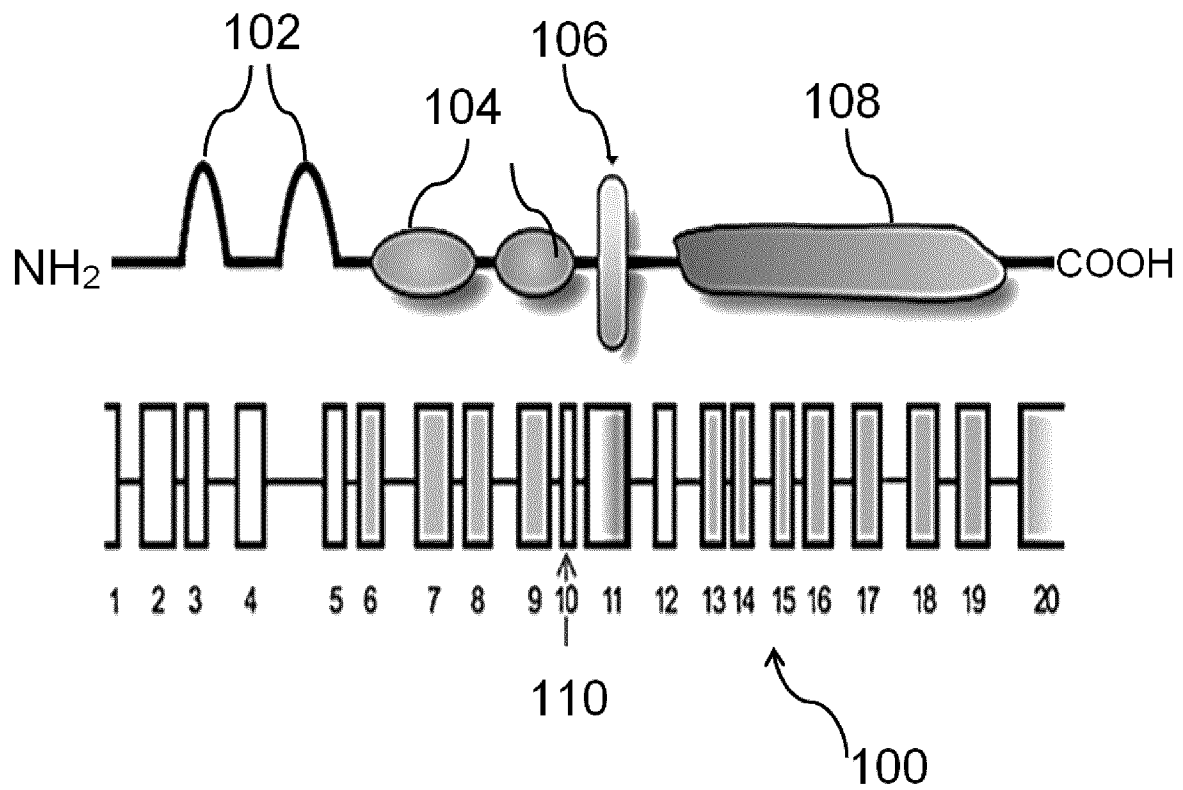
60. Use of the antibody of any one of claims 1 to 24 for preparation of a medicament
20 comprising an anti-cancer antibody drug conjugate, for treating cancer in a subject in need thereof.

61. Use of an anti-cancer antibody drug conjugate comprising the antibody of any one of claims 1 to 24 for treating cancer in a subject in need thereof.

25

62. Use of the antibody of any one of claims 1 to 24 for preparation of a cellular therapy, a chimeric antigen receptor (CAR-T cell) therapy, or an oncolytic virus.

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Prior Art

FIG. 1

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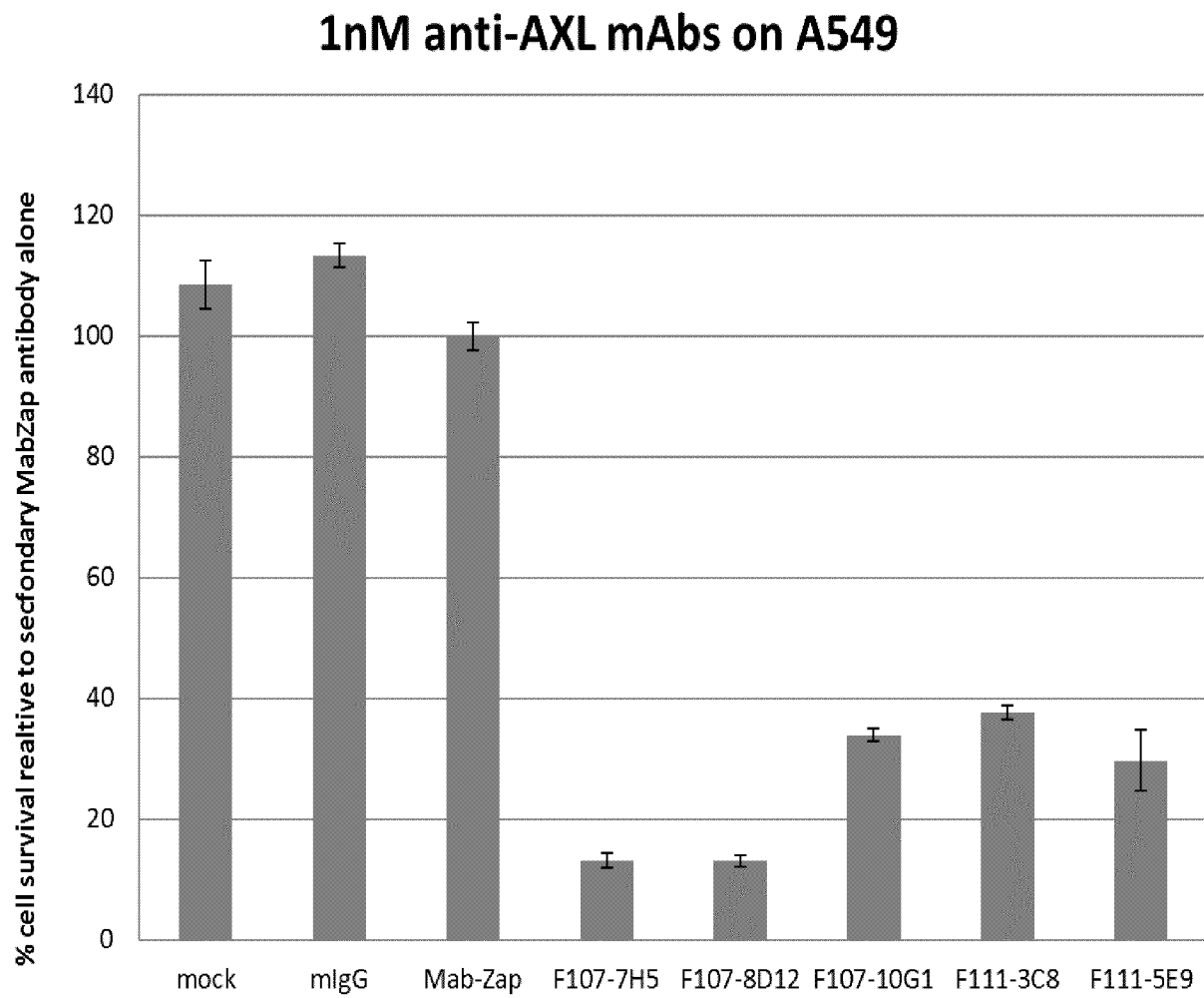


FIG. 2

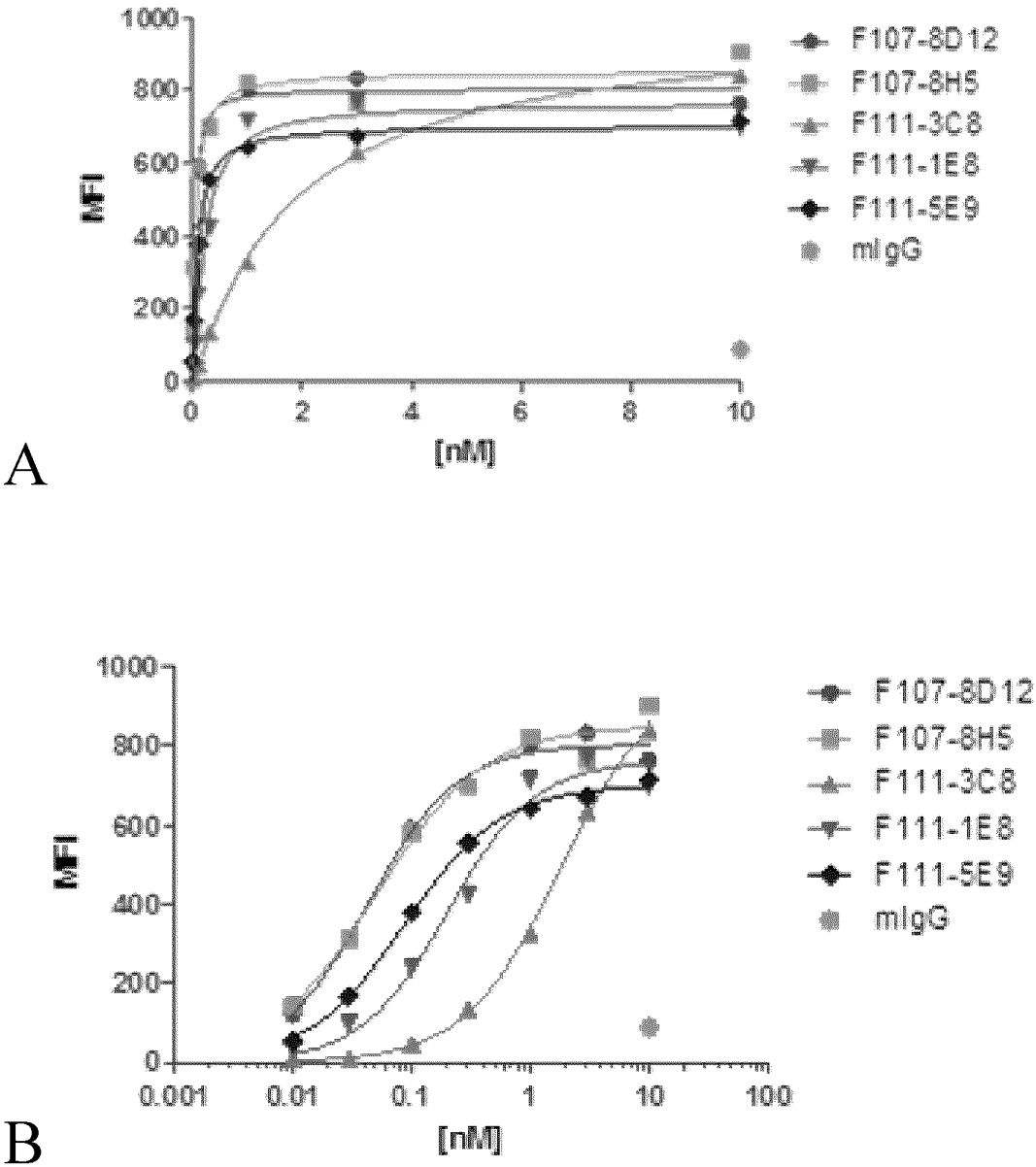


FIG. 3

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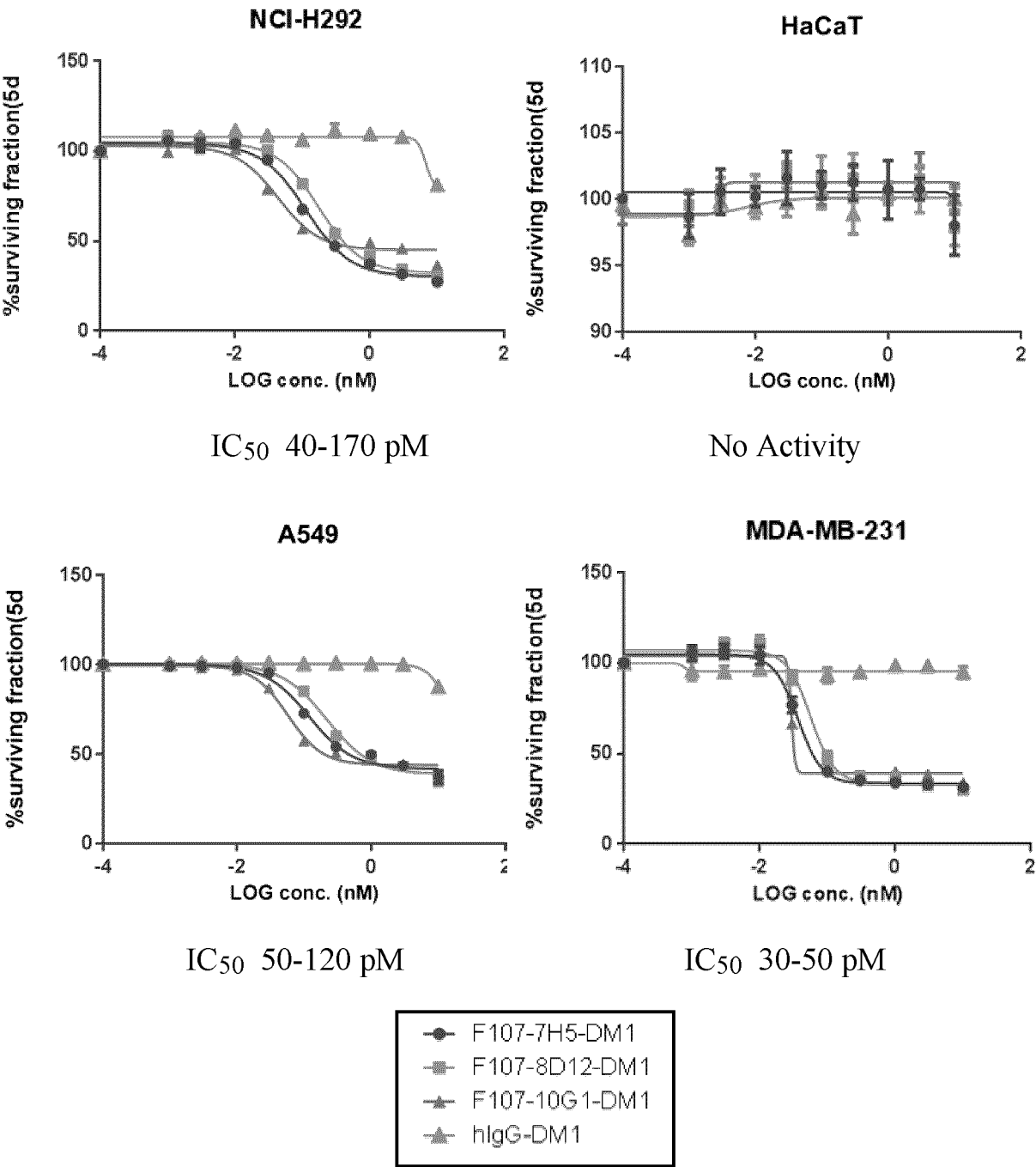
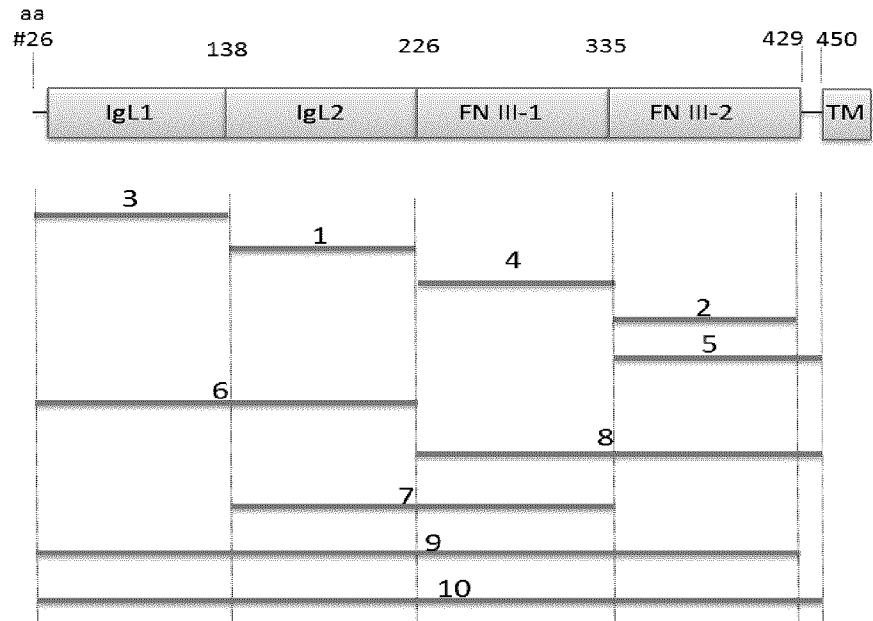


FIG. 4

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AXL protein ectodomain



AXL fragment #	AXL amino acids	AXL domain	F107-8D12-2	F107-7H5-2	F107-10G1-3	F111-3C8-2	F111-5E9-2	F138-9H4-2	F138-9G4-2
1	135-226	IgL2	-	-	-	++	+	-	+
2	321-429	FN III-2	-	-	-	-	-	-	-
3	26-138	IgL1	+++	+++	+++	-	-	++	-
4	219-335	FN III-1	-	-	-	-	-	-	-
5	321-450	FN III-2+	-	-	-	-	-	-	-
6	26-226	IgL1+IgL2	++++	++++	+++	++	+	+++	+
7	135-335	IgL2+FN III-1	-	-	-	+++	++	-	+
8	219-450	FN III-1+FN III-2+	-	-	-	-	-	-	-
9	26-429	IgL1+IgL2+FN III-1+FN III-2	++++	+++	++	+++	++	++	+
10	26-450	IgL1+IgL2+FN III-1+FN III-2+	++++	++++	+++	++++	++	+++	+
None	-----	-----	-	-	-	-	-	-	-

FIG. 5

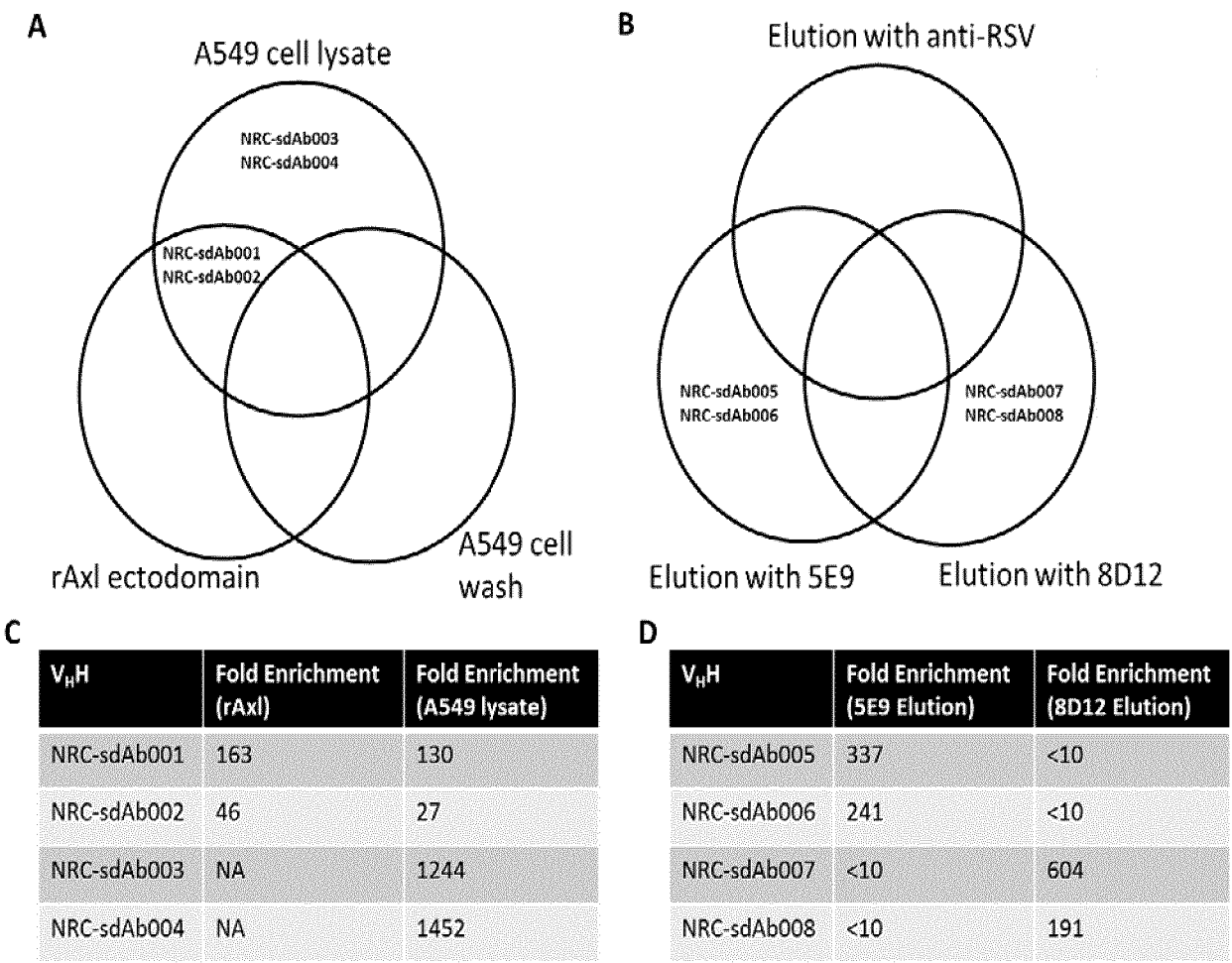


FIG. 6

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>F107.10G1(heavy)>EVNLVESGGGVVKPGASLKLSCEASGFTFSNYGMSWVRQTSDKREWVASISGGGGRTYLD
NVKGRFISRENAKNTLYLQMSSLKSEDTALFYCARGARASYFAMDYWGQGSSVTVSS (SEQ ID:47, 77-79, 35-37)

>F107.10G1(kappa)>VIVMTQSHKFMSTSVGDRVSITCKASQDVTTAVAWYQQKPGQSPKLLIYWASTRHTGVPDR
FTGSGSGTDYSLTISNVQTEDLAFYCQQHFTTPLTFGAGTKLELK (SEQ ID NO:46, NO: 80-82 and NO:32-34)

>F111.5E9(heavy)>QIQLVQSGPELKKPGETVKISCKASGYTFTKYGMNWVKQAPGKLKWMGWINTYTGEPTYAD
DFKGRFASLETSASTAYLQINNLTTEDMVTFCAKGGYSNPIPMDYWGQGTSVTVSS (SEQ:45, 83-85, 23-25)

>F111.5E9(kappa)>DIQMTQTSSLSASLGDRVTISCRASQDINNYLNWYQQKPDGTVKLLIYYISRLHSGVPSRFSGS
GSGTDYSLTISNLEDVATFCQQGNTLPFTFGSGTKLEIK (SEQ ID NO:44, NO: 86-88 and NO:20-22)

>F107.8D12(heavy)>QVQLQQPGAELVKPGASVQLSCKASGYTFISFWINWVKRPGQGLEWMGNIFPGSSSTNYN
EKFKSKATLTVDKSSTAYMQLSSLTSEDSAVFCARDYYGGSPDYWGQGTTLTVSS (SEQ ID NO:41, 89-91, 17-19)

>F107.8D12(kappa)>DIVMSQSPSSLAVSAGERVTMSCKSSQSLLNTRTRKNYLAWYQQKPGQSPKLLIYWASTRES
GVPDRFTGSGSGTDFTLISSVQAEDLAVYCKQSYNLWTFGGGTKLEIK (SEQ ID NO:40, NO: 92-94, 15,16,11)

>F107.7H5(heavy)>QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWINWVKRPGQGLEWIGNIYPDSSSTNYNEK
FKSKATLTVDKSTTAYIQFSSLTSEDSAVYCTRDTYGGSPDYWGQGTTLTVSS (SEQ ID NO:39, 95-97, 12-14)

>F107.7H5(kappa)>DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLNSRTRKIYLAWYQQKPGQSPKLLIYWASTRQSGVP
DRFTGSGSGTDFTLISSVQAEDLAVYCKQSYNLWTFGGGTKLEIK (SEQ ID NO:38, NO: 98-100, 9-11)

>F155.3C7(heavy)>QVQLQQPGTELVKPGASVKLSCKASGYIFTNFWINWVKRPGQGLEWIGNIFPGSNSSNYNEK
FKNKATLTVDKSSTAYMHLSSLTSEDSAVYCVRDYYGGSPDYWGQGTTLTVSS (SEQ NO:73, 101-103, 151-153)

>F155.3C7(kappa)>DIVMSQSPSSLAVSAGEKVTMSCKSSQSLLNSKTRKNYLAWYQQKPGQSPKLLIYWASTRESGV
PARFTGSGSGTDFTLISSVQAEDLAIYCKHSYNLWTFGGGTKLEIR (SEQ:74, 104-106, 154-156)

>F111.3C8(heavy)>QVQLQQPGAELGKPGTSVKLSCKASGYTFTSYWMHWVKRVPGQGLEWIGNINPNSTSADYN
EKFKRKATLTVDKSSTAYMQLSTLTSEDSAVYCTRPLMGPYWYFDVWGTGTTVTVSS (SEQ:43, 107-109, 29-31)

>F111.3C8(kappa)>QIVLTQSPAIMASAPGEKVTISCSASSSSVSYMYWYQQKPGSSPKPWIYRTSNLASGVPARFSGS
GTSYSLTISSMEAEDAATYCQQYHNPPTFGGGTKLEIK (SEQ ID NO:42, 110-112, and 26-28)

>F149.4G4(heavy)>QIQLVQSGPELKKPGETVKISCKTSGYTFTYYGINWVKQAPGKLEWMGWINTYLGEPTYAD
DFKGRFASLETSASTAYLQINNLRDEDMATFCTRGTMSYSFDYWGQGTALTVSS (SEQ:75, 113-115, 157-159)

>F149.4G4(kappa)>QNVLTQSPAIMASAPGEEVTMTCRASSSSSVSSSYLHWYQQKSGASPKLWIYSTSKLASGVPARFS
GSGSGTSYSLTISSVEAEDAATYCHQYSGDPLTFGSGTKLEVK (SEQ ID NO:76, NO: 116-118, 160-162)

FIG. 7

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>NRC-sdAb001

QVKLEESGGGLVQAGGSLRLSCTAS**ASISSFDI**MGWYRQAPGKQRELVA**AITL**DIANYRDSVKGRFTISRDNAKNTVY
 LQMDSLKPEDTARYHC**AAFOSDQNY**WGQGTQVTSS (SEQ ID NO: 119, NO: **127-129**, 163-165)

>NRC-sdAb002

QVQLVDSGGGLVQAGGSLRLSCAT**STRTVSSAV**MAWFRQAPEKVRDFVG**FI**TNSG**NI**LYDDSVKGRFTISRDNAQNT
 VYLQMNSLKPEDTAVYYC**AAKWSFSSGYDLRRAAMYDY**WGQGTQVTSS (SEQ NO:120, **130-132**, 166-168)

>NRC-sdAb003

QVQLVESGGGLVQAGGSLRLSCAAS**GVTL**DYTAIGWFRQAPGKERELVA**AITSGGNT**DYAESAKGRFRISRDNASKNTIY
 LQMNSLKPEDTGVYYC**AARRGGARGEYDY**WDQGTQVTSS (SEQ ID NO:121, NO: **133-135**, 169-171)

>NRC-sdAb004

QVQLVESGGGVVQAGGSLRLSCAF**S**RGA**FD**TYEIGWFRQAPGKEREFVA**AVTRNGDSV**VYADSLKARFTASRNNAVN
 TAYLHMNILQPEDTATYYC**AANWRPLRTSSGADDYAD**WGQGTQVTSS (SEQ ID NO:122, **136-138**, 172-174)

>NRC-sdAb005

QVKLEESGGGLAQAGGSLRLSCAAS**GSISSINT**IGWFRQAPGKQRELVA**ASDSGANR**NYADSVKGRFTISRDNAKNTV
 YLQMNNLKPEDTAIYYC**RAWGTGTISTMY**WGQGTQVTSS (SEQ ID NO:123, NO: **139-141**, and 175-177)

>NRC-sdAb006

QVKLEESGGGLVQAGASRLSCVASE**SIFGFNT**MGWYRQAPGNERELVAS**ISNSKRT**MYADSVKGRFTISRDNAKNTV
 NLQMNNLKPEDTAVYYC**RAWGIITSATVY**WGQGTQVTSS (SEQ ID NO:124, NO: **142-144**, 178-180)

>NRC-sdAb007

QVKLEESGGGLVQAGGSLRLSCAT**STRTVSSAV**MAWFRQAPEKERDFVG**FISNSGSV**YYDDSVKGRFTISRDNAQNTV
 YLQMNSLKPEDTAVYYC**AIIWRTSDLTGRFNT**WGQGTQVTSS (SEQ ID NO:125, NO: **145-147**, 181-183)

>NRC-sdAb008

QVKLEESGGGLVQAGGSLRLSCAAS**GSSGMINT**MGWYRQAPGKQRELVA**RRSTGGTT**NYADSVKGRFTISRDDANN
 TVYLQMNSLKPEDTAVYYC**AIIWRTSDLTGRFNT**WGQGTQVTSS (SEQ ID NO:126, NO: **148-150**, 184-186)

FIG. 8

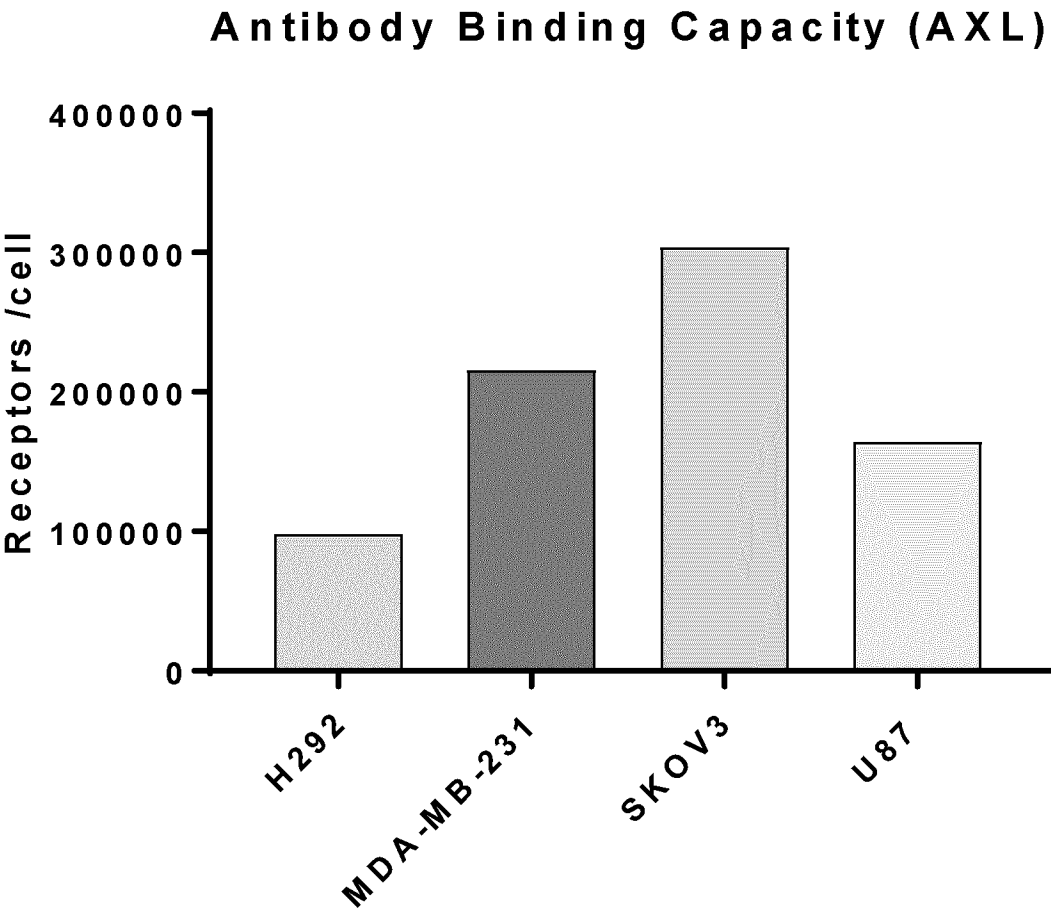


FIG. 9

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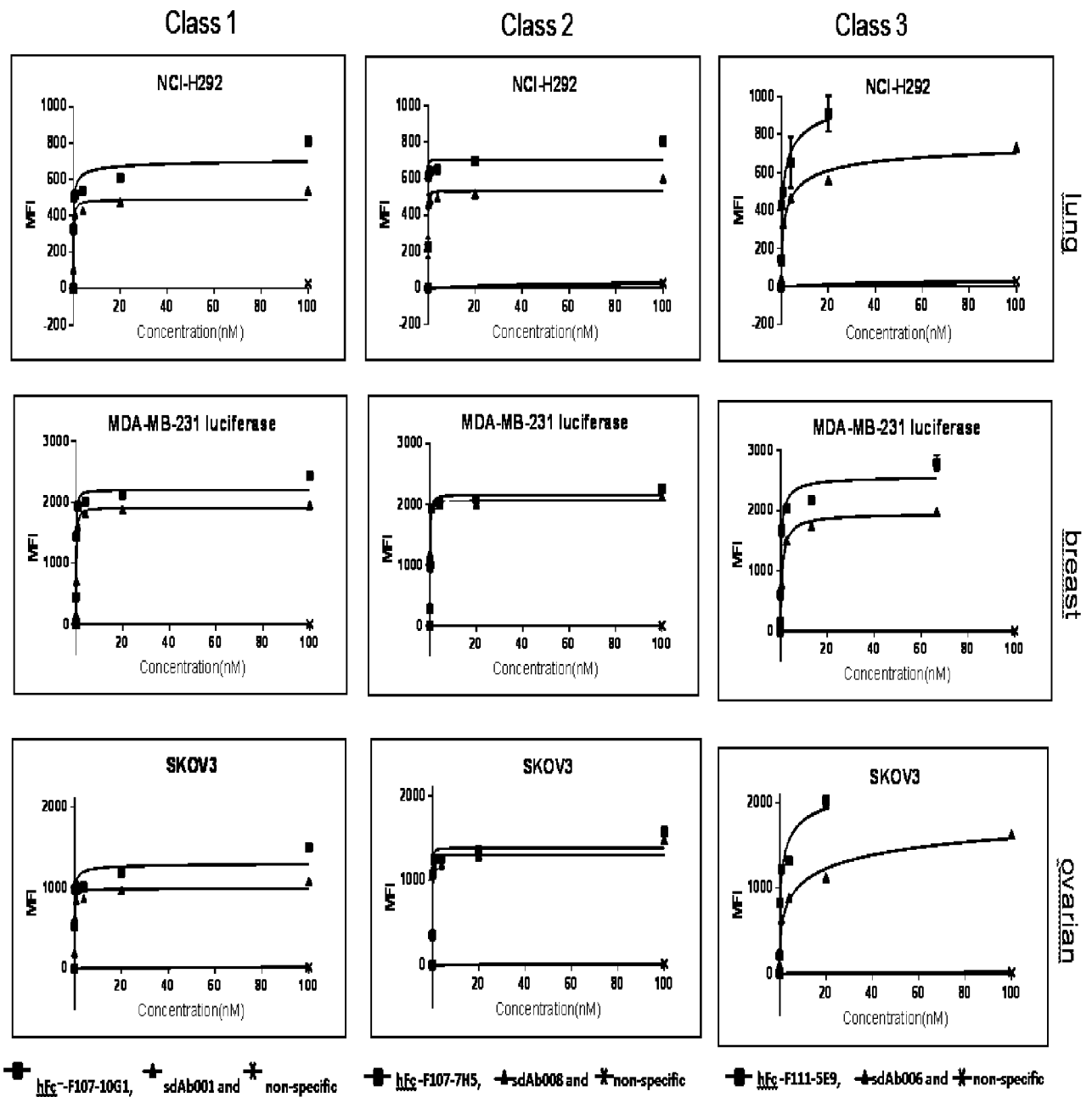


FIG. 10

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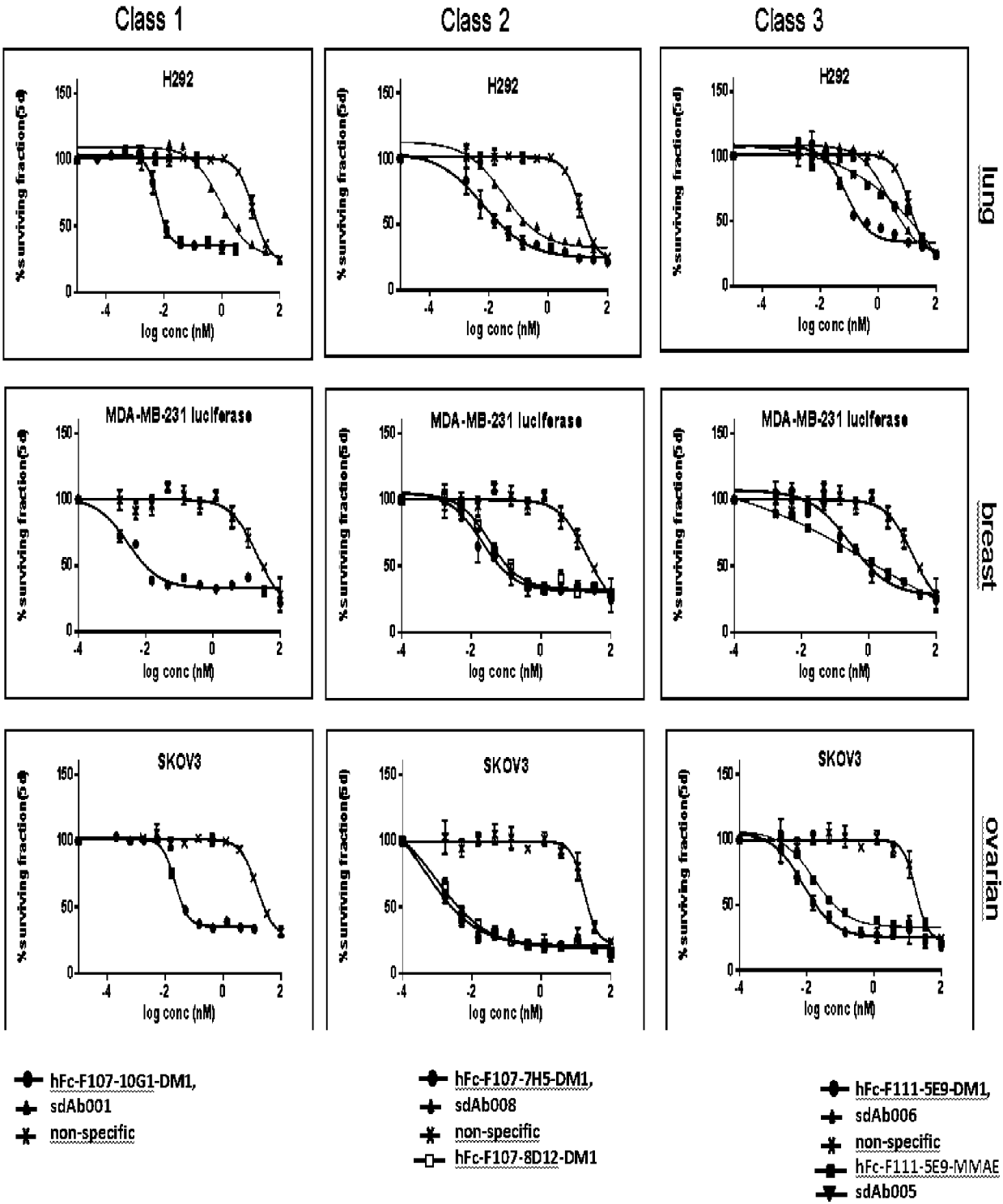


FIG. 11

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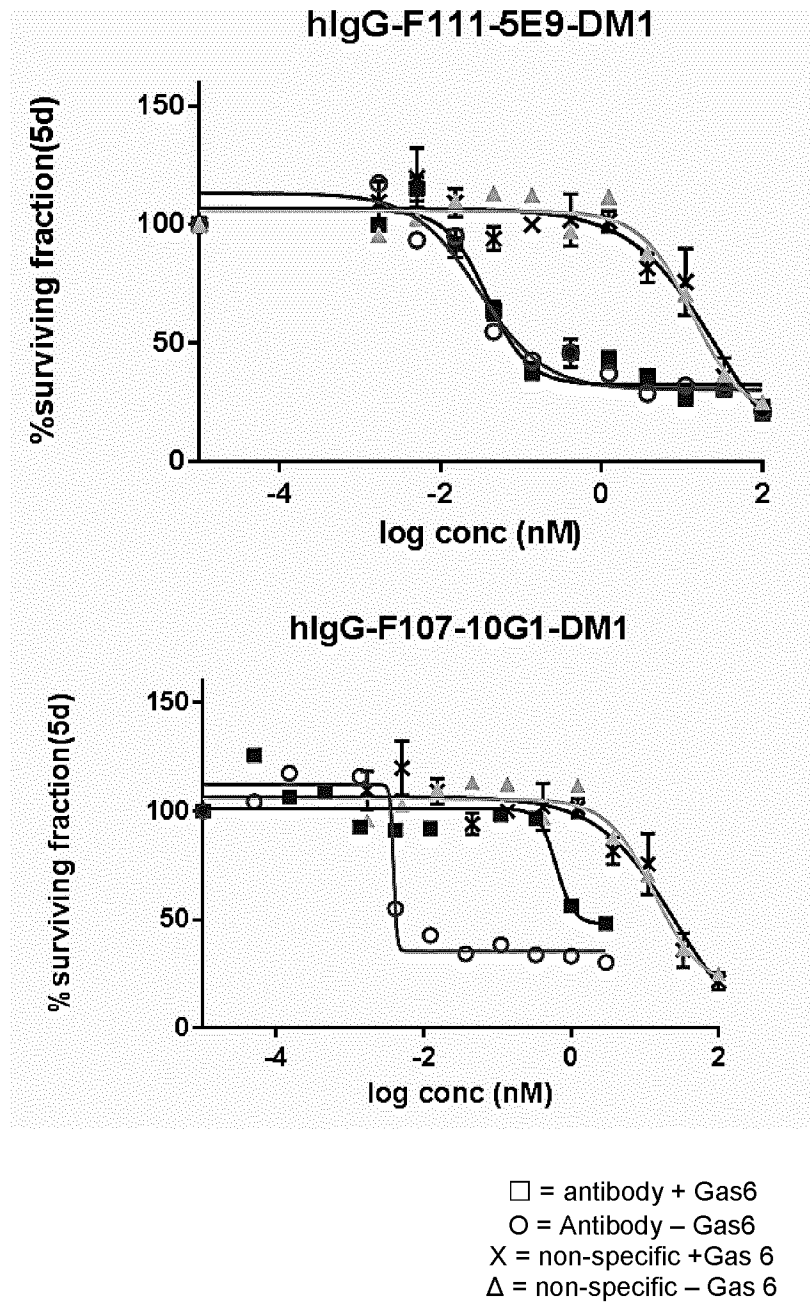


FIG. 12

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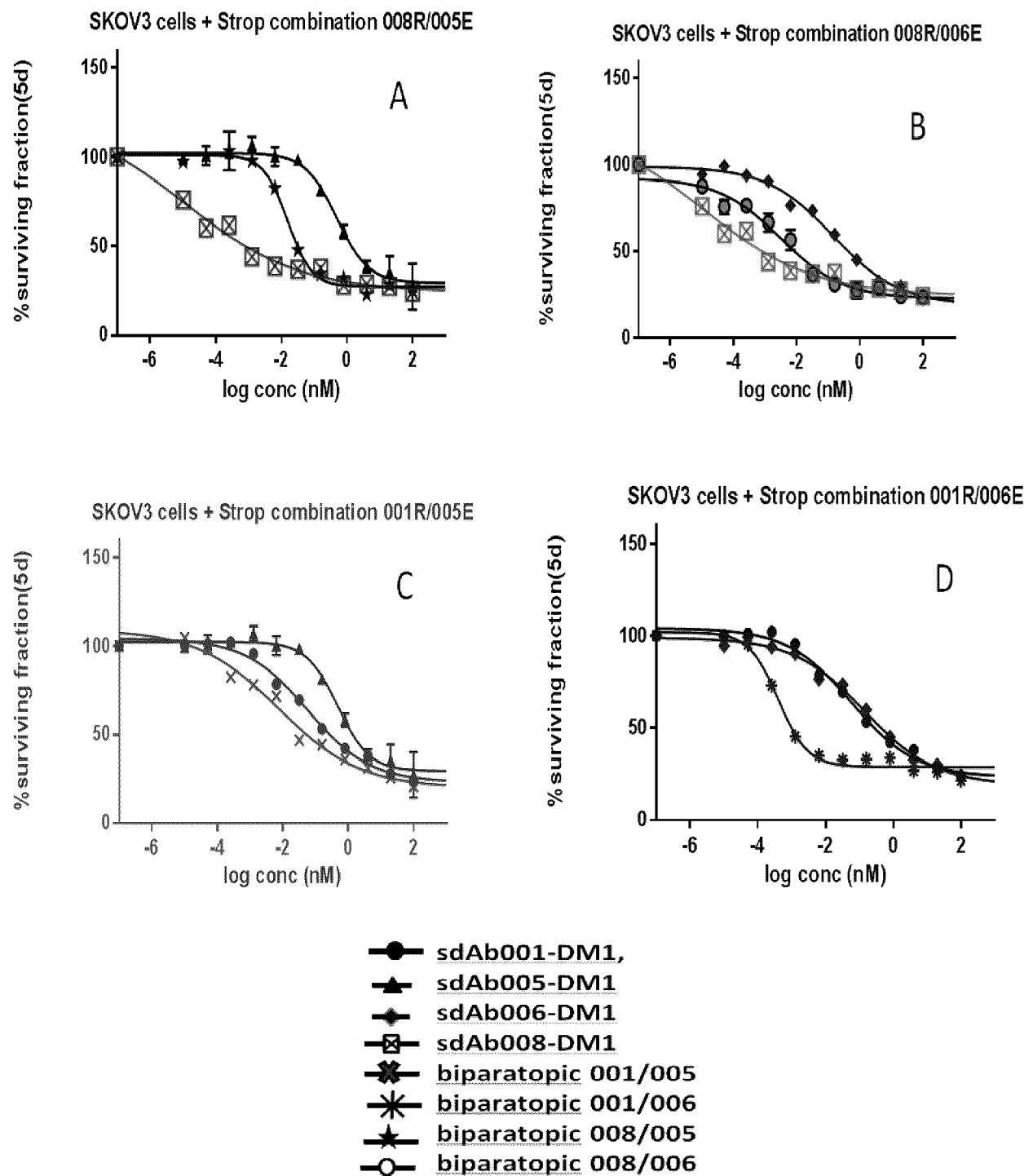


FIG. 13

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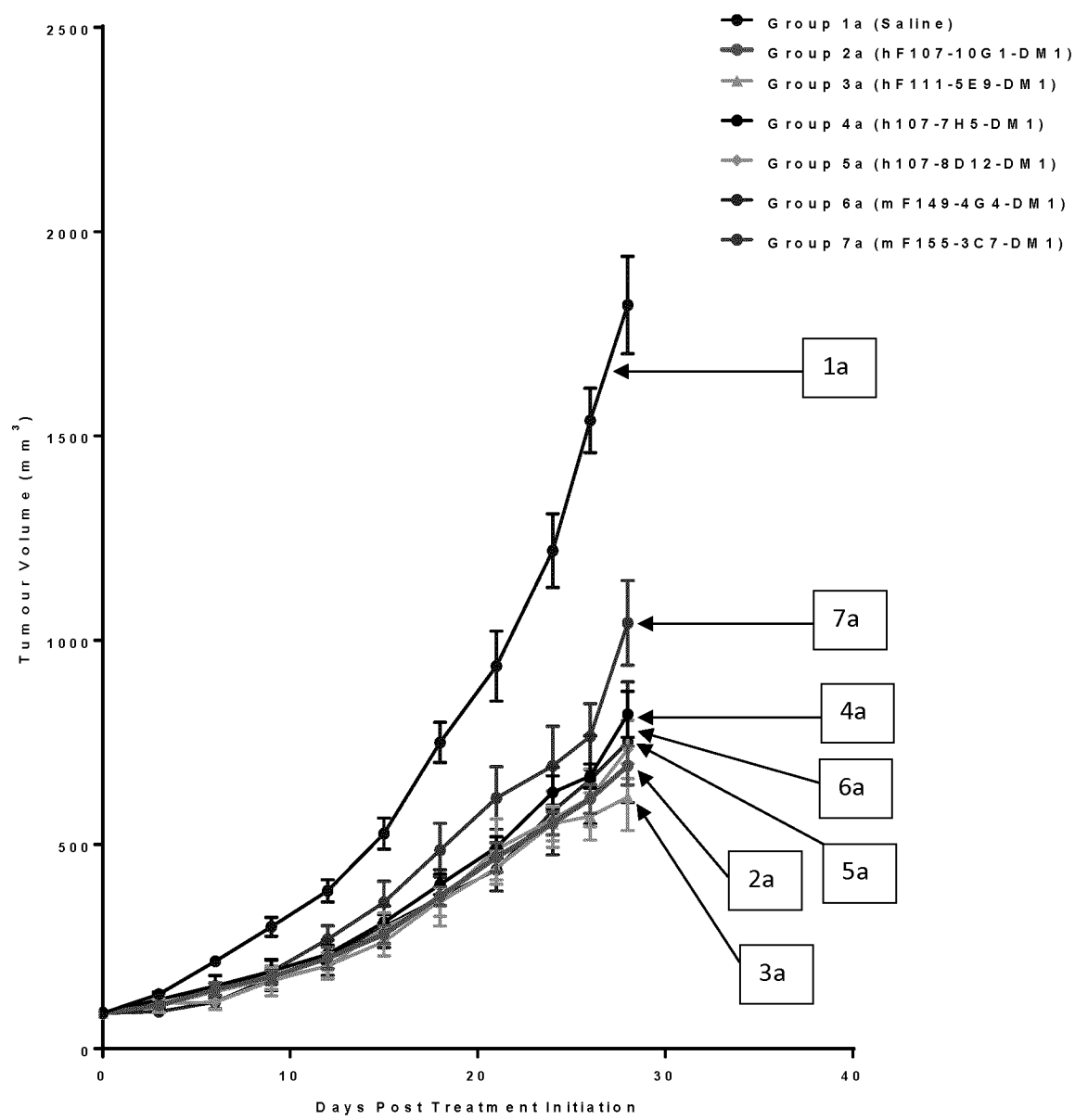


FIG. 14

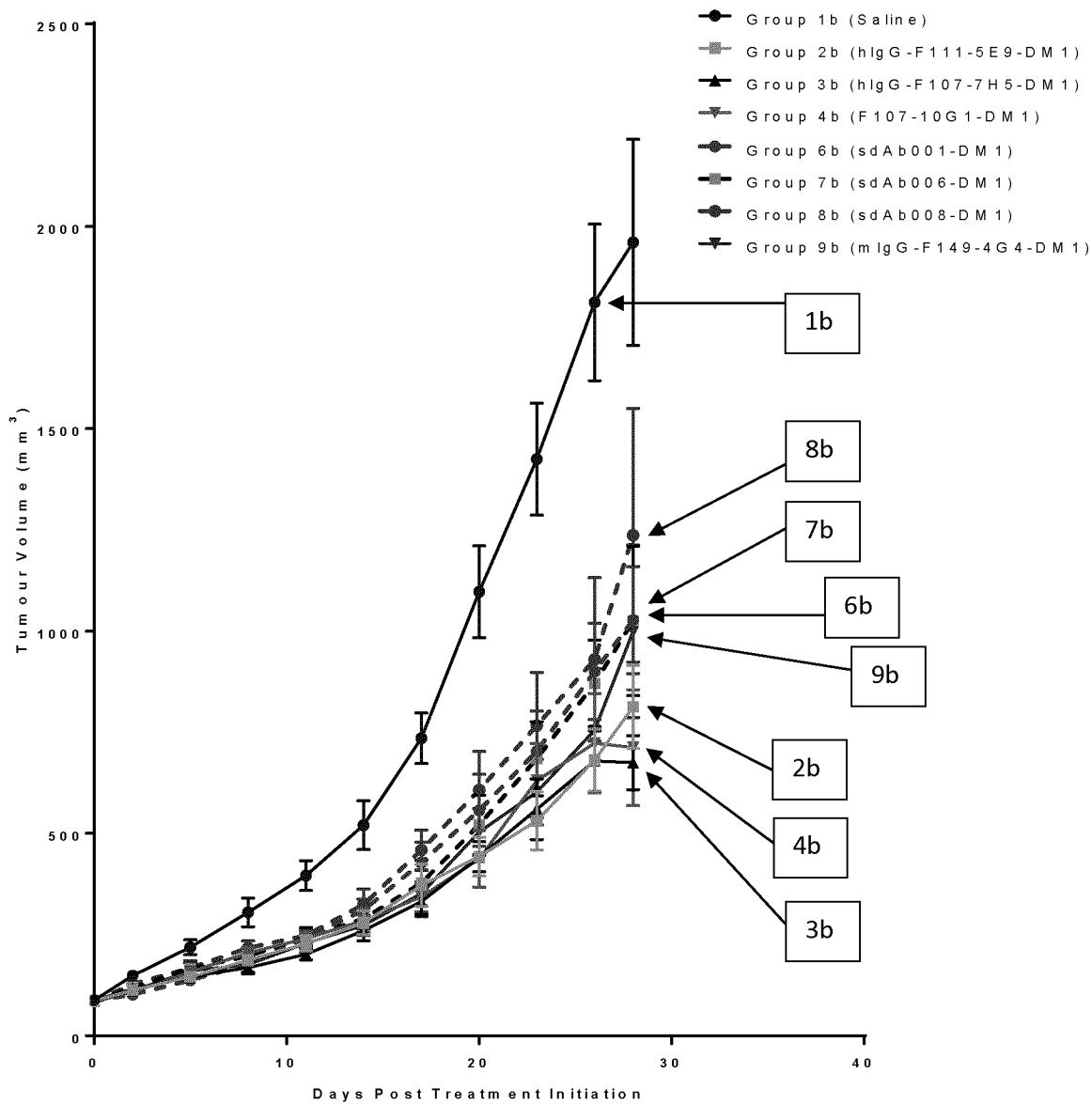
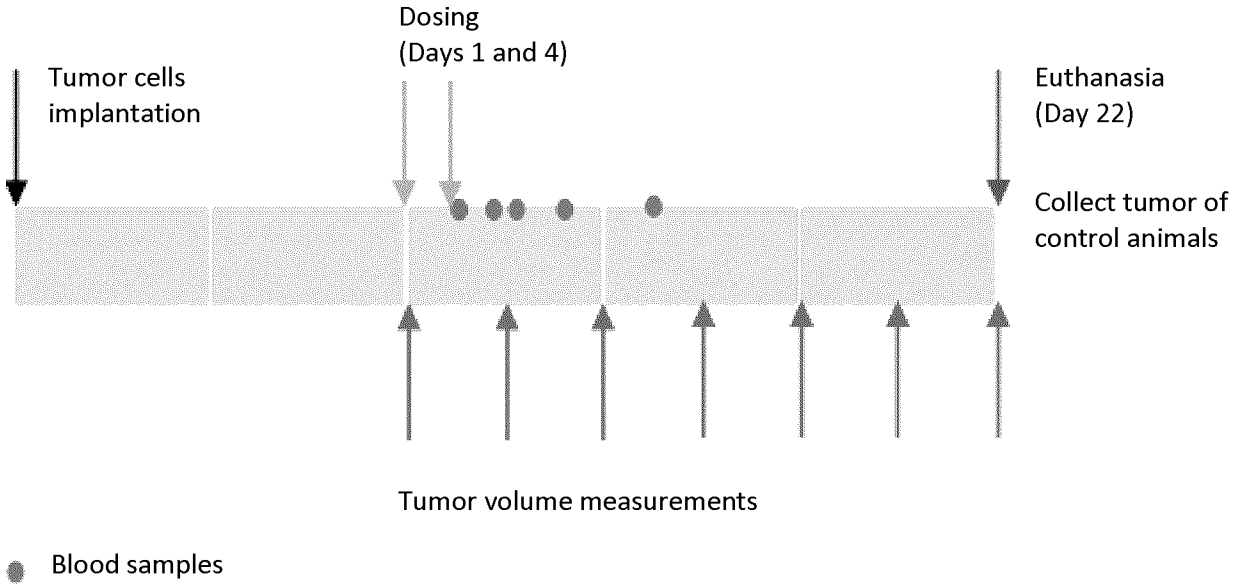


FIG. 15

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Groups	No. of animals/Time Point [®]	Pre 2 nd dose	Post 2 nd dose											
			1 h	2 h	4 h	6 h	24 h	48 h	96 h	120 h	168 h	216 h	264 h	336 h
2 to 3	1	x				x				x				x
	1	x				x				x				x
	1	x				x				x				x
	1		x				x				x			
	1		x				x				x			
	1		x				x				x			
	1			x				x				x		
	1			x				x				x		
	1			x				x				x		
	1				x				x				x	
	1				x				x				x	
	1				x				x				x	

FIG. 16

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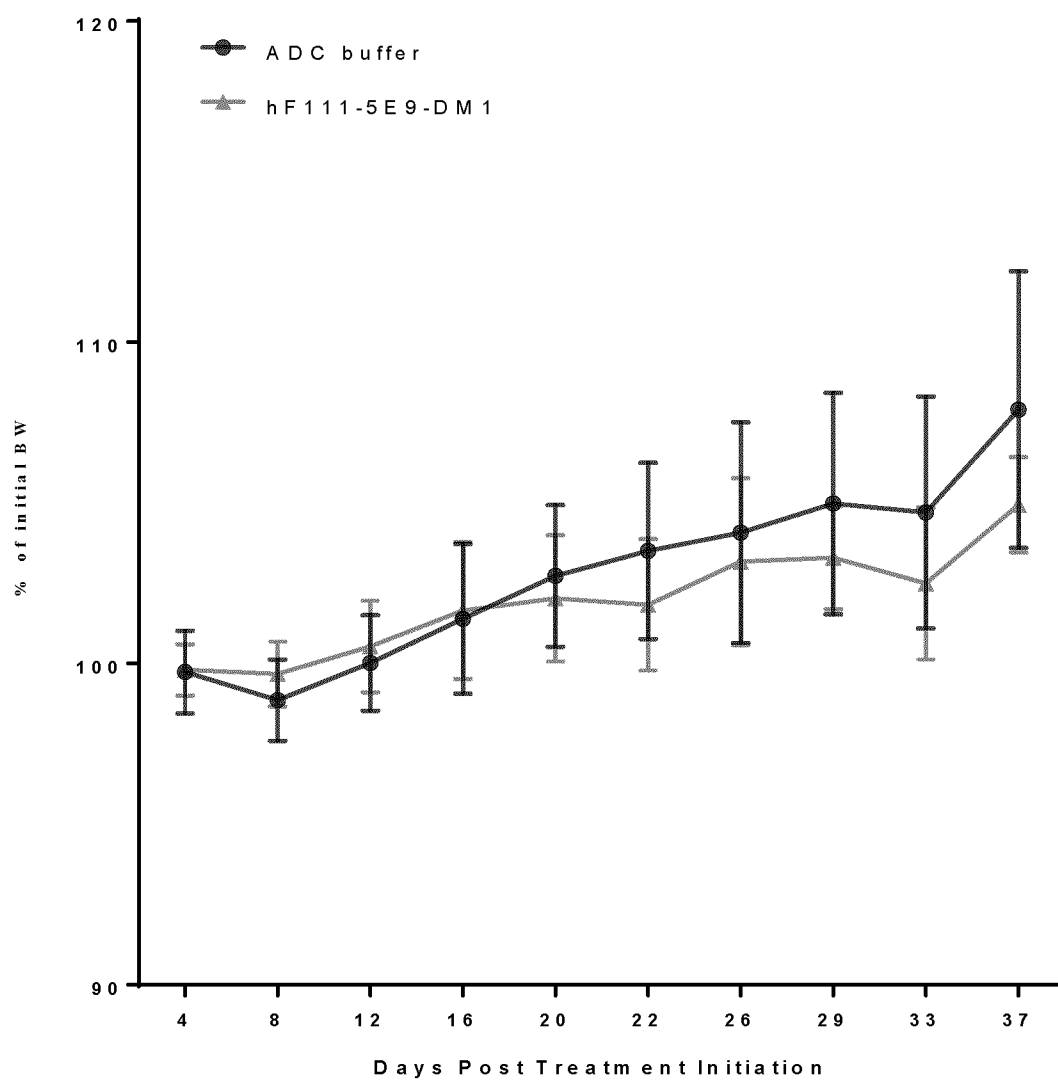


FIG. 17

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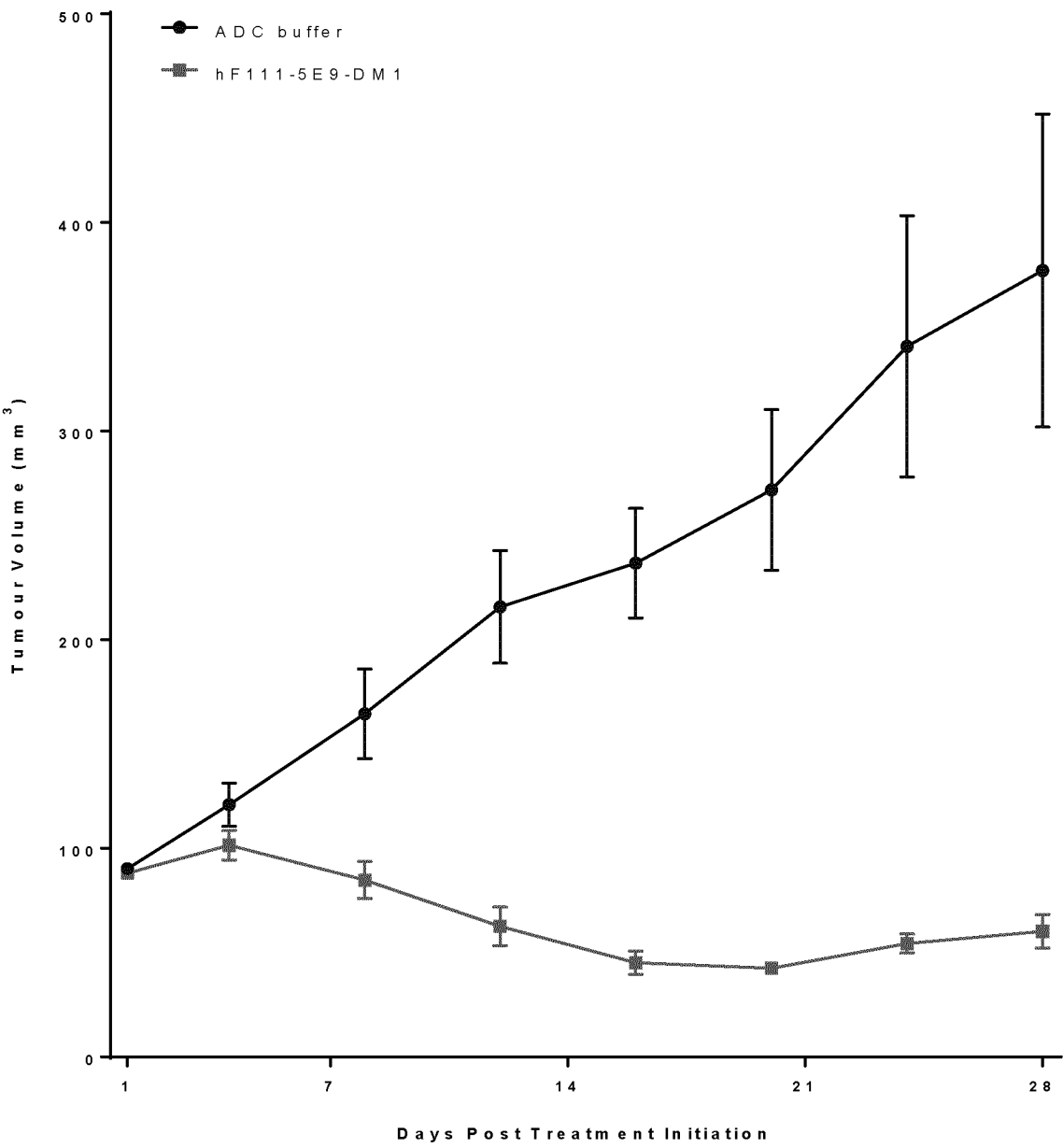


FIG. 18

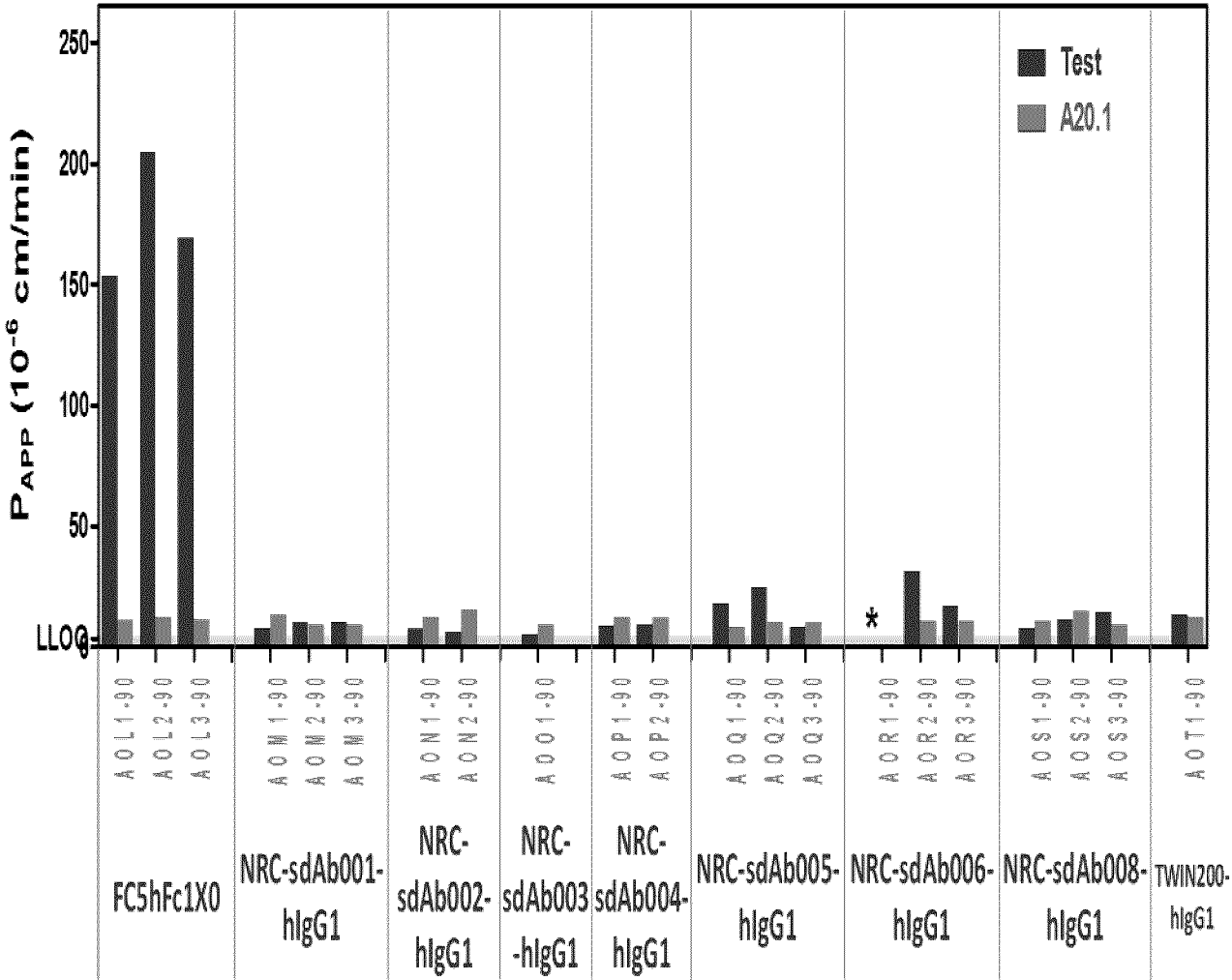


FIG. 19

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2018/051108

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07K 16/28** (2006.01), **A61K 39/395** (2006.01), **A61K 47/68** (2017.01), **A61K 49/00** (2006.01), **A61P 35/00** (2006.01), **C07K 14/705** (2006.01) (more IPCs on the last page)

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)
IPC: ALL(2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Databases: Canadian Patent Database, Questel Orbit, Scopus, Google Patent, Google Scholar, Genomequest

Keywords: AXL, antibody, ectodomain, immunoglobulin-like domain, IgL1, Ig1

Sequences: SEQ ID NO: 1-19, 38-47, 73-150

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/005593 A1 BREIJ <i>et al.</i> 14 January 2016 (14-01-2016) ***whole document***	1-62
X	WO 2017/009258 A1 BOSHUIZEN <i>et al.</i> 19 January 2017 (19-01-2017) ***whole document***	1-62

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"T" "X" "Y" "&"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention 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 document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
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Date of the actual completion of the international search
06 November 2018 (06-11-2018)

Date of mailing of the international search report
03 December 2018 (03-12-2018)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 819-953-2476

Authorized officer

Scott Gurd (819) 639-7793

Additional IPCs form Box A (second sheet):

C07K 19/00 (2006.01), *C12N 15/113* (2010.01), *C12N 15/13* (2006.01), *C12N 5/10* (2006.01),
C12N 7/01 (2006.01), *C12P 21/08* (2006.01), *G01N 33/53* (2006.01), *G01N 33/543* (2006.01)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2018/051108**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see Extra Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box III:

The claims are directed to a plurality of inventive concepts as follows:

Group A - Claims 1-16, 19, 20-22, 25-62 (partially) and claim 23 (completely) are directed to the F107-7H5 antibody which specifically binds to an epitope in the immunoglobulin-like (IgL) domain of human AXL, antibody drug conjugates comprising said antibody, and uses thereof;

Group B - Claims 1-16, 19, 20-22, 25-62 (partially) and claim 24 (completely) are directed to the F107-8D12 antibody which specifically binds to an epitope in the immunoglobulin-like (IgL) domain of human AXL, antibody drug conjugates comprising said antibody, and uses thereof;

Group C - Claims 1-16, 19, 20, and 25-62 (all partially) are directed to the F111-3C8 antibody which specifically binds to an epitope in the immunoglobulin-like (IgL) domain of human AXL, antibody drug conjugates comprising said antibody, and uses thereof;

Group D - Claims 1-16, 19, 20, and 25-62 (all partially) are directed to the F111-5E9 antibody which specifically binds to an epitope in the immunoglobulin-like (IgL) domain of human AXL, antibody drug conjugates comprising said antibody, and uses thereof;

Group E - Claims 1-16, 19, 20, and 25-62 (all partially) are directed to the F107-10G1 antibody which specifically binds to an epitope in the immunoglobulin-like (IgL) domain of human AXL, antibody drug conjugates comprising said antibody, and uses thereof;

Group F - Claims 1-16, 19, 20, and 25-62 (all partially) are directed to the F155-3C7 antibody which specifically binds to an epitope in the immunoglobulin-like (IgL) domain of human AXL, antibody drug conjugates comprising said antibody, and uses thereof;

Group G - Claims 1-16, 19, 20, and 25-62 (all partially) are directed to the F149-4G4 antibody which specifically binds to an epitope in the immunoglobulin-like (IgL) domain of human AXL, antibody drug conjugates comprising said antibody, and uses thereof;

Group H - Claims 1-4, 17-20, and 25-62 (all partially) are directed to the sdAB001 single domain antibody, antibody drug conjugates comprising said antibody, and uses thereof;

Group I - Claims 1-4, 17-20, and 25-62 (all partially) are directed to the sdAB002 single domain antibody, antibody drug conjugates comprising said antibody, and uses thereof;

Group J - Claims 1-4, 17-20, and 25-62 (all partially) are directed to the sdAB003 single domain antibody, antibody drug conjugates comprising said antibody, and uses thereof;

Group K - Claims 1-4, 17-20, and 25-62 (all partially) are directed to the sdAB004 single domain antibody, antibody drug conjugates comprising said antibody, and uses thereof;

Group L - Claims 1-4, 17-20, and 25-62 (all partially) are directed to the sdAB005 single domain antibody, antibody drug conjugates comprising said antibody, and uses thereof;

Group M - Claims 1-4, 17-20, and 25-62 (all partially) are directed to the sdAB006 single domain antibody, antibody drug conjugates comprising said antibody, and uses thereof;

Group N - Claims 1-4, 17-20, and 25-62 (all partially) are directed to the sdAB007 single domain antibody, antibody drug conjugates comprising said antibody, and uses thereof; and

Group O - Claims 1-4, 17-20, and 25-62 (all partially) are directed to the sdAB008 single domain antibody, antibody drug conjugates comprising said antibody, and uses thereof.

The groups of alleged inventions are each directed to an antibody which specifically binds to an IgL domain of human AXL. However, antibodies which specifically bind to the IgL domain of AXL, which are internalized into cells expressing AXL, and which do not compete with the Gas6 ligand are known in the art (See D1 and D2). Therefore, there is no single inventive feature which is common to the groups of alleged inventions.

As indicated above, all claims were searched without the request for additional fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2018/051108

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
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