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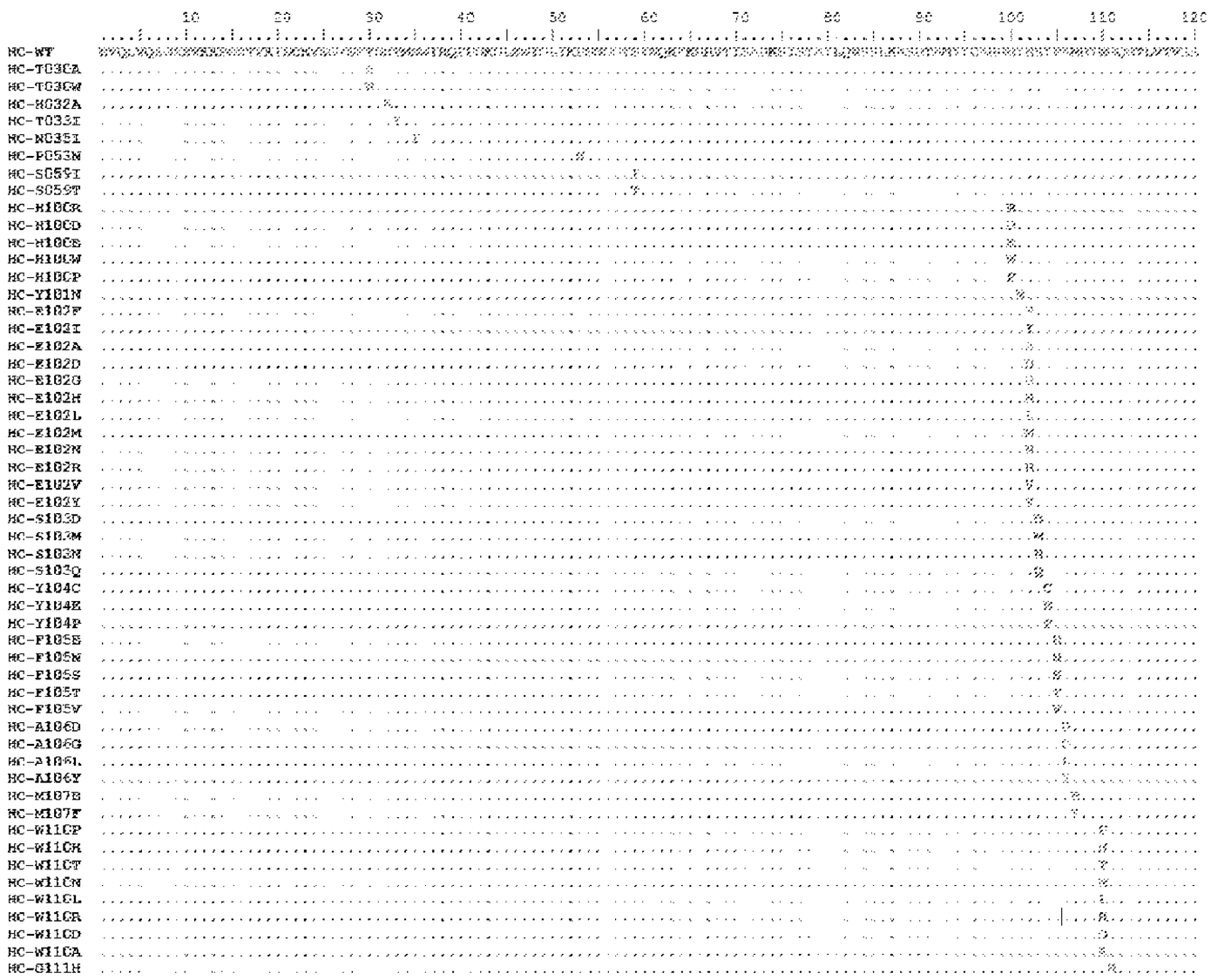


FIG. 1A

(57) Abstract: Methods for treatment of Axl-expressing cancers are provided. The methods involve administering to a subject in need thereof, a polypeptide having a heavy chain variable region and/or light chain variable region that specifically binds to Axl protein, antibodies or antibody fragments containing the polypeptide, and/or an immunoconjugate compound thereof. The immunoconjugate compound is a Conditionally Active Biologic (CAB) anti-Axl antibody conjugated to one or more drug(s) via a cleavable linker (CAB-Axl-ADC). The immunoconjugate is a mAbBA3011-cleavable linker-$MMAE_{(n)}$ or pharmaceutically acceptable salt thereof, in which the mAbBA3011 is an antibody or antibody fragment, the MMAE is monomethyl auristatin E, and the (n) is an integer between 1 and 4.

[Continued on next page]

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Pharmaceutical compositions and kits comprising the polypeptide or antibodies and antibody fragments containing the polypeptide are also provided.

METHODS OF TREATING AXL-EXPRESSING CANCERS WITH ANTI-AXL ANTIBODIES, ANTIBODY FRAGMENTS AND THEIR IMMUNOCONJUGATES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 63/113,040, filed November 12, 2020, the entire disclosure of which is specifically incorporated herein by reference.

REFERENCE TO A SEQUENCE LISTING

This application includes a sequence listing submitted herewith as a text file named "BIAT-1034_Sequence Listing" created on October 14, 2020 and containing 12,000 bytes. The material contained in this text file is incorporated herein by reference.

FIELD OF THE DISCLOSURE

[0001] This disclosure relates to methods for treatment of Axl-expressing cancers. The methods comprise administering to a subject in need thereof, a weekly dose of from about 0.3 mg/kg to about 1.8 mg/kg of anti-Axl antibodies, antibody fragments and/or immunoconjugates of such antibodies and antibody fragments.

BACKGROUND OF THE DISCLOSURE

[0002] Axl protein (also known as Ark, UFO, Tyro-7) is a receptor tyrosine kinase in the Tyro-3 family of kinases. The Tyro-3 receptor kinases are characterized by a combination of two immunoglobin-like domains and dual fibronectin type III repeats in the extracellular region and a cytoplasmic kinase domain. The ligands for Tyro-3 receptor kinases are Gas6 (growth-arrest-specific 6) and protein S, two vitamin-K dependent proteins that show 43% amino acid sequence identity and share similar domain structures. Each protein has an N-terminal GIa domain containing 11 g-carboxyglutamic acid residues, followed by four epidermal growth factor (EGF)-like modules, and a C-terminal sex hormone-binding globlin (SHBG)-like structure consisting of two tandem laminin G domains. The SHBG domain is both necessary and sufficient for Tyro-3 receptor kinase binding and activation, whereas the GIa domain binds the negatively charged membrane phospholipids and plays an important role in Tyro-3 kinase-mediated phagocytosis of apoptotic cells.

[0003] Axl activation leads to signaling through PI-3-kinase/Akt (Franke et al., *Oncogene*, vol. 22, pp. 8983-8998, 2003) and other major pathways like Ras/Erk and β-catenin/TCF

(Goruppi et al., *Mol. Cell. Biol.*, vol. 21, pp. 902-915, 2001). Axl is weakly expressed in a range of normal tissues, including brain, heart, skeletal muscle, the organ capsules and connective tissues of several other organs, and in monocytes, but not lymphocytes. Akt phosphorylation induced by Axl has been described in survival of fibroblasts (Goruppi et al., *Mol. Cell. Biol.*, vol. 17, pp. 4442-4453 1997), endothelial cells (Hasanbasic et al., *Am J Physiol Heart Circ Physiol*, vol. 287, H1207-H1213, 2004), vascular smooth muscle cells (Melaragno et al., *J. Mol. Cell. Cardiol.*, vol. 37, pp. 881-887, 2004) and neurons (Allen et al., *Mol. Endocrinol.*, vol. 13, pp.191-201, 1999). Furthermore, Axl plays a role in cell-adhesion and chemotaxis because Axl knockout animals display impaired platelet aggregate stabilization and thrombus formation as a result of reduced activation of the platelet integrin IIb3.

[0004] Dysregulation of Axl or its ligand Gas6 is implicated in the pathogenesis of a variety of human cancers. Axl overexpression has been demonstrated in various cancer types, e.g. breast (Meric et al., *Clin. Cancer Res.*, vol. 8, pp. 361-367, 2002; Berclaz et al., *Ann. Oncol.*, vol. 12, pp. 819-824, 2001), colon (Chen et al., *Int. J. Cancer*, vol. 83, pp. 579-584, 1999; Craven et al., *Int. J. Cancer,* vol. 60, pp. 791-797, 1995), prostate (Jacob et al., *Cancer Detect. Prev.*, vol. 23, pp. 325-332, 1999), lung (Wimmel et al., *Eur J Cancer*, vol. 37, pp. 2264-2274, 2001), gastric (Wu et al., *Anticancer Res.*, vol. 22, pp. 1071-1078, 2002), ovarian (Sun et al., *Oncology*, vol. 66, pp. 450-457, 2004), endometrial (Sun et al., *Ann. Oncol.*, vol. 14, pp. 898-906, 2003), renal (Chung et al., *DNA Cell Biol.*, vol. 22, pp. 533-540, 2003), hepatocellular (Tsou et al., *Genomics*, vol. 50, pp. 331-340, 1998), thyroid (Ito et al., *Thyroid*, vol. 12, pp. 971-975, 2002; Ito et al., *Thyroid*, vol. 9, pp. 563-567, 1999), and furthermore in chronic myelogenous leukemia (Janssen et al., *Oncogene*, vol. 6, pp. 2113-2120, 1991; Braunger et al., *Oncogene*, vol. 14, pp. 2619-2631 1997; O'Bryan et al., *Mol. Cell. Biol.*, vol. 11, pp. 5016-5031, 1991), acute myeloid leukemia (Rochlitz et al., *Leukemia*, vol. 13, pp. 1352-1358, 1999), osteosarcoma (Nakano et al., *J. Biol. Chem.*, vol. 270, pp. 5702-5705, 2003), melanoma (van Ginkel et al., *Cancer Res.*, vol. 64, pp. 128-134, 2004), and in head and neck squamous cell carcinoma (Green et al., *Br J. Cancer.*, vol. 94, pp. 1446-5, 2006).

[0005] Recently, by profiling of phosphotyrosine signaling, activated Axl was detected in about 5% of primary tumors of NSCLC (Rikova et al, *Cell*, vol. 131, pp. 1190-1203, 2007). Axl expression is induced by targeted chemotherapy drugs and drug-induced Axl expression confers resistance to chemotherapy in acute myeloid leukemia (Hong et al, *Cancer Letters*, vol. 268, pp. 314-324, 2008), as well as resistance to imatinib and Lapatinib/Herceptin in

gastrointestinal stromal tumors (Mehadevan, et al, *Oncogene*, vol. 26, pp.3909-3919, 2007) and breast cancer (Liu et al, Cancer Research, vol. 281, pp. 6871-6878, 2009), respectively.

[0006] Moreover Axl has been identified to be related to tumor metastasis because Axl is upregulated in aggressive breast cancer cell lines compared to non-invasive cells. *In vitro*, Axl activity was found to be required for migration and invasion, and this activity could be inhibited by antibody treatment (WO 04/008147). Similarly, abrogation of Axl activity *in vivo*, either via expression of a dominant negative version of Axl (Vajkoczy, P., et al., *Proc. Natl. Acad. Science U.S.A.*, vol. 103, pp. 5799-5804, 2005) or by siRNA mediated downregulation of Axl (Holland et al., *Cancer Res.*, vol. 65, pp. 9294-9303, 2005) prevented subcutaneous and orthotopic cell growth in murine xenograft experiments.

[0007] Accordingly, anti-Axl monoclonal antibodies have been described for use in the treatment of cancers. For example publications relating to anti-Axl antibodies include WO 2009/063965, WO 2009/062690, WO 2011/014457, US 2014/0227283, and U.S. Patent No. 8,853,369. US 2014/0227283 discloses monoclonal anti-Axl antibodies and uses thereof in diagnostic and therapeutic methods. WO 2009/062690 discloses antibodies that bind to the extracellular domain of the Axl protein and can at least partially inhibit Axl activity.

[0008] These monoclonal anti-Axl antibodies will bind to Axl at any location of a patient's body with similar affinity, including at locations of the tumors they are intended to treat. The binding of such antibodies to Axl in non-tumor environments is expected to have an adverse effect on the normal functioning of Axl in these environments and thus may cause significant side-effects. The present invention provides conditionally active anti-Axl antibodies and antibody fragments that have a higher binding affinity to Axl in a tumor microenvironment in comparison with their binding affinities to Axl in a non-tumor environment. The anti-Axl antibodies and antibody fragments of the present invention are expected to have comparable or greater anti-cancer efficacy with reduced side-effects, in comparison with the monoclonal anti-Axl antibodies known in the art. This may also permit administration of higher dosages of the anti-Axl antibodies and antibody fragments or more frequent treatment, thus providing a more effective therapeutic option.

[0009] Cancer continues to be a major global health burden. In the United States (US), it is the second most common cause of death after heart disease, accounting for nearly 1 in every 4 deaths (American Cancer Society 2011). The treatment of solid tumors poses a particular challenge. For example, lung cancer has been the most common cancer in the world for several decades, and by 2008, there were an estimated 1.61 million new cases, representing 12.7% of all new cancers. It was also the most common cause of death from cancer, with

1.38 million deaths (18.2% of the total) (GLOBOCAN 2008). Non-small-cell lung cancer (NSCLC) represents approximately 80% to 85% of all lung cancers. With recent progress in immunotherapies (programmed death ligand 1 antibodies) and targeted therapies (such as epidermal growth factor receptor, anaplastic lymphoma kinase inhibitors, etc.), still only a limited portion of patients with NSCLC benefit from the therapies.

[0010] Soft tissue sarcomas (STSs) are mesenchymal-derived cancers which have more than 100 histological subtypes according to the most recent World Health Organization (WHO) classification (WHO 2013). The management of STSs is also a challenging problem, as treatment is essentially palliative and the potential for cure decreases drastically. The reported median overall survival period is approximately 12 to 18 months (Cioffi 2012; Martin-Liberal 2014). Unfortunately, despite progress in the treatment of cancer, there continues to be an unmet medical need for more effective and less toxic therapies, especially for those patients with advanced disease that do not respond to, or have become resistant to, existing therapies.

SUMMARY OF THE DISCLOSURE

[0011] In one aspect, the present invention provides an isolated polypeptide that specifically binds to the Axl protein and uses of the polypeptide in methods of treating an Axl-expressing tumor that involve administering the polypeptide to a human in need of such treatment.

[0012] The polypeptide of the present invention includes six complementarity determining regions H1, H2, H3, L1, L2 and L3, wherein:

the H1 sequence is $X_1GX_2X_3MX_4$ (SEQ ID NO: 1) (X_1, X_2, X_3 and X_4 each independently represent an amino acid): wherein

X_1 is T or A or W,

X_2 is H or A,

X_3 is T or I, and

X_4 is N or I;

the H2 sequence is $LIKX_5SNGGTX_6YNQKFKG$ (SEQ ID NO: 2) (X_5 and X_6 each independently respresent an amino acid): wherein

X_5 is P or N, and

X_6 is S or I or T; and

the H3 sequence is $GX_7X_8X_9X_{10}X_{11}X_{12}X_{13}X_{14}DYX_{15}X_{16}$ (SEQ ID NO: 3) (X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, and X_{16}, each independently represent an amino acid): wherein

X_7 is H or D or E or P or R or W,

X_8 is Y or N,

X_9 is E or A or D or F or G or H or I or L or M or N or R or V or Y,

X_{10} is S or D or M or N or Q,

X_{11} is Y or C or E or P,

X_{12} is F or E or N or S or T or V,

X_{13} is A or D or G or L or Y,

X_{14} is M or E or F,

X_{15} is W or A or D or H or L or N or P or R or T, and

X_{16} is G or H,

the L1 sequence is $KASQDX_{17}X_{18}SX_{19}VX_{20}$ (SEQ ID NO: 4) (X_{17}, X_{18}, X_{19}, and X_{20} each independently represent an amino acid): wherein

X_{17} is V or D or G or N or W,

X_{18} is S or V,

X_{19} is A or L or M, and

X_{20} is A or D or N or Q;

the L2 sequence is $X_{21}X_{22}X_{23}TRX_{24}T$ (SEQ ID NO: 5) (X_{21}, X_{22}, X_{23}, and X_{24}, each independently represent an amino acid): wherein

X_{21} is W or F,

X_{22} is A or I or N or P or Q,

X_{23} is S or D, and

X_{24} is H or D; and

the L3 sequence is $QEX_{25}X_{26}SX_{27}X_{28}X_{29}X_{30}$ (SEQ ID NO: 6) (X_{25}, X_{26}, X_{27}, X_{28}, X_{29}, and X_{30}, each independently represent an amino acid): wherein

X_{25} is H or C or F or I or L or Q or S or T or V or Y,

X_{26} is F or C or D or E or G or N or S,

X_{27} is T or C or P,

X_{28} is P or A or C or D or E or H or K or S or T or V or W,

X_{29} is L or G or R, and

X_{30} is T or I or R, and

the polypeptide of the present invention excludes the parent polypeptide which has the following six CDRs:

H1 = TGHTMN,

H2 = LIKPSNGGTSYNQKFKG,

H3 = GHYESYFAMDYWG,

L1 = KASQDVSSAVA,

L2 = WASTRHT, and

L3 = QEHFSTPLT.

[0013] In another aspect, the present invention provides an isolated polypeptide as described above which has up to one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide and up to one substitution in CDRs L1, L2 and Le, relative to the parent polypeptide. This includes isolated polypeptides with one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide, isolated polypeptides with one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide, and isolated polypeptides with one substitution in CDRs H1, H2 and H3, and one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide, relative to the parent polypeptide. The possible combinations of CDRs H1, H2 and H3 are shown in Fig. 1A and the possible combinations of CDRs L1, L2 and L3 are shown in Fig. 1B.

[0014] In another aspect, the present invent provides an isolated polypeptide that specifically binds to the Axl protein and uses of the polypeptide in methods of treating an Axl-expressing tumor that involve administering the polypeptide to a human in need of such treatment wherein the isolated polypeptide comprises a heavy chain variable region selected from SEQ ID NO: 20 and a light chain variable region selected from SEQ ID NO: 21.

[0015] In yet another aspect, the present invention provides anti-Axl antibodies or antibody fragments, or immunoconjugates that include at least one said isolated polypeptide of the invention

[0016] In another aspect, the present invention provides a method of using the above-described anti-Axl antibodies or antibody fragments, or immunoconjugates for treatment of an Axl-expressing tumor.

[0017] In yet another aspect, the present invention provides an immunoconjugates that include the antibodies or antibody fragments of the invention, optionally conjugated to an agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent, as well as provides methods of treating an Axl-expressing tumor that involve administering such immunoconjugates.

[0018] In one aspect, the immunoconjugate is an antibody-drug conjugate (ADC) in which a Conditionally Active Biologic (CAB) anti-Axl antibody is conjugated to one or more heterologous molecule(s) via a cleavable linker (CAB-Axl-ADC). The CAB-Axl-ADC may bemAbBA3011-cleavable linker-$MMAE_{(n)}$, in which the heterologous molecule is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0019] In yet another aspect, the present invention provides methods of treating an Axl-expressing tumor that includes administering to a human subject in need of such treatment, a mAbBA301-cleavable linker-$MMAE_{(n)}$, where the mAbBA301 is an antibody or antibody fragment having a heavy chain variable region that includes a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region that includes a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18, and a lcCDR3 of SEQ ID NO. 19; MMAE is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0020] In yet another aspect, the present invention provides a pharmaceutical composition that includes the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the invention, together with a pharmaceutically acceptable carrier.

[0021] In yet another aspect, the present invention provides a kit for diagnosis or treatment including the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the present invention, with instructions for use to diagnose or treat Axl-expressing tumors.

[0022] In another aspect, the present invention provides a method of treating an Axl-expressing tumor that includes administering to a human subject in need of such treatment, a pharmaceutical composition containing a mAbBA301-cleavable linker-$MMAE_{(n)}$ and a pharmaceutically acceptable carrier, in which the pharmaceutical composition is administered at a dose of 1.8 mg/kg of the human subject weight on days 1 and 8 every 21 days by intravenous infusion. The mAbBA301 is an antibody or antibody fragment having a heavy chain variable region that includes a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region that includes a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18, and a lcCDR3 of SEQ ID NO. 19; and (n) is an integer between 1 and 4, inclusive, preferably, (n) equals 4.

[0023] In one aspect, the heavy chain variable region of the mAbBA301 includes SEQ ID NO. 20 and the light chain variable region of the mAbBA301 includes SEQ ID NO. 21.

[0024] In a further aspect, the cleavable linker is mc-vc-PAB.

[0025] In a further aspect, the Axl-expressing tumor is a sarcoma, an adenocarcinoma, or a non-small lung cell cancer, preferably, the Axl-expressing tumor is a sarcoma.

[0026] In a further aspect, the methods further include administering a programmed death receptor-1 (PD-1) blocking antibody.

[0027] In a further aspect, the Axl-expressing tumor has a tumor membrane P score of at least 70.

[0028] In a further aspect, the methods further include administering a granulocyte colony stimulating factor or an analog thereof.

[0029] In a further aspect, the pharmaceutically acceptable carrier has a pH of 6.0 and comprises 20 mM histidine-HCl, 70 mg/mL sucrose and 0.5 mg/mL polysorbate 80.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] FIGS. 1A-1B show sequence alignments of the heavy chain variable regions and the light chain variable regions, respectively, of anti-Axl antibodies of the present invention.

[0031] FIG. 2 shows binding (OD450) of various conditionally active antibodies of the invention to Axl's extracellular domain at pH 6.0 and pH 7.4. These conditionally active antibodies were more active at pH 6.0 than at pH 7.4.

[0032] FIG. 3 shows the selectivity of various conditionally active antibodies of the invention to Axl's extracellular domain. The selectivity was measured as the ratio of the binding affinity to a binding partner at pH6.0 to the binding affinity to the same binding partner at pH 7.4.

[0033] FIG. 4 shows by size exclusion chromatograph indicating that conditionally active antibodies of the invention do not aggregate, as described in Example 1.

[0034] FIG. 5 shows thermostability of conditionally active antibodies of the invention before and after heat shock as measured by an ELISA assay, as described in Example 1.

[0035] FIGS. 6A-6B show selectivity of conditionally active antibodies of the invention as measured by SPR assay in Example 1.

[0036] FIG. 7 shows pH dependent binding profiles for binding of anti-Axl antibodies of the present invention to Axl in KREBS buffer.

[0037] FIGS. 8A-8E show results of another cell killing study using A549 cells wherein anti-Axl antibodies of the present invention were employed for cell killing at pH 6.0 and pH 7.4 and at different antibody concentrations.

[0038] FIGS. 9A-9D show binding affinity to human Axl and cynomolgus Axl for anti-Axl antibodies of the present invention in different buffers and at different pH levels.

[0039] FIGS. 10A-10H show cell killing of different cell lines at different pH levels by anti-Axl antibodies of the present invention that were conjugated to duomycin.

[0040] FIG. 11 shows cell killing of A549 cells at different pH levels by anti-Axl antibodies of the present invention that were conjugated to gemcitabine.

[0041] FIG. 12 shows effects on tumor volume of treatment of xenografted mice with a duomycin-conjugated anti-Axl antibody of the present invention.

[0042] FIGS. 13A-13B show the detected presence of the duomycin-conjugated anti-Axl antibody of the present invention in the blood of *cynomolgus* monkeys over time after injection of the conjugate.

[0043] FIG. 14A shows the detected presence of Aspartate transaminase (AST) in the blood of *cynomolgus* monkeys over time starting just prior to injection (pre (D-3)) until 3 days afer injection (post (D-3)) of the conjugate.

[0044] FIG. 14B shows the detected presence of Alanine Aspartate transaminase (ALT) in the blood of *cynomolgus* monkeys over time starting just prior to injection (pre (D-3)) until 3 days afer injection (post (D-3)) of the conjugate.

[0045] FIG. 15 shows the lymphocyte count over time in the blood of cynomolgus monkeys after injection of the conjugate.

[0046] FIGS. 16A-16B show *in vivo* treatment of mice receiving LCLC103H and DU145 respectively.

[0047] FIGS. 17A-D show the anti-tumor efficacy of BA3011 in xenograft mouse models. The anti-tumor efficacy of BA3011 was demonstrated *in vivo* using human tumor cell line derived xenograft tumors expressing Axl in immune-deficient animals. Tumor cell lines representing NSCLC (LCLC103H; FIG. 17A), prostate (DU145; FIG. 17B), and pancreatic tumor (MIAPaCa2; FIG. 17C) were tested in this *in vivo* mouse model system.

[0048] FIG. 18 is an exemplary dose escalation flow chart.

[0049] FIG. 19 shows exemplary dosing schedules.

[0050] FIG. 20 shows changes in the sum of target lesions in patients administered 1.8 mg/kg Q3W or 2Q3W BA3011-CAB-Axl-ADC-MMAE. Plasma Membrane Scoring of AXL in Tumor membrane Percent Scores (TmPS) are calculated by summing the percentages of intensities at either $\geq$ 1+, $\geq$ 2+, or $\geq$ 3+. Thus, scores range from 0 to 100. Patients for which the tumor membrane score cannot be distinguished from the tumor cytoplasmic score are excluded.

[0051] FIG. 21 shows the average Axl plasma membrane H-Scores by cancer indication.

[0052] FIG. 22 shows the percent change in sum of target lesions (best response) in sarcoma patients in Phase 1 with Axl TmPS $\geq$ 70 at a dose of 1.8 mg/kg Q3W (d1) or 2Q3W (d1,8).

[0053] Fig. 23 shows the percent change in sum of target lesions (best response) by Axl TmPS category in evaluable sarcoma patients in Phase 1 at all doses tested.

[0054] Fig. 24 shows percent change in sum of target lesions by visit and Axl TmPS category in evaluable sarcoma patients in Phase 1 at all doses tested.

DEFINITIONS

[0055] In order to facilitate understanding of the examples provided herein, certain frequently occurring terms are defined herein.

[0056] In connection with a measured quantity, the term "about" as used herein refers to the normal variation in that measured quantity that would be expected by a skilled person making the measurement and exercising a level of care commensurate with the objective of the measurement and the precision of the measuring equipment used. Unless otherwise indicated, "about" refers to a variation of +/- 10% of the value provided.

[0057] The term “affinity” as used herein refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, “binding affinity” refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0058] The term “affinity matured” when used in reference to an antibody refers to an antibody or antibody fragment with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody or antibody fragment which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody or antibody fragment for an antigen.

[0059] The term "amino acid" as used herein refers to any organic compound that contains an amino group (--NH_2) and a carboxyl group (--COOH); preferably either as free groups or alternatively after condensation as part of peptide bonds. The "twenty naturally encoded polypeptide-forming alpha-amino acids" are understood in the art and refer to: alanine (ala or A), arginine (arg or R), asparagine (asn or N), aspartic acid (asp or D), cysteine (cys or C), glutamic acid (glu or E), glutamine (gin or Q), glycine (gly or G), histidine (his or H), isoleucine (ile or I), leucine (leu or L), lysine (lys or K), methionine (met or M), phenylalanine (phe or F), proline (pro or P), serine (ser or S), threonine (thr or T), tryptophan (tip or W), tyrosine (tyr or Y), and valine (val or V).

[0060] The term "agent" as used herein means an element, compound, or molecular entity, including, e.g., a pharmaceutical, therapeutic, or pharmacologic compound. Agents can be natural or synthetic or a combination thereof.

[0061] An "anti-cancer agent" is an agent that exerts a cytotoxic or cytostatic effect on cancer cells either alone or in combination with another agent as part of a treatment regimen. For example, an anti-cancer agent is an agent that can inhibit tumor growth, arrest tumor growth, and/or cause the regression of already existing tumors.

[0062] The term "anti-angiogenic agent" as used herein refers to a compound which blocks, or interferes with to some degree, the development of blood vessels. An anti-angiogenic agent may, for instance, be a small molecule or antibody that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. In one embodiment, an anti-angiogenic agent is an antibody or or antibody fragment that binds to vascular endothelial growth factor (VEGF), such as bevacizumab (AVASTIN®).

[0063] "Cytotoxic effect" means an inhibition of cell proliferation. A "cytotoxic agent" means an agent that has a cytotoxic or cytostatic effect on a cell, thereby depleting or inhibiting the growth of, respectively, cells within a cell population.The term "antibody fragment" as used herein refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab′, Fab′-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv). These antibody fragments, which retain some ability to selectively bind to an antigen (e.g., a polypeptide antigen) of the antibody from which they are derived, can be made using well known methods in the art (see, e.g., Harlow and Lane, supra).

[0064] The term "antibody" as used herein refers to intact immunoglobulin molecules. Antibodies or antibody fragments can be used to isolate preparative quantities of an antigen by immunoaffinity chromatography. Various other uses of such antibodies or antibody fragments are to diagnose and/or stage disease (e.g., neoplasia) and for therapeutic application to treat disease, such as for example: neoplasia, autoimmune disease, AIDS, cardiovascular disease, infections, and the like. Chimeric, human-like, humanized or fully human antibodies or antibody fragments are particularly useful for administration to human patients.

[0065] An Fab fragment consists of a monovalent antigen-binding fragment of an antibody molecule, and can be produced by digestion of a whole antibody molecule with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain.

[0066] An Fab' fragment of an antibody molecule can be obtained by treating a whole antibody molecule with pepsin, followed by reduction, to yield a molecule consisting of an intact light chain and a portion of a heavy chain. Two Fab' fragments are obtained per antibody molecule treated in this manner.

[0067] An (Fab')2 fragment of an antibody can be obtained by treating a whole antibody molecule with the enzyme pepsin, without subsequent reduction. A (Fab')2 fragment is a dimer of two Fab' fragments, held together by two disulfide bonds.

[0068] An Fv fragment is defined as a genetically engineered fragment containing the variable region of a light chain and the variable region of a heavy chain expressed as two chains.

[0069] The terms "anti-Axl antibody", anti-Axl antibody fragment and "an antibody or antibody fragment that binds to Axl" as used herein refer to an antibody or antibody fragment that is capable of binding Axl with sufficient affinity such that the antibody or antibody fragment is useful as a diagnostic and/or therapeutic agent in targeting Axl. In one embodiment, the extent of binding of an anti-Axl antibody or antibody fragment to an unrelated, non-Axl protein is less than about 10% of the binding of the antibody or antibody fragment to Axl as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody or antibody fragment that binds to Axl has a dissociation constant (Kd) of ≦1 μM, ≦100 nM, ≦10 nM, ≦1 nM, ≦0.1 nM, ≦0.01 nM, or ≦0.001 nM (e.g. 10^{-8}M or less, or from 10^{-8}M to 10^{-13}M, or from 10^{-9}M to 10^{-13} M). In certain embodiments, an anti-Axl antibody or antibody fragment binds to an epitope of Axl that is conserved among Axl from different species.

[0070] The term "angiogenic disorder" as used herein refers to any dysregulation of angiogenesis, including both non-neoplastic and neoplastic conditions. Neoplastic conditions include but are not limited those described below (see, e.g., "Cancer"). Non-neoplastic disorders include but are not limited to undesired or aberrant hypertrophy, arthritis, rheumatoid arthritis (RA), psoriasis, psoriatic plaques, sarcoidosis, atherosclerosis, atherosclerotic plaques, diabetic and other proliferative retinopathies including retinopathy of prematurity, retrolental fibroplasia, neovascular glaucoma, age-related macular degeneration, diabetic macular edema, corneal neovascularization, corneal graft neovascularization, corneal graft rejection, retinal/choroidal neovascularization, neovascularization of the angle (rubeosis), ocular neovascular disease, vascular restenosis, arteriovenous malformations (AVM), meningioma, hemangioma, angiofibroma, thyroid hyperplasias (including Grave's disease), corneal and other tissue transplantation, chronic inflammation, lung inflammation,

acute lung injury/ARDS, sepsis, primary pulmonary hypertension, malignant pulmonary effusions, cerebral edema (e.g., associated with acute stroke/closed head injury/trauma), synovial inflammation, pannus formation in RA, myositis ossificans, hypertropic bone formation, osteoarthritis (OA), refractory ascites, polycystic ovarian disease, endometriosis, 3rd spacing of fluid diseases (pancreatitis, compartment syndrome, burns, bowel disease), uterine fibroids, premature labor, chronic inflammation such as IBD (Crohn's disease and ulcerative colitis), renal allograft rejection, inflammatory bowel disease, nephrotic syndrome, undesired or aberrant tissue mass growth (non-cancer), hemophilic joints, hypertrophic scars, inhibition of hair growth, Osler-Weber syndrome, pyogenic granuloma retrolental fibroplasias, scleroderma, trachoma, vascular adhesions, synovitis, dermatitis, preeclampsia, ascites, pericardial effusion (such as that associated with pericarditis), and pleural effusion.

[0071] The term "angiogenesis" as used herein refers to all Axl-involving processes that contribute to the growth of new blood vessels from pre-existing vessels, in particular but not limited to new tumor supplying blood vessels. These processes include multiple cellular events such as proliferation, survival, migration and sprouting of vascular endothelial cells, attraction and migration of pericytes as well as basal membrane formation for vessel stabilization, vessel perfusion, or secretion of angiogenic factors by stromal or neoplastic cells, and shall be stimulated or mediated by non-catalytic or catalytic activities of Axl, preferably including Axl phosphorylation and/or Axl-mediated signal transduction.

[0072] The term "Axl" as used herein, refers to any native Axl from any vertebrate source, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed Axl as well as any form of Axl that results from processing in the cell. The term also encompasses naturally occurring variants of Axl, e.g., splice variants or allelic variants. The amino acid sequence of human Axl is well-known in the art and available from public databases such as GenBank.

[0073] The term "Axl activation" as used herein refers to activation, or phosphorylation, of the Axl receptor. Generally, Axl activation results in signal transduction (e.g. that caused by an intracellular kinase domain of an Axl receptor phosphorylating tyrosine residues in Axl or a substrate polypeptide). Axl activation may be mediated by Axl ligand (Gas6) binding to an Axl receptor of interest. Gas6 binding to Axl may activate a kinase domain of Axl and thereby result in phosphorylation of tyrosine residues in the Axl and/or phosphorylation of tyrosine residues in additional substrate polypeptides(s).

[0074] The term "Axl mediated anti-apoptosis" as used herein refers to all Axl-involving processes that prevent human cells, preferably but not limited to human cancer cells from

programmed cell death (apoptosis). In particular, it refers to processes that prevent human cells, preferably but not limited to human cancer cells from induction of apoptosis through growth factor withdrawal, hypoxia, exposure to chemotherapeutic agents or radiation, or initiation of the Fas/Apo-1 receptor-mediated signaling, and are stimulated or mediated by non-catalytic or catalytic activities of Axl, preferably including Axl phosphorylation and/or Axl-mediated signal transduction.

[0075] The term "binding" as used herein refers to interaction of the variable region or an Fv of an antibody with an antigen with the interaction depending upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the antigen. For example, an antibody variable region or Fv recognizes and binds to a specific protein structure rather than to proteins generally. As used herein, the term "specifically binding" or "binding specifically" means that an antibody variable region or Fv binds to or associates with more frequently, more rapidly, with greater duration and/or with greater affinity with a particular antigen than with other proteins. For example, an antibody variable region or Fv specifically binds to its antigen with greater affinity, avidity, more readily, and/or with greater duration than it binds to other antigens. For another example, an antibody variable region or Fv binds to a cell surface protein (antigen) with materially greater affinity than it does to related proteins or other cell surface proteins or to antigens commonly recognized by polyreactive natural antibodies (i.e., by naturally occurring antibodies known to bind a variety of antigens naturally found in humans). However, "specifically binding" does not necessarily require exclusive binding or non-detectable binding of another antigen, this is meant by the term "selective binding". In one example, "specific binding" of an antibody variable region or Fv (or other binding region) binds to an antigen, means that the antibody variable region or Fv binds to the antigen with an equilibrium constant (KD) of l00 nM or less, such as 50nM or less, for example 20nM or less, such as, 15nM or less, or 10 nM or less, or 5nM or less, 2 nM or less, or 1 nM or less.

[0076] The terms “cancer” and “cancerous” as used herein refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer include, but are not limited to, carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast

cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

[0077] The terms "cell proliferative disorder" and "proliferative disorder" as used herein refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

[0078] The term "chemotherapeutic agent" as used herein refers to a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclosphosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopolectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimnustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gamma1I and calicheamicin omegaI1 (see, e.g., Nicolaou et al., Angew. Chem. Intl. Ed. Engl., 33: 183-186 (1994)); CDP323, an oral alpha-4 integrin inhibitor; dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabicin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®),

liposomal doxorubicin TLC D-99 (MYOCET®), peglylated liposomal doxorubicin (CAELYX®), and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate, gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2′,2′-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside (“Ara-C”); thiotepa; taxoid, e.g., paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and docetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; platinum agents such as cisplatin, oxaliplatin (e.g., ELOXATIN®), and carboplatin; vincas, which prevent tubulin polymerization from forming microtubules, including vinblastine (VELBAN®), vincristine (ONCOVIN®), vindesine (ELDISINE®, FILDESIN®), and vinorelbine (NAVELBINE®); etoposide (VP-16); ifosfamide; mitoxantrone; leucovorin; novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMF®); retinoids such as retinoic acid, including bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); troxacitabine (a 1,3-

dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase 1 inhibitor (e.g., LURTOTECAN®); rmRH (e.g., ABARELIX®); BAY439006 (sorafenib; Bayer); SU-11248 (sunitinib, SUTENT®, Pfizer); perifosine, COX-2 inhibitor (e.g. celecoxib or etoricoxib), proteosome inhibitor (e.g. PS341); bortezomib (VELCADE®); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; EGFR inhibitors (see definition below); tyrosine kinase inhibitors (see definition below); serine-threonine kinase inhibitors such as rapamycin (sirolimus, RAPAMUNE®); farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

[0079] Chemotherapeutic agents as defined herein include “anti-hormonal agents” or “endocrine therapeutics” which act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer. They may be hormones themselves, including, but not limited to: anti-estrogens with mixed agonist/antagonist profile, including, tamoxifen (NOLVADEX®), 4-hydroxytamoxifen, toremifene (FARESTON®), idoxifene, droloxifene, raloxifene (EVISTA®), trioxifene, keoxifene, and selective estrogen receptor modulators (SERMs) such as SERM3; pure anti-estrogens without agonist properties, such as fulvestrant (FASLODEX®), and EM800 (such agents may block estrogen receptor (ER) dimerization, inhibit DNA binding, increase ER turnover, and/or suppress ER levels); aromatase inhibitors, including steroidal aromatase inhibitors such as formestane and exemestane (AROMASIN®), and nonsteroidal aromatase inhibitors such as anastrazole (ARIMIDEX®), letrozole (FEMARA®) and aminoglutethimide, and other aromatase inhibitors include vorozole (RIVISOR®), megestrol acetate (MEGASE®), fadrozole, and 4(5)-imidazoles; lutenizing hormone-releaseing hormone agonists, including leuprolide (LUPRON® and ELIGARD®), goserelin, buserelin, and tripterelin; sex steroids, including progestines such as megestrol acetate and medroxyprogesterone acetate, estrogens such as diethylstilbestrol and premarin, and androgens/retinoids such as fluoxymesterone, all

transretionic acid and fenretinide; onapristone; anti-progesterones; estrogen receptor down-regulators (ERDs); anti-androgens such as flutamide, nilutamide and bicalutamide; and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above.

[0080] The term “chimeric” antibody as used herein refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0081] The term “class” of an antibody as used herein refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[0082] The terms "conditionally active antibody" and “conditionally active antibody fragment” as used herein refer to an antibody or antibody fragment which is more active at a value of a condition in a tumor microenvironment compared to a different value of the same condition in a non-tumor microenvironment. As compared to the conditions in the non-tumor microenvironment, the conditions in the tumor microenvironment may include a lower pH, a higher concentration of lactate and/or pyruvate, hypoxia, a lower concentration of glucose, and a slightly higher temperature. For example, in one embodiment a conditionally active antibody or antibody fragment may be virtually inactive at a normal body temperature, but active at a higher temperature that may be encountered in a tumor microenvironment. In yet another embodiment, the conditionally active antibody or antibody fragment may be less active in normal oxygenated blood than in a less oxygenated environment that may exist in a tumor microenvironment. There are other conditions in the tumor microenvironment known to a person skilled in the field that may also be selected for use as the condition in the present invention which may trigger the anti-Axl antibodies or antibody fragments to have different activities at different values of that condition.

[0083] The term “constitutive” as used herein, as for example applied to Axl activity, refers to continuous signaling activity of the receptor kinase that is not dependent on the presence of a ligand or other activating molecules. Depending on the nature of the receptor kinase, all of the activity may be constitutive or the activity of the receptor may be further activated by the binding of other molecules (e.g. ligands). Cellular events that lead to activation of receptor kinase are well known among those of ordinary skill in the art. For example, activation may

include oligomerization, e.g., dimerization, trimerization, etc., into higher order receptor complexes. Complexes may comprise a single species of protein, i.e., a homomeric complex. Alternatively, complexes may comprise at least two different protein species, i.e., a heteromeric complex. Complex formation may be caused by, for example, overexpression of normal or mutant forms of receptor on the surface of a cell. Complex formation may also be caused by a specific mutation or mutations in a receptor.

[0084] The term "cytostatic agent" as used herein refers to a compound or composition which arrests growth of a cell either *in vitro* or *in vivo*. Thus, a cytostatic agent may be one which significantly reduces the percentage of cells in S phase. Further examples of cytostatic agents include agents that block cell cycle progression by inducing G0/G1 arrest or M-phase arrest. The humanized anti-Her2 antibody trastuzumab (HERCEPTIN®) is an example of a cytostatic agent that induces G0/G1 arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Certain agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., The Molecular Basis of Cancer, Chapter 1, entitled "Cell cycle regulation, oncogenes, and antineoplastic drugs" by Murakami et al. (W.B. Saunders, Philadelphia, 1995), e.g., p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0085] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to radioactive isotopes (e.g., At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamicin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[0086] The term “diabodies” as used herein refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

[0087] The term “detectably label” as used herein refers to any substance whose detection or measurement, either directly or indirectly, by physical or chemical means, is indicative of the presence of the CTCs in a sample. Representative examples of useful detectable labels, include, but are not limited to the following: molecules or ions directly or indirectly detectable based on light absorbance, fluorescence, reflectance, light scatter, phosphorescence, or luminescence properties; molecules or ions detectable by their radioactive properties; molecules or ions detectable by their nuclear magnetic resonance or paramagnetic properties. Included among the group of molecules indirectly detectable based on light absorbance or fluorescence, for example, are various enzymes which cause appropriate substrates to convert, e.g., from non-light absorbing to light absorbing molecules, or from non-fluorescent to fluorescent molecules.

[0088] The term "diagnostics" as used herein refers to determination of a subject's susceptibility to a disease or disorder, determination as to whether a subject is presently affected by a disease or disorder, prognosis of a subject affected by a disease or disorder (e. g., identification of pre- metastatic or metastatic cancerous states, stages of cancer, or responsiveness of cancer to therapy), and therametrics (e. g., monitoring a subject's condition to provide information as to the effect or efficacy of therapy). In some embodiments, the diagnostic method of this invention is particularly useful in detecting early stage cancers.

[0089] The term "diagnostic agent" as used herein refers to a molecule which can be directly or indirectly detected and is used for diagnostic purposes. The diagnostic agent may be administered to a subject or a sample. The diagnostic agent can be provided per se or may be conjugated to a vehicle such as a conditionally active antibody.

[0090] The term “effector functions” as used herein refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

[0091] The term "effective amount" of an agent as used herein, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic or prophylactic result.

[0092] The term "Fc region" as used herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

[0093] The term "framework" or "FR" as used herein refers to variable domain residues other than hypervariable region (HVR or H1-3 in the heavy chain and L1-3 in the light chain) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in V_H (or V_L): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0094] The term "full length antibody," "intact antibody," or "whole antibody" refers to an antibody which comprises an antigen-binding variable region (V_H or V_L) as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variants thereof. Depending on the amino acid sequence of the constant domain of their heavy chains, full length antibodies can be assigned to different "classes". There are five major classes of full length antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0095] The terms "host cell," "host cell line," and "host cell culture" as used herein are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid

content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0096] The term "human antibody" as used herein is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0097] The term "human consensus framework" as used herein is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin V_L or V_H framework sequences. Generally, the selection of human immunoglobulin V_L or V_H sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda Md. (1991), vols. 1-3. In one embodiment, for the V_L, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the V_H, the subgroup is subgroup III as in Kabat et al., supra.

[0098] The term "humanized" antibody as used herein refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[0099] The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the V_H (H1, H2, H3), and three in the V_L (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.*, vol. 196, pp. 901-917 1987) Exemplary CDRs (CDR-

L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3 (Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. 1991). With the exception of CDR1 in V_H, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.*, vol. 13, pp.1619-1633, 2008). Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., supra.

[0100] The term "immunoconjugate" as used herein is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0101] The term "individual" or "subject" as used herein refers to a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[0102] "Adverse Event" (AE) (also referred to as an adverse experience) means any unfavorable and unintended sign (e.g, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality. An AE can arise with any use of a drug (eg, off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose. An AE is considered "serious" if it results in any of the following outcomes:

- Death (excluding death due to underlying disease)
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Is an important medical event - Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon

appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition.

[0103] The terms "inhibit" or "inhibition of" as used herein means to reduce by a measurable amount, or to prevent entirely.

[0104] The term "inhibiting cell growth or proliferation" as used herein means decreasing a cell's growth or proliferation by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, and includes inducing cell death.

[0105] The term "isolated" antibody as used herein is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B*, vol. 848, pp. 79-87, 2007.

[0106] The term "isolated" nucleic acid as used herein refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0107] The term "isolated nucleic acid encoding an anti-Axl antibody" as used herein refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0108] The term "ligand-independent" as used herein, as for example applied to receptor signaling activity, refers to signaling activity that is not dependent on the presence of a ligand. A receptor having ligand-independent kinase activity will not necessarily preclude the binding of ligand to that receptor to produce additional activation of the kinase activity.

[0109] The term "metastasis" as used herein refers to all Axl-involving processes that support cancer cells to disperse from a primary tumor, penetrate into lymphatic and/or blood vessels, circulate through the bloodstream, and grow in a distant focus (metastasis) in normal tissues elsewhere in the body. In particular, it refers to cellular events of tumor cells such as proliferation, migration, anchorage independence, evasion of apoptosis, or secretion of angiogenic factors, that underlie metastasis and are stimulated or mediated by non-catalytic or catalytic activities of Axl, preferably including Axl phosphorylation and/or Axl-mediated signal transduction.

[0110] The term "microenvironment" as used herein means any portion or region of a tissue or body that has constant or temporal, physical or chemical differences from other regions of the tissue or regions of the body. For tumors, the term “tumor microenvironment” as used herein refers to the environment in which a tumor exists, which is the non-cellular area within the tumor and the area directly outside the tumorous tissue but does not pertain to the intracellular compartment of the cancer cell itself. The tumor and the tumor microenvironment are closely related and interact constantly. A tumor can change its microenvironment, and the microenvironment can affect how a tumor grows and spreads. Typically, the tumor microenvironment has a low pH in the range of 5.8 to 7.0, more commonly in the range of 6.2 to 6.8, most commonly in the range of 6.4-6.8. On the other hand, a normal physiological pH is typically in the range of 7.2-7.8. The tumor microenvironment is also known to have lower concentration of glucose and other nutrients, but higher concentration of lactic acid, in comparison with blood plasma. Furthermore, the tumor microenvironment can have a temperature that is 0.3 to 1 °C higher than the normal physiological temperature. The tumor microenvironment has been discussed in Gillies et al., “MRI of the Tumor Microenvironment,” *Journal of Magnetic Resonance Imaging*, vol. 16, pp.430-450, 2002. The term “non-tumor microenvironment” refers to a microenvironment at a site other than a tumor.

[0111] The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[0112] The term "naked antibody" as used herein refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

[0113] The term "native antibodies" as used herein refers to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (V_H), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (V_L), also called a variable light domain or a light chain variable domain, followed by a constant light (C_L) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0114] The term "package insert" as used herein is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0115] The term "percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence as used herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the

source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0116] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 * (X/Y)$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[0117] The term “pharmaceutical formulation” as used herein refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0118] The term “pharmaceutically acceptable carrier” as used herein refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject., A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0119] The terms “purified” and “isolated” used herein refer to an antibody according to the invention or to a nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term “purified” as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present. An “isolated” nucleic acid molecule which encodes a particular polypeptide refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the polypeptide;

however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition.

[0120] The term "recombinant antibody" as used herein refers to an antibody (e.g. a chimeric, humanized, or human antibody or antigen-binding fragment thereof) that is expressed by a recombinant host cell comprising nucleic acid encoding the antibody. Examples of "host cells" for producing recombinant antibodies include: (1) mammalian cells, for example, Chinese Hamster Ovary (CHO), COS, myeloma cells (including Y0 and NS0 cells), baby hamster kidney (BHK), Hela and Vero cells; (2) insect cells, for example, sf9, sf21 and Tn5; (3) plant cells, for example plants belonging to the genus *Nicotiana* (e.g. *Nicotiana tabacum*); (4) yeast cells, for example, those belonging to the genus *Saccharomyces* (e.g. *Saccharomyces cerevisiae*) or the genus *Aspergillus* (e.g. *Aspergillus niger*); (5) bacterial cells, for example *Escherichia. coli* cells or *Bacillus subtilis* cells, etc.

[0121] The term "therapeutically effective amount" of the antibody or antibody fragment of the invention means a sufficient amount of the antibody or antibody fragment to treat a disease or illness, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the antibodies or antibody fragments and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific antibody or antibody fragment employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific antibody or antibody fragment employed; the duration of the treatment; drugs used in combination or coincidental with the specific antibody employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

[0122] The term "single chain Fv" ("scFv") as used herein is a covalently linked V_H::V_L heterodimer which is usually expressed from a gene fusion including V_H and V_L encoding genes linked by a peptide-encoding linker. "dsFv" is a V_H::V_L heterodimer stabilised by a disulfide bond. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)2.

[0123] The term "treatment," "treat," or "treating" as used herein refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies or antibody fragments of the invention are used to delay development of a disease or to slow the progression of a disease. In particular embodiments, antibodies or antibody fragments of the invention are used to prevent occurrence or recurrence of tumor proliferation, alleviate symptoms related to tumor progression or regression, diminish any direct or indirect pathological consequences related to cancer, prevent tumor metastasis, enhance tumor regression, decrease or inhibit tumor progression, and induce remission or improved prognosis.

[0124] The term "tumor" as used herein refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms "cancer," "cancerous," "cell proliferative disorder," "proliferative disorder" and "tumor" are not mutually exclusive as referred to herein.

[0125] The term "variable region" or "variable domain" as used herein refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (V_H and V_L, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies or antibody fragments that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. See, e.g., Portolano et al., *J. Immunol.*, vol. 150, pp. 880-887, 1993; Clarkson et al., *Nature*, vol. 352, pp. 624-628, 1991.

[0126] The term "vector" as used herein refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression

of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

DETAILED DESCRIPTION

[0127] For illustrative purposes, the principles of the present invention are described by referencing various exemplary embodiments. Although certain embodiments of the invention are specifically described herein, one of ordinary skill in the art will readily recognize that the same principles are equally applicable to, and can be employed in, other systems and methods. Before explaining the disclosed embodiments of the present invention in detail, it is to be understood that the invention is not limited in its application to the details of any particular embodiment shown. Additionally, the terminology used herein is for the purpose of description and not for limitation. Furthermore, although certain methods are described with reference to steps that are presented herein in a certain order, in many instances, these steps can be performed in any order as may be appreciated by one skilled in the art; the novel method is therefore not limited to the particular arrangement of steps disclosed herein.

[0128] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise. Furthermore, the terms "a" (or "an"), "one or more", and "at least one" can be used interchangeably herein. The terms "comprising", "including", "having" and "constructed from" can also be used interchangeably.

[0129] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, percent, ratio, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about," whether or not the term "about" is present. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained by the present disclosure. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0130] It is to be understood that each component, compound, substituent, or parameter disclosed herein is to be interpreted as being disclosed for use alone or in combination with one or more of each and every other component, compound, substituent, or parameter disclosed herein.

[0131] It is also to be understood that each amount/value or range of amounts/values for each component, compound, substituent, or parameter disclosed herein is to be interpreted as also being disclosed in combination with each amount/value or range of amounts/values disclosed for any other component(s), compounds(s), substituent(s), or parameter(s) disclosed herein and that any combination of amounts/values or ranges of amounts/values for two or more component(s), compounds(s), substituent(s), or parameters disclosed herein are thus also disclosed in combination with each other for the purposes of this description.

[0132] It is further understood that each lower limit of each range disclosed herein is to be interpreted as disclosed in combination with each upper limit of each range disclosed herein for the same component, compounds, substituent, or parameter. Thus, a disclosure of two ranges is to be interpreted as a disclosure of four ranges derived by combining each lower limit of each range with each upper limit of each range. A disclosure of three ranges is to be interpreted as a disclosure of nine ranges derived by combining each lower limit of each range with each upper limit of each range, etc. Furthermore, specific amounts/values of a component, compound, substituent, or parameter disclosed in the description or an example is to be interpreted as a disclosure of either a lower or an upper limit of a range and thus can be combined with any other lower or upper limit of a range or specific amount/value for the same component, compound, substituent, or parameter disclosed elsewhere in the application to form a range for that component, compound, substituent, or parameter.

Regions of Anti-Axl Antibodies or Antibody Fragments

[0133] In one aspect, the present invention provides an isolated polypeptide that specifically binds to the Axl protein and uses of the polypeptide in methods of treating an Axl-expressing tumor that involve administering the polypeptide to a human in need of such treatment.

[0134] The polypeptide of the present invention includes six complementarity determining regions H1, H2, H3, L1, L2 and L3, wherein:

the H1 sequence is $X_1GX_2X_3MX_4$ (SEQ ID NO: 1) (X_1, X_2, X_3 and X_4 each independently represent an amino acid): wherein

X_1 is T or A or W,

X_2 is H or A,

X_3 is T or I, and

X_4 is N or I;

the H2 sequence is LIKX$_5$SNGGTX$_6$YNQKFKG (SEQ ID NO: 2) (X_5 and X_6 each independently represent an amino acid): wherein

X_5 is P or N, and

X_6 is S or I or T; and

the H3 sequence is GX$_7$X$_8$X$_9$X$_{10}$X$_{11}$X$_{12}$X$_{13}$X$_{14}$DYX$_{15}$X$_{16}$ (SEQ ID NO: 3) (X_7, X_8, X_9, X_{10}, X_{11}, X_{12}, X_{13}, X_{14}, X_{15}, and X_{16}, each independently represent an amino acid): wherein

X_7 is H or D or E or P or R or W,

X_8 is Y or N,

X_9 is E or A or D or F or G or H or I or L or M or N or R or V or Y,

X_{10} is S or D or M or N or Q,

X_{11} is Y or C or E or P,

X_{12} is F or E or N or S or T or V,

X_{13} is A or D or G or L or Y,

X_{14} is M or E or F,

X_{15} is W or A or D or H or L or N or P or R or T, and

X_{16} is G or H,

the L1 sequence is KASQDX$_{17}$X$_{18}$SX$_{19}$VX$_{20}$ (SEQ ID NO: 4) (X_{17}, X_{18}, X_{19}, and X_{20} each independently represent an amino acid): wherein

X_{17} is V or D or G or N or W,

X_{18} is S or V,

X_{19} is A or L or M, and

X_{20} is A or D or N or Q;

the L2 sequence is X$_{21}$X$_{22}$X$_{23}$TRX$_{24}$T (SEQ ID NO: 5) (X_{21}, X_{22}, X_{23}, and X_{24}, each independently represent an amino acid): wherein

X_{21} is W or F,

X_{22} is A or I or N or P or Q,

X_{23} is S or D, and

X_{24} is H or D; and

the L3 sequence is QEX$_{25}$X$_{26}$SX$_{27}$X$_{28}$X$_{29}$X$_{30}$ (SEQ ID NO: 6) (X_{25}, X_{26}, X_{27}, X_{28}, X_{29}, and X_{30}, each independently represent an amino acid): wherein

X_{25} is H or C or F or I or L or Q or S or T or V or Y,

X_{26} is F or C or D or E or G or N or S,

X_{27} is T or C or P,

X_{28} is P or A or C or D or E or H or K or S or T or V or W,

X_{29} is L or G or R, and

X_{30} is T or I or R, and

the polypeptide of the present invention excludes the parent polypeptide which has the following six CDRs:

H1 = TGHTMN,

H2 = LIKPSNGGTSYNQKFKG,

H3 = GHYESYFAMDYWG,

L1 = KASQDVSSAVA,

L2 = WASTRHT, and

L3 = QEHFSTPLT.

[0135] In another aspect, the present invention provides an isolated polypeptide as described above which has up to one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide and up to one substitution in CDRs L1, L2 and Le, relative to the parent polypeptide. This includes isolated polypeptides with one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide, isolated polypeptides with one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide, and isolated polypeptides with one substitution in CDRs H1, H2 and H3, and one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide, relative to the parent polypeptide. The possible combinations of CDRs H1, H2 and H3 are shown in Fig. 1A and the possible combinations of CDRs L1, L2 and L3 are shown in Fig. 1B.

[0136] The alignment of the heavy chain variable regions is shown in FIG. 1A, where the complementarity determining regions H1, H2, and H3 are boxed.

[0137] The alignment of the light chain variable regions is shown in FIG. 1B, where the complementarity determining regions L1, L2, and L3 are boxed.

[0138] The present invention identified these isolated heavy chain variable region polypeptides and isolated light chain variable region polypeptides from a parent antibody using a method disclosed in U.S. Patent No. 8,709,755. The heavy chain variable region and the light chain variable region of the parent antibody (063-hum10F10) are also aligned in FIGS. 1A-1B to show the mutations in the isolated heavy chain variable region polypeptides and isolated light chain variable region polypeptides.

[0139] The DNA encoding the wild-type antibody was evolved to generate a mutant antibody library using Comprehensive Positional Evolution (CPE), which each position in the template

antibody is randomized one at a time. Each mutant antibody in the library has only one single point mutation. The mutant antibodies in the library were generated by simultaneously screening for selective binding affinity to Axl at pH 6.0 over pH 7.4 by ELISA. Two mutant antibody dilutions were used: 1:3 and 1:9 dilutions. The mutant antibodies that have at least 1.5 ratio of binding affinity at pH 6.0 to at pH 7.4 under either 1:3 or 1:9 dilution are selected as conditionally active antibodies, with the single point mutations indicated in each of the heavy chain and light chain variable region (Tables 1 and 2).

Table 1: Conditionally active anti-Axl antibody heavy chain variable regions

Mutant	Affinity ELISA CPE mutant ratio (1:3, pH 6.0/7.4)	ratio (1:9, pH 6.0/7.4)	Mutant	Affinity ELISA CPE mutant ratio (1:3, pH 6.0/7.4)	ratio (1:9, pH 6.0/7.4)
HC-T030A	1.292	1.762	HC-E103L	3.266	2.698
HC-T033I	1.594	1.005	HC-E102M	2.079	2.363
HC-N035I	2.614	1.121	HC-E102N	1.920	1.567
HC-F053N	1.403	1.689	HC-E102R	3.701	3.416
HC-S059I	30.402	2.055	HC-E102V	1.931	2.507
HC-S059T	1.031	1.926	HC-E102Y	2.610	1.990
HC-R100R	1.877	2.507	HC-S103D	4.237	2.230
HC-R100D	2.416	3.094	HC-S103M	1.784	1.806
HC-R100E	2.775	3.623	HC-S103N	1.075	3.179
HC-R100W	4.037	5.908	HC-S103Q	0.912	2.256
HC-R100F	2.575	1.179	HC-Y104C	1.845	1.741
HC-Y101N	2.174	2.907	HC-Y104E	1.321	1.796
HC-E102F	1.879	1.363	HC-Y104F	2.535	2.416
HC-E102I	2.016	1.696	HC-F105N	2.364	2.642
HC-E102A	1.536	1.687	HC-F105S	3.898	4.545
HC-E102D	2.674	2.853	HC-F105T	3.463	4.021
HC-E102G	1.798	2.677	HC-F105V	2.429	2.801
HC-E102H	2.076	2.315	HC-A106D	3.389	2.506
HC-W110T	7.469	1.372	HC-A106G	1.519	2.196
HC-W110N	4.304	0.900	HC-A106L	21.538	1.613
HC-W110L	8.220	0.924	HC-A106Y	3.293	3.060
HC-W110R	5.991	1.063	HC-M107E	2.164	1.357
HC-W110D	3.716	1.112	HC-M107F	20.579	1.090
HC-W110A	7.909	1.198	HC-W110P	6.490	2.148
HC-G111H	1.058	1.786	HC-W110H	7.993	0.932

Table 2: Conditionally active anti-Axl antibody light chain variable regions

Mutant	Affinity ELISA CPE mutant ratio (1:3, pH 6.0/7.4)	ratio (1:9, pH 6.0/7.4)	Mutant	Affinity ELISA CPE mutant ratio (1:3, pH 6.0/7.4)	ratio (1:9, pH 6.0/7.4)
LC-V029D	2.882	2.679	LC-H091S	1.758	1.336
LC-V029G	1.939	1.803	LC-H091T	3.779	3.781
LC-V029M	2.595	2.652	LC-H091V	4.133	1.171
LC-V029W	2.310	2.353	LC-H091Y	3.931	5.126
LC-A032L	1.982	2.272	LC-F092C	3.862	4.576
LC-A032M	4.757	2.920	LC-F092D	2.969	2.940
LC-A034D	3.005	2.599	LC-F092E	1.759	1.999
LC-A034M	2.626	2.403	LC-F092G	3.692	4.758
LC-A034Q	1.999	1.409	LC-F092M	1.933	3.004
LC-W050F	2.245	3.504	LC-F092S	3.179	2.937
LC-A051I	2.241	2.139	LC-T094C	1.423	1.768
LC-A051N	1.412	2.200	LC-P095A	2.523	2.987
LC-A051P	1.920	1.569	LC-P095C	2.350	2.630
LC-H091C	4.003	3.025	LC-P095D	3.949	2.089
LC-H091F	1.603	2.116	LC-P095E	7.121	7.511
LC-H091I	1.550	2.154	LC-P095H	2.304	2.734
LC-H091L	2.798	2.081	LC-P095K	3.840	3.466
LC-H091Q	1.770	2.010	LC-P095S	2.841	3.512
LC-L096G	2.165	2.436	LC-P095T	2.497	2.086
LC-L096R	2.876	2.176	LC-P095V	1.871	2.132
LC-T097I	3.086	4.049	LC-P095W	2.148	2.263

[0140] In another aspect, the present invention identified the heavy chain variable regions as represented in FIG. 1A and the light chain variable regions as presented in FIG. 1B. Some heavy chain variable regions are encoded by DNA sequences with SEQ ID NOS: 11-13. Some light chain variable regions are encoded by DNA sequences with SEQ ID NOS: 7-10. These heavy and light chain variable regions can specifically bind to Axl. Antibodies comprising one of these heavy and light chain variable regions have been found to have a higher binding affinity to Axl at a pH found in the tumor microenvironment than at a pH in a non-tumor microenvironment.

[0141] The present invention also includes variants of the heavy and light chain variable regions presented in FIGS. 1A-1B and encoded by DNA sequences with SEQ ID NOS: 9-13 that can specifically bind to Axl. In order to derive these variants, it was determined that the complementarity determining regions (CDRs) of the heavy chain variable regions (H1-H3) and the CDRs of the light chain variable regions (L1-L3) should remain intact.

[0142] In deriving these variants, one is guided by the process as described herein. The variants of these heavy and light chain variable regions may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the heavy and light chain variable regions, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody or antibody fragment. Any combination of deletion(s), insertion(s), and substitution(s) can be made to arrive at the final construct, provided that the final construct possesses at least one of the desired characteristics, e.g., antigen-binding.

Substitution, Insertion, and Deletion Variants

[0143] In certain embodiments, antibody or antibody fragment variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the CDRs and framework regions (FRs). Conservative substitutions are shown in Table 3 under the heading of "conservative substitutions." More substantial changes are provided in Table 3 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody or antibody fragment of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, or decreased immunogenicity.

Table 3: Amino acid substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0144] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0145] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0146] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0147] Alterations (e.g., substitutions) may be made in CDRs, e.g., to improve antibody affinity. Such alterations may be made in CDR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.*, vol. 207, pp. 179-196, 2008), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology*, vol. 178, pp. 1-37, 2001). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves CDR-directed approaches, in which several CDR residues (e.g., 4-6 residues at a time) are randomized. CDR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0148] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody or antibody fragment to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in CDRs. Such alterations may be outside of CDR "hotspots" or SDRs. In

certain embodiments of the variant V_H and V_L sequences provided above, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0149] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells, *Science*, vol. 244, pp. 1081-1085, 1989. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody or antibody fragment with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody or antibody fragment and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0150] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

[0151] Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. It is known that when a humanized antibody is produced by simply grafting only CDRs in VH and VL of an antibody derived from a non-human animal in FRs of the V_H and V_L of a human antibody, the antigen binding activity is reduced in comparison with that of the original antibody derived from a non-human animal. It is considered that several amino acid residues of the VH and VL of the non-human antibody, not only in CDRs but also in FRs, are directly or indirectly associated with the antigen binding activity. Hence, substitution of these amino acid residues with different amino acid residues derived from FRs of the VH and VL of the human antibody would reduce of the binding activity. In order to resolve the problem, in antibodies grafted with human CDR, attempts have to be made to identify, among amino acid sequences of the FR of the VH and VL of human antibodies, an amino acid residue which is directly associated with binding to

the antibody, or which interacts with an amino acid residue of CDR, or which maintains the three-dimensional structure of the antibody and which is directly associated with binding to the antigen. The reduced antigen binding activity could be increased by replacing the identified amino acids with amino acid residues of the original antibody derived from a non-human animal.

[0152] Modifications and changes may be made in the structure of the antibodies of the present invention, and in the DNA sequences encoding them, and still obtain a functional molecule that encodes an antibody with desirable characteristics.

[0153] In making the changes in the amino sequences, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (−0.4); threonine (−0.7); serine (−0.8); tryptophan (−0.9); tyrosine (−1.3); proline (−1.6); histidine (−3.2); glutamate (−3.5); glutamine (−3.5); aspartate (−3.5); asparagine (−3.5); lysine (−3.9); and arginine (−4.5).

[0154] A further object of the present invention also encompasses function-conservative variants of the antibodies of the present invention.

[0155] "Function-conservative variants" are those in which a given amino acid residue in a protein or enzyme has been changed without altering the overall conformation and function of the polypeptide, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, hydrophobic, aromatic, and the like). Amino acids other than those indicated as conserved may differ in a protein so that the percent protein or amino acid sequence similarity between any two proteins of similar function may vary and may be, for example, from 70% to 99% as determined according to an alignment scheme such as by the Cluster Method, wherein similarity is based on the MEGALIGN algorithm. A "function-conservative variant" also includes a polypeptide which has at least 60% amino acid identity as determined by BLAST or FASTA algorithms, preferably at least 75%, more preferably at least 85%, still preferably at least 90%, and even more preferably at least 95%, and which has the same or

substantially similar properties or functions as the native or parent protein to which it is compared.

[0156] Two amino acid sequences are "substantially homologous" or "substantially similar" when greater than 80%, preferably greater than 85%, preferably greater than 90% of the amino acids are identical, or greater than about 90%, preferably greater than 95%, are similar (functionally identical) over the whole length of the shorter sequence. Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wis.) pileup program, or any of sequence comparison algorithms such as BLAST, FASTA, etc.

[0157] For example, certain amino acids may be substituted by other amino acids in a protein structure without appreciable loss of activity. Since the interactive capacity and nature of a protein define the protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and, of course, in its DNA encoding sequence, while nevertheless obtaining a protein with like properties. It is thus contemplated that various changes may be made in the sequences of the antibodies or antibody fragments of the invention, or corresponding DNA sequences which encode said antibodies or antibody fragments, without appreciable loss of their biological activity.

[0158] It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e. still obtain a biological functionally equivalent protein.

[0159] As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

Glycosylation Variants

[0160] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0161] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2

domain of the Fc region. See, e.g., Wright et al. *TIBTECH*, vol. 15, pp. 26-32, 1997. The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0162] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ±3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.*, vol. 336, pp. 1239-1249, 2004; Yamane-Ohnuki et al. *Biotech. Bioeng.*, vol. 87, pp. 614-622, 2004. Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.*, vol. 249, pp. 533-545, 1986; US Pat Appl No US 2003/0157108 A; and WO 2004/056312 A1, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.*, vol. 87, pp. 614-622, 2004; Kanda, Y. et al., *Biotechnol. Bioeng.*, vol. 94, pp. 680-688, 2006; and WO2003/085107).

[0163] Antibody variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function.

Examples of such antibody variants are described, e.g., in WO 2003/011878; U.S. Pat. No. 6,602,684; and US 2005/0123546. Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

Fc Region Variants

[0164] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0165] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 5 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol*., vol. 9, pp. 457-492, 1991. Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see also, e.g. Hellstrom et al. *Proc. Nat'l Acad. Sci. USA*, vol. 83, pp. 7059-7063, 1986) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA*, vol. 82, pp. 1499-1502, 1985; U.S. Pat. No. 5,821,337 (see also Bruggemann et al., *J. Exp. Med.*, vol. 166, pp. 1351-1361, 1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA*, vol. 95, pp. 652-656, 1998. C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in

WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods*, vol. 202, pp.163-171, 1996; Cragg, M. S. et al., *Blood*, vol. 101, pp. 1045-1052, 2003; and Cragg, M. S, and M. J. Glennie, *Blood*, vol. 103, pp. 2738-2743, 2004). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S. B. et al.,*Int'l. Immunol.*, vol. 18, pp. 1759-1769, 2006).

[0166] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0167] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.*, vol. 9, pp. 6591-6604, 2001).

[0168] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0169] In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.*, vol. 164, pp. 4178-4184, 2000.

[0170] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.*, vol. 117, pp. 587-593, 1976 and Kim et al., *J. Immunol.*, vol. 24, p. 249, 1994), are described in US2005/0014934. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include/e those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826). See also Duncan & Winter, *Nature*, vol. 322, pp. 738-740, 1988; U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

Cysteine Engineered Antibody Variants

[0171] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine

residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and 5400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Pat. No. 7,521,541.

Antibody Derivatives

[0172] In certain embodiments, an antibody or antibody fragment provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody or antibody fragment include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody or antibody fragment may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody or antibody fragment to be improved, whether the derivative will be used in a therapy under defined conditions, etc.

[0173] In another embodiment, conjugates of an antibody or antibody fragment and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA*, vol. 102, pp. 11600-11605, 2005). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells,

but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

[0174] In another aspect, the present invention provides an anti-Axl antibody or antibody fragment including the isolated heavy chain variable region polypeptides or isolated light chain variable region polypeptides. The isolated heavy chain variable region polypeptides comprise the H1, H2, and H3 regions with SEQ ID NOS: 1-3 respectively. The isolated light chain variable region polypeptides comprise the L1, L2, and L3 regions with SEQ ID NOS: 4-6 respectively.

[0175] The anti-Axl antibody or antibody fragment of the invention has a higher binding affinity to Axl under a condition in tumor microenvironment than under a condition in a non-tumor microenvironment. In one embodiment, the condition in tumor microenvironment and the condition in a non-tumor microenvironment are both pH. The anti-Axl antibodies or antibody fragments of the invention thus can selectively bind to Axl at a pH t about 5.of-6.8 but will have a lower binding affinity to Axl at a pH of 7.2-7.8 encountered in a normal physiological environment. As shown Examples 3-4, the anti-Axl antibodies or antibody fragments have higher binding affinity at pH 6.0 that at pH 7.4.

[0176] In certain embodiments, the anti-Axl antibodies or antibody fragments of the present invention have a dissociation constant (Kd) with Axl under a condition in tumor microenvironment of about ≦1 μM, ≦100 nM, ≦10 nM, ≦1 nM, ≦0.1 nM, ≦0.01 nM, or ≦0.001 nM (e.g. 10^{-8}M or less, or from 10^{-8}M to 10^{-13}M, or from 10^{-9}M to 10^{-13} M). In one embodiment, the ratio of the Kd of the antibody or antibody fragment with Axl at a value of the condition in tumor microenvironment to the Kd at a different value of the same condition in non-tumor microenvironment is at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.

[0177] In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen using the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.*293:865-881 (1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and

subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23 °C.). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., Cancer Res. 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 µl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0178] According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, N.J.) at 25° C. with immobilized antigen CM5 chips at about 10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with N-ethyl-N′-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25° C. at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass) at 25° C. of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv

Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

[0179] The anti-Axl antibodies of the invention may be a chimeric, humanized or human antibody. In one embodiment, an anti-Axl antibody fragment is employed, e.g., a Fv, Fab, Fab′, Fab′-SH, scFv, a diabody, a triabody, a tetrabody or an $F(ab')_2$ fragment and multispecific antibodies formed from antibody fragments. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG antibody or other antibody class or isotype as defined herein. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.*, vol. 9, pp. 129-134, 2003. For a review of scFv fragments, see, e.g., Pluckthün, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Pat. Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Pat. No. 5,869,046.

[0180] The diabodies of the invention may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., Nat. Med. 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, vol. 90, pp. 6444-6448, 1993 for examples of diabodies. Examples of triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.*, vol. 9, pp. 129-134, 2003.

[0181] In some embodiments, the invention comprises single-domain antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, Mass.; see, e.g., U.S. Pat. No. 6,248,516 B1).

[0182] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

[0183] In some embodiments, the anti-Axl antibodies of the invention may be chimeric antibodies. Certain chimeric antibodies are described, e.g., in U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA,* vol. 81, pp. 6851-6855, 1984). In one example, the chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, the chimeric antibody is a "class switched" antibody in

which the class or subclass of the antibody has been changed relative to the class or subclass of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0184] In certain embodiments, the chimeric antibody of the invention is a humanized antibody. Typically, such a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which CDRs (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody may optionally also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0185] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.,* vol. 13, pp. 1619-1633, 2008, and are further described, e.g., in Riechmann et al., *Nature*, vol. 332, pp. 323-329, 1988; Queen et al., *Proc. Nat'l Acad. Sci. USA*, vol. 86, pp. 10029-10033, 1989; U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., *Methods*, vol. 36, pp. 25-34, 2005 (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.*, vol. 28, pp. 489-498, 1991 (describing "resurfacing"); Dall'Acqua et al., *Methods*, vol. 36, pp. 43-60, 2005 (describing "FR shuffling"); and Osbourn et al., *Methods*, vol. 36, pp. 61-68, 2005 and Klimka et al., *Br. J. Cancer,* vol. 83, pp. 252-260, 2000 (describing the "guided selection" approach to FR shuffling).

[0186] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, e.g., Sims et al. *J. Immunol.*, vol. 151, p. 2296, 1993); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA,* vol. 89, p. 4285, 1992; and Presta et al. *J. Immunol.,* vol. 151, p. 2623, 1993); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.*, vol. 13, pp. 1619-1633, 2008); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.*, vol. 272, pp. 10678-10684, 1997 and Rosok et al., *J. Biol. Chem.*, vol. 271, pp. 22611-22618, 1996).

[0187] In some embodiments, the anti-Axl antibodies of the invention are multispecific, e.g. bispecific antibodies. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding

specificities is for Axl and the other is for another antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of Axl. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express Axl. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0188] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature*, vol. 305, pp. 537-540, 1983), WO 93/08829, and Traunecker et al., *EMBO J.* vol. 10, pp. 3655-3659, 1991), and "knob-in-hole" engineering (see, e.g., U.S. Pat. No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., U.S. Pat. No. 4,676,980, and Brennan et al., *Science*, vol. 229, pp. 81-83, 1985); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, vol. 148, pp. 1547-1553, 1992); using "diabody" technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA,* vol. 90, pp. 6444-6448, 1993); and using single-chain Fv (scFv) dimers (see, e.g. Gruber et al., *J. Immunol.*, vol. 152, pp. 5368-5374, 1994); and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.*, vol. 147, pp. 60-69, 1991.

[0189] Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

[0190] The antibody or antibody fragment may also include a "Dual Acting Fab" or "DAF" comprising an antigen binding site that binds to Axl as well as another, different antigen (see, US 2008/0069820, for example).

[0191] The anti-Axl antibodies or antibody fragments of the invention may be produced using recombinant methods and compositions, which are described in detail in US 2016/0017040.

[0192] The physical/chemical properties and/or biological activities of the anti-Axl antibodies or antibody fragments of the invention may be tested and measured by various assays known in the art. Some of these assays are described in U.S. Patent No. 8,853,369.

Immunoconjugates

[0193] In another aspect, the invention also provides immunoconjugates comprising an anti-Axl antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active

toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0194] In one embodiment, the immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Pat. Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Pat. Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Pat. Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.*, vol. 53, pp. 3336-3342, 1993; and Lode et al., *Cancer Res.*, vol. 58, pp. 2925-2928, 1998); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.*, vol. 13, pp. 477-523, 2006; Jeffrey et al., *Bioorganic & Med. Chem.* Letters, vol. 16, pp. 358-362, 2006; Torgov et al., *Bioconj. Chem.*, vol. 16, pp. 717-721, 2005; Nagy et al., *Proc. Natl. Acad. Sci. USA*, vol. 97, pp. 829-834, 2000; Dubowchik et al., *Bioorg. & Med. Chem. Letters*, vol. 12, vol. 1529-1532, 2002; King et al., *J. Med. Chem.*, vol. 45, pp. 4336-4343, 2002; and U.S. Pat. No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin,*Aleurites fordii* proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia*inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0195] In another embodiment, an immunoconjugate comprises an antibody as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or

[0196] Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate

(SPDP), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science*, vol. 238, pp. 1098-, 1987. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.*, vol. 52, pp. 127-131, 1992; U.S. Pat. No. 5,208,020) may be used.

[0197] The immunoconjugates herein expressly contemplate, but are not limited to conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SLAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, Ill., U.S.A).

[0198] An exemplary embodiment of an ADC comprises an antibody (Ab) which targets a tumor cell, a drug moiety (D), and a linker moiety (L) that attaches Ab to D. In some embodiments, the antibody is attached to the linker moiety (L) through one or more amino acid residues, such as lysine and/or cysteine.

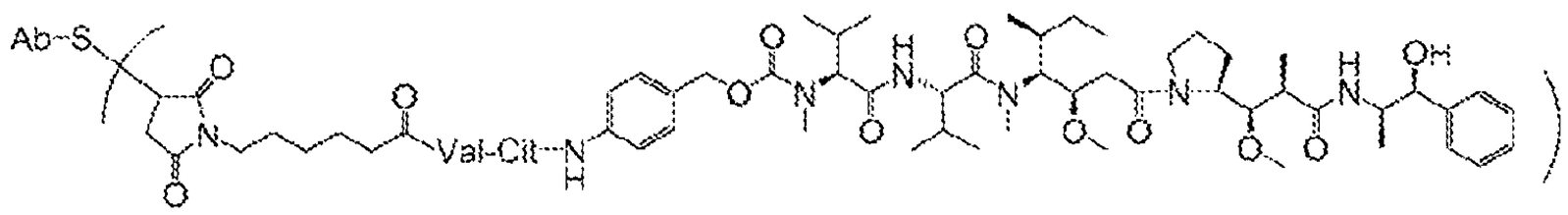
[0199] An exemplary ADC has Formula I as Ab-$(L\text{-}D)_p$, where p is 1 to about 20. In some embodiments, the number of drug moieties that can be conjugated to an antibody is limited by the number of free cysteine residues. In some embodiments, free cysteine residues are introduced into the antibody amino acid sequence by the methods described herein. Exemplary ADC of Formula I include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon et al., *Methods in Enzym.*, vol. 502, pp. 123-138, 2012). In some embodiments, one or more free cysteine residues are already present in an antibody, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the antibody to a drug. In some embodiments, an antibody is exposed to reducing conditions prior to conjugation of the antibody in order to generate one or more free cysteine residues.

[0200] The present invention encompasses antibody-drug conjugates (ADCs) comprising a Conditionally Active Biologic (CAB) anti-Axl antibody conjugated to at least one drug moiety via a cleavable linker (CAB-Axl-ADC) and use of the same for treatment of Axl-expressing tumors. Such ADCs are delivered to a subject in need of treatment, preferably, in a pharmaceutical composition or kit. In some embodiments, the drug moiety is at least one cytotoxic agent such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF). The CAB-Axl antibody may be chemically conjugated to cysteines in the heavy and light chains of the antibody via a cleavable peptide linker coupled with MMAE (a synthetic analog to dolastatin 10, an anti-tubulin agent), and with a drug to antibody ratio (DAR) close to 4. In some embodiments, the antibody drug conjugates comprising an anti-Axl antibody is covalently linked to MMAE through a vc-PAB linker. Following binding, the CAB-Axl-ADC is internalized into the tumor cell where the peptide linker is cleaved by proteases to release MMAE. Delivery of the MMAE specifically to tumor cells expressing Axl is expected to prevent further tumor cell proliferation and result in tumor shrinkage.

[0201] In some embodiments, the antibody drug conjugates are delivered to the subject as a pharmaceutical composition.

[0202] In some embodiments, the CAB-Axl-ADC is Ab-cleavable linker-MMAE$_{(n)}$, in which the MMAE is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive. An exemplary CAB-Axl-ADC of the present invention has the following chemical structure:

Ab-MC-vc-PAB-MMAE:



or pharmaceutically acceptable salts thereof, wherein Ab is an anti-Axl antibody and S is a sulfur atom of the antibody.

[0203] In some embodiments, the CAB-Axl-ADC comprises several distinct parts including a mAb as the Ab portion, a cysteine conjugated maleimide (MC), and a cleavable peptide linker containing valine and citrulline (vc) followed by MMAE. For example, the CAB-Axl-ADC is mAb-cleavable linker-MMAE$_{(n)}$. Preferably, mAbBA3011-MC-vc-PAB-MMAE antibody-drug conjugate which is a Conditionally Active Biologic (CAB) anti-Axl humanized monoclonal antibody (mAb) (immunoglobulin IgG1) conjugated to monomethyl auristatin E (MMAE) via a cleavable linker comprising the dipeptide valine-citrulline (vc), which in turn is attached to a para-aminobenzyl alcohol (PAB) (self-immolative moiety)

(CAB-Axl-ADC). The antibody portion (mAb), BA3011, is attached to MC-vc-PAB-MMAE via a sulfhydryl bond, and is specific for the Axl tyrosine kinase growth factor inhibitor, and specifically and reversibly binds to Axl in conditions found within the tumor microenvironment (TME) but have reduced binding to Axl outside the TME; thus conferring a selectivity advantage for tumor over normal cells.

[0204] Antibody drug conjugates (ADCs), which for example, attach potent cytotoxic agents or toxins to a mAb, represent an advance over treatment with a naked antibody because they offer the potential to enhance efficacy without increasing toxicity. Clinical utility has been demonstrated by licensing of gemtuzumab ozogamicin (Mylotarg®) for the treatment of CD33-positive acute myeloid leukemia. Two other ADCs, brentuximab vedotin (Adcetris®) and ado-trastuzumab emtansine (Kadcyla®), are currently approved by the US Food and Drug Administration (FDA).

[0205] The present invention provides CAB antibodies (as well as other biologics) that preferentially bind under defined physiological conditions to target tissues (such as tumors) associated with different diseases and tissues. Conditional and reversible binding by CABs is designed to reduce off-tumor toxicity and immunogenicity, avoid tissue-mediated drug deposition, and improve pharmacokinetics (PK). For example, in cancer, the unique cell metabolism described by Warburg contributes to a characteristic microenvironment such as, low pH, and high lactate (Warburg 1924; Warburg 1956). Mecbotamab vedotin (BA3011) is a conditionally active biologic anti-AXL antibody-drug conjugate (CAB-AXL-ADC) developed as an anticancer therapy for patients with advanced solid tumors. BA3011 takes advantage of the unique TME and preferentially binds to its target when in close proximity to a tumor expressing Axl; however, BA3011 has reduced binding to Axl in conditions that lack the appropriate environment. AXL is a cell-surface transmembrane receptor protein tyrosine kinase highly expressed in several tumor types including sarcoma. Increased AXL expression has been associated with tumor resistance to chemotherapy, programmed death-1 (PD-1) inhibitors, molecular targeted therapy, and radiation therapy. The activated binding property of CABs like BA3011 is reversible, such that there are no permanent changes as it transitions from diseased to normal to diseased tissue microenvironments. BA3011 is a humanized mAb without the addition of non-antibody sequences to achieve the CAB properties.

[0206] BA3011 may also be administered in combination with checkpoint inhibitors such as anti-programmed death-1 (PD-1) and anti-programmed death ligand-1 (PD-L1) therapeutic antibodies. In general, many ADCs, particularly those using MMAE as a cytotoxic payload, have been tested in combination with immune-oncology (IO) therapies (Gerber 2016).

Immunogenic cell death (ICD) of tumor cells is induced by certain classes of cytotoxic compounds and represents a potent stimulator of effector T-cell recruitment to tumors. In addition, several cytotoxic drugs directly stimulate dendritic cell activation and maturation, resulting in improved anti-tumor immune responses when combined with IO compounds. Among them, several cytotoxic agents are currently utilized as payloads for ADCs. Therefore, combination regimens with ADC and IO compounds holds strong promise to overcome the current limitations of immune checkpoint inhibitors, by increasing the recruitment of $CD8_+$ effector T-cells to the tumor core. With respect to clinical studies, ADC-IO combinations may have a broader impact on oncology drug development, as synergistic activities between IO compounds and ADCs may increase the formation of tumor specific immunological memory, ultimately leading to durable responses in a larger fraction of cancer patients (Coats 2019). With respect to BA3011 combination with checkpoint inhibitors, transcriptome analysis of PD-1 therapy-resistant melanoma patients reveal a set of upregulated genes involved in immunosuppression, angiogenesis, macrophage chemotaxis, extracellular matrix remodeling and epithelial-mesenchymal transition (EMT). Among the genes that are upregulated is the BA3011 target, Axl (Hugo 2016; Bu 2016). The upregulation of Axl in PD-1 resistant tumor strongly suggests its role in resistance and recurrence in this population and provide ample rationale for combining BA3011 in combination with PD-1 inhibitors, such as nivolumab.

Exemplary Linkers

[0207] A "Linker" (L) is a bifunctional or multifunctional moiety that can be used to link one or more moieties such as drug moieties (D) to an antibody (Ab) to form an immunoconjugate such as an ADC of the Formula I. In some embodiments, ADCs can be prepared using a Linker having reactive functionalities for covalently attaching to the drug and to the antibody. For example, in some embodiments, a cysteine thiol of an antibody (Ab) can form a bond with a reactive functional group of a linker or a drug-linker intermediate to make an ADC.

[0208] In one aspect, a linker has a functionality that is capable of reacting with a free cysteine present on an antibody to form a covalent bond. Nonlimiting exemplary such reactive functionalities include maleimide, haloacetamides, α-haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates, and isothiocyanates. See, e.g., the conjugation method at page 766 of Klussman, et al, *Bioconjugate Chemistry*, vol. 15, pp. 765-773, 2004.

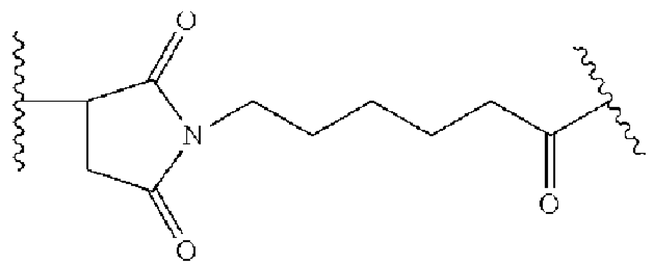
[0209] In some embodiments, a linker has a functionality that is capable of reacting with an electrophilic group present on an antibody. Exemplary such electrophilic groups include, but are not limited to, aldehyde and ketone carbonyl groups. In some embodiments, a heteroatom of the reactive functionality of the linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Nonlimiting exemplary such reactive functionalities include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

[0210] A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl (a "PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), and 4-(N-maleimidomethyl)cyclohexane-1 carboxylate ("MCC"). Various linker components are known in the art, some of which are described below.

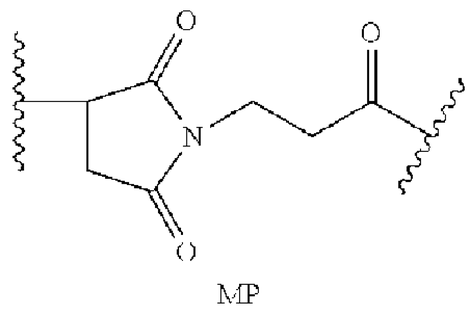
[0211] A linker may be a "cleavable linker," facilitating release of a drug. Nonlimiting exemplary cleavable linkers include acid-labile linkers (e.g., comprising hydrazone), protease-sensitive (e.g., peptidase-sensitive) linkers, photolabile linkers, or disulfide-containing linkers (Chari et al., *Cancer Research*, vol. 52, pp. 127-131, 1992; U.S. Pat. No. 5,208,020).

[0212] In certain embodiments, a linker has the following Formula II as —A_a—W_w—Y_y—, wherein A is a "stretcher unit", and a is an integer from 0 to 1; W is an "amino acid unit", and w is an integer from 0 to 12; Y is a "spacer unit", and y is 0, 1, or 2. An ADC comprising the linker of Formula II has the Formula I(A): Ab-(A_a—W_w—Y_y-D)$_p$, wherein Ab, D, and p are defined as above for Formula I. Exemplary embodiments of such linkers are described in U.S. Pat. No. 7,498,298.

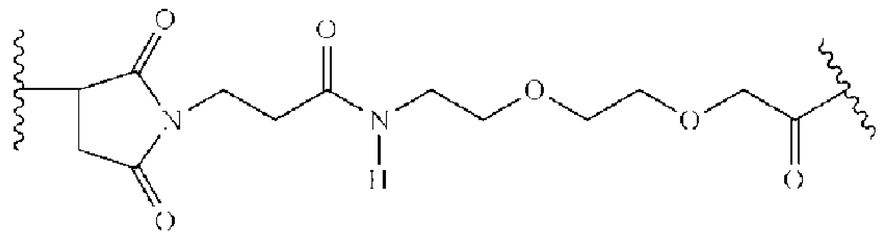
[0213] In some embodiments, a linker component comprises a "stretcher unit" (A) that links an antibody to another linker component or to a drug moiety. Nonlimiting exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody, drug, or additional linker components):



MC



MP



mPEG

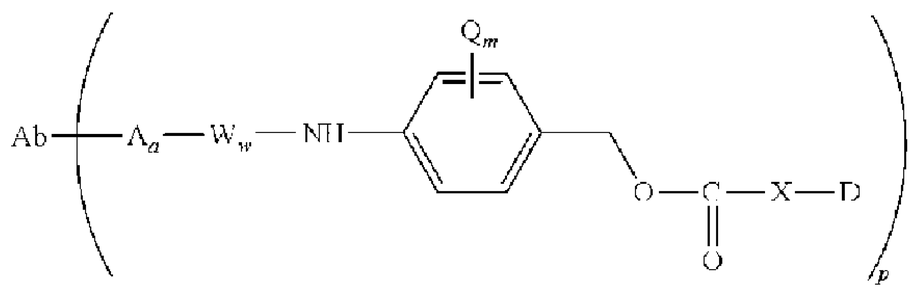
[0214] In some embodiments, a linker component comprises an "amino acid unit" (W). In some such embodiments, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular proteases, such as lysosomal enzymes (Doronina et al., *Nat. Biotechnol.*, vol. 21, pp. 778-784, 2003). Exemplary amino acid units include, but are not limited to, dipeptides, tripeptides, tetrapeptides, and pentapeptides. Exemplary dipeptides include, but are not limited to, valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe); phenylalanine-lysine (fk or phe-lys); phenylalanine-homolysine (phe-homolys); and N-methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include, but are not limited to, glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). An amino acid unit may comprise amino acid residues that occur naturally and/or minor amino acids and/or non-naturally occurring amino acid analogs, such as citrulline Amino acid units can be designed and optimized for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

[0215] Typically, peptide-type linkers can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to a liquid phase synthesis method (e.g., E. Schroder and K. Lübke (1965) "The Peptides", volume 1, pp 76-136, Academic Press).

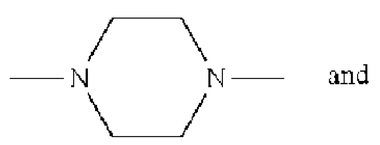
[0216] In some embodiments, a linker component comprises a "spacer unit" (Y) that links the antibody to a drug moiety, either directly or through a stretcher unit and/or an amino acid unit. A spacer unit may be "self-immolative" or a "non-self-immolative." A "non-self-

immolative" spacer unit is one in which part or all of the spacer unit remains bound to the drug moiety upon cleavage of the ADC. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. In some embodiments, enzymatic cleavage of an ADC containing a glycine-glycine spacer unit by a tumor-cell associated protease results in release of a glycine-glycine-drug moiety from the remainder of the ADC. In some such embodiments, the glycine-glycine-drug moiety is subjected to a hydrolysis step in the tumor cell, thus cleaving the glycine-glycine spacer unit from the drug moiety.

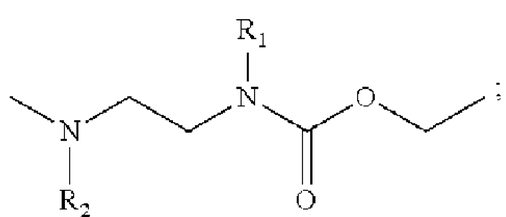
[0217] A "self-immolative" spacer unit allows for release of the drug moiety. In certain embodiments, a spacer unit of a linker comprises a p-aminobenzyl unit. In some such embodiments, a p-aminobenzyl alcohol is attached to an amino acid unit via an amide bond, and a carbamate, methylcarbamate, or carbonate is made between the benzyl alcohol and the drug (Hamann et al. *Expert Opin. Ther. Patents*, vol. 15, pp. 1087-1103, 2005). In some embodiments, the spacer unit comprises p-aminobenzyloxycarbonyl (PAB). In some embodiments, an ADC comprising a self-immolative linker has the structure:



wherein Q is —C_1-C_8 alkyl, —O—(C_1-C_8 alkyl), -halogen, -nitro, or -cyano; m is an integer ranging from 0 to 4; X may be one or more additional spacer units or may be absent; and p ranges from 1 to about 20. In some embodiments, p ranges from 1 to 10, 1 to 7, 1 to 5, or 1 to 4. Nonlimiting exemplary X spacer units include:



and



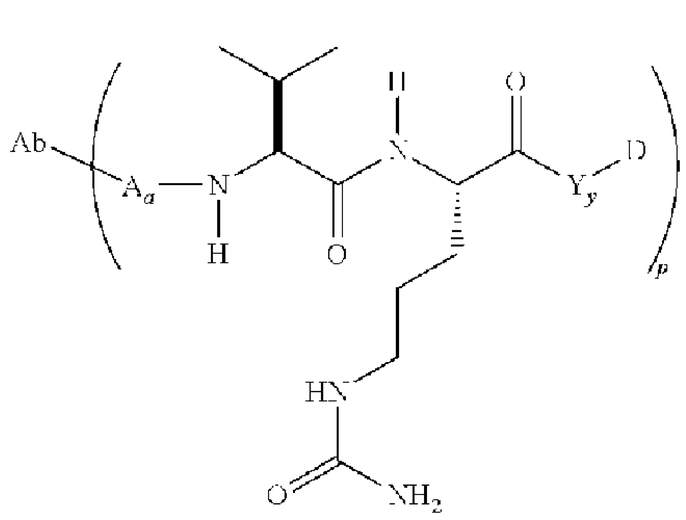
wherein R_1 and R_2 are independently selected from H and C_1-C_6 alkyl. In some embodiments, R_1 and R_2 are each —CH_3.

[0218] Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group, such as 2-aminoimidazol-5-methanol derivatives (U.S. Pat. No. 7,375,078; Hay et al., *Bioorg. Med. Chem. Lett.*, vol. 9, p. 2237-, 1999) and ortho- or para-aminobenzylacetals. In some embodiments, spacers can be

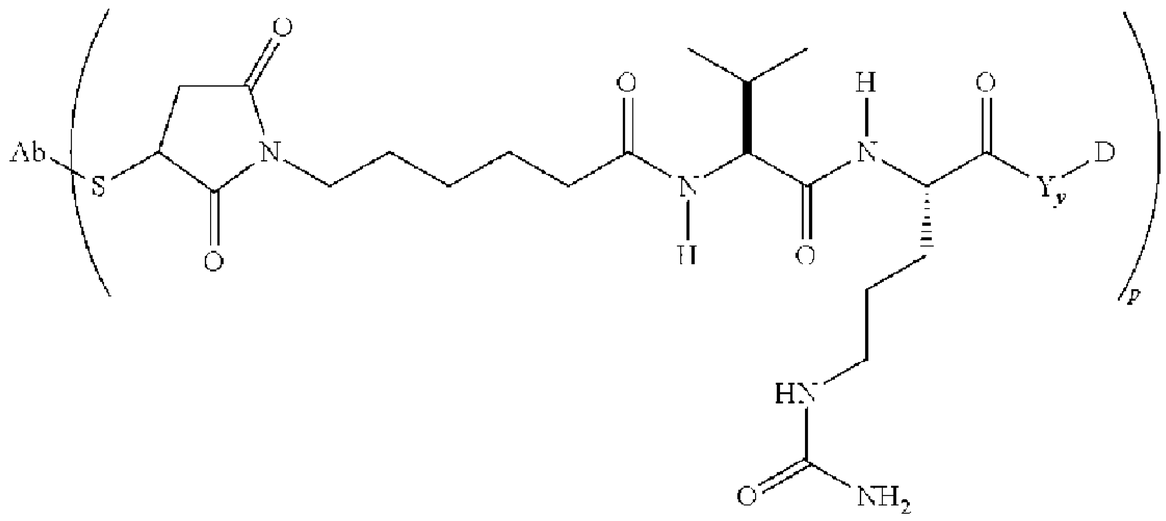
used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al., *Chemistry Biology*, vol. 2, pp. 223-, 1995), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm et al., *J. Amer. Chem. Soc.*, vol. 94, p. 5815-, 1972) and 2-aminophenylpropionic acid amides (Amsberry et al, *J. Org. Chem.*, vol. 55, p. 5867, 1990). Linkage of a drug to the α-carbon of a glycine residue is another example of a self-immolative spacer that may be useful in ADCs (Kingsbury et al., *J. Med. Chem.*, vol. 27, p.1447, 1984).

[0219] In some embodiments, linker L may be a dendritic type linker for covalent attachment of more than one drug moiety to an antibody through a branching, multifunctional linker moiety (Sun et al. *Bioorganic & Medicinal Chemistry Letters*, vol. 12, pp. 2213-2215, 2002; Sun et al., *Bioorganic & Medicinal Chemistry*, vol.11, pp. 1761-1768, 2003). Dendritic linkers can increase the molar ratio of drug to antibody, i.e. loading, which is related to the potency of the ADC. Thus, where an antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

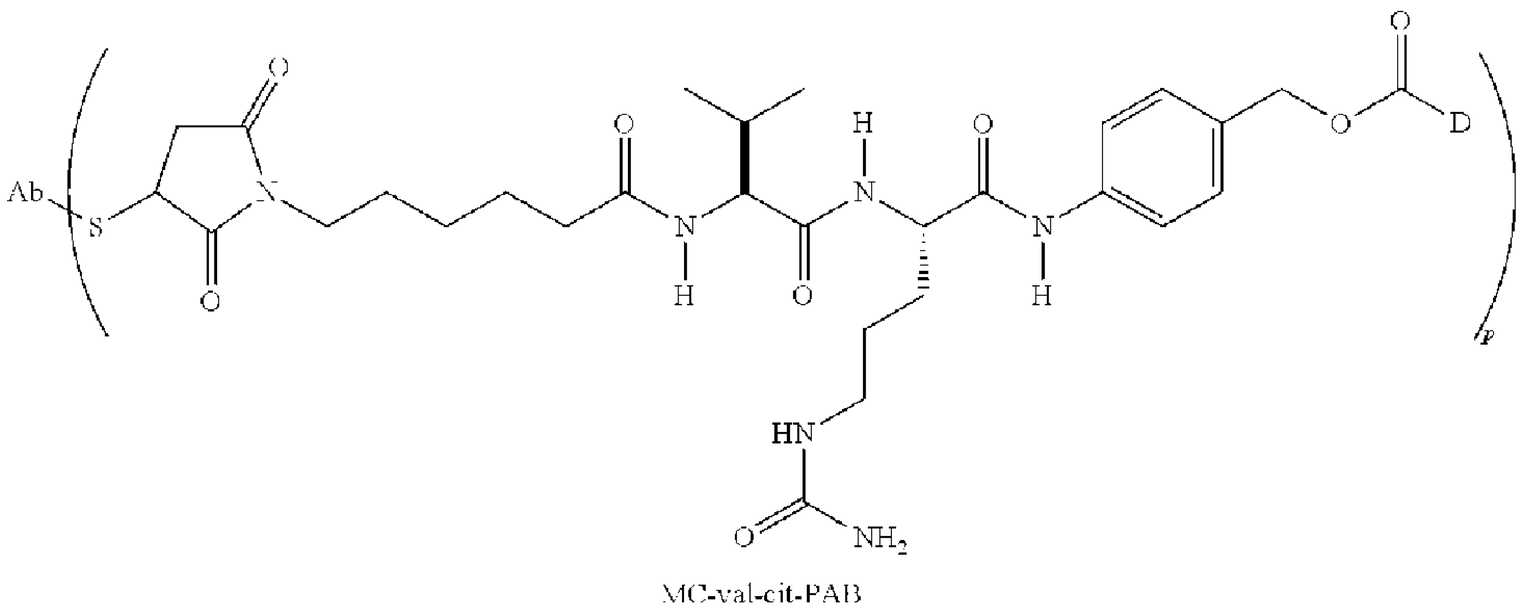
[0220] Nonlimiting exemplary linkers are shown below in the context of an ADC of Formula I:



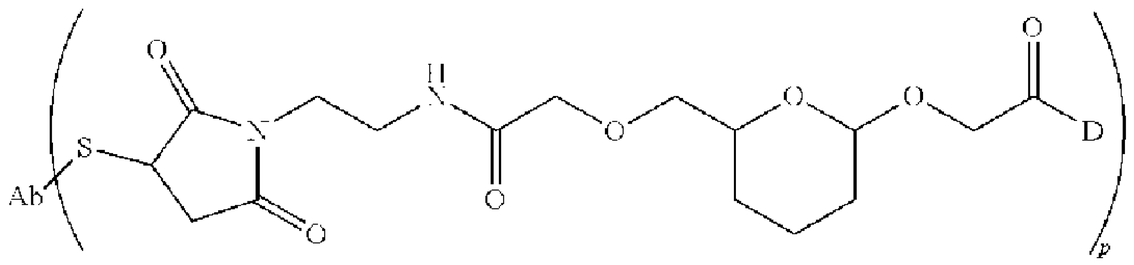
val-cit



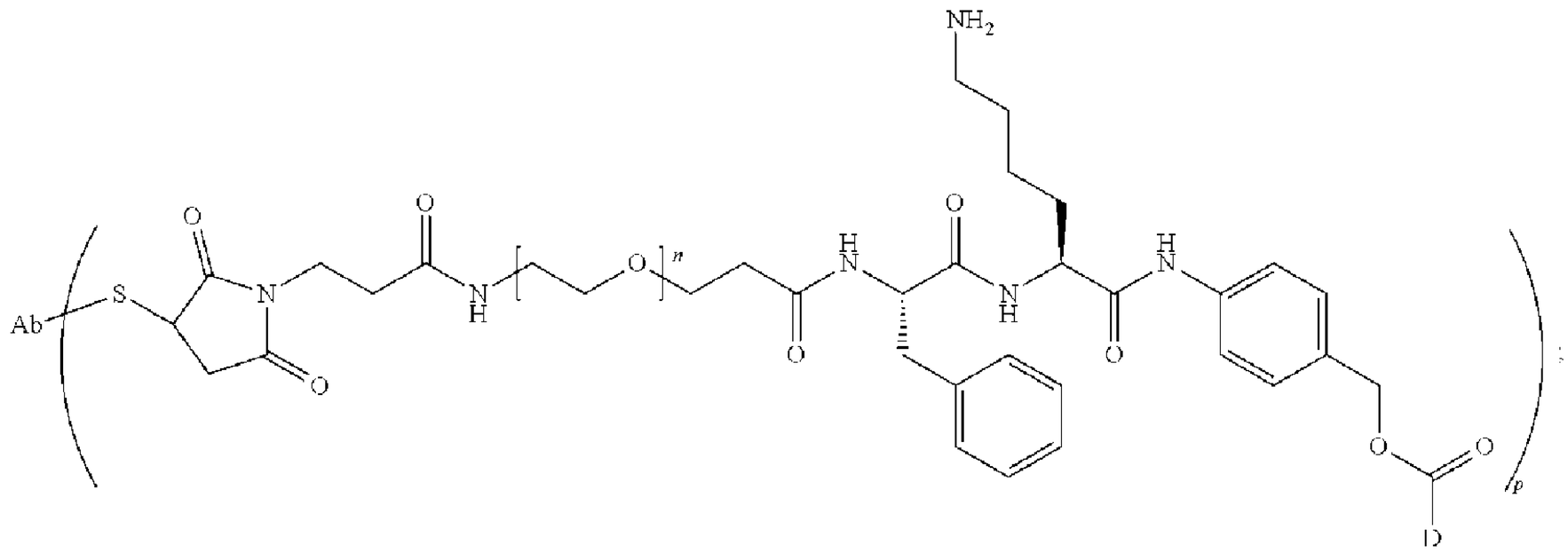
MC-val-cit



MC-val-cit-PAB



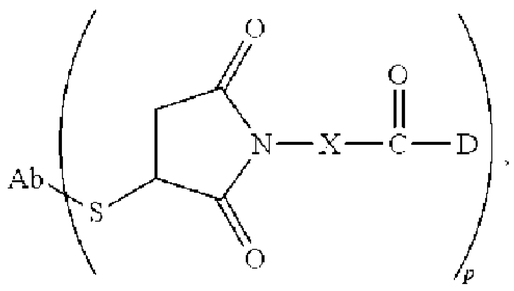
wherein R_1 and R_2 are independently selected from H and C_1-C_6 alkyl. In some embodiments, R_1 and R_2 are each —CH_3.

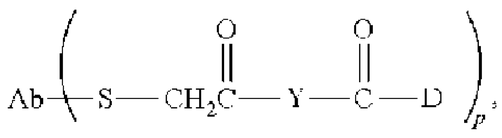


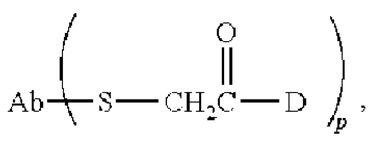
Phe-Lys-PAB-Ab

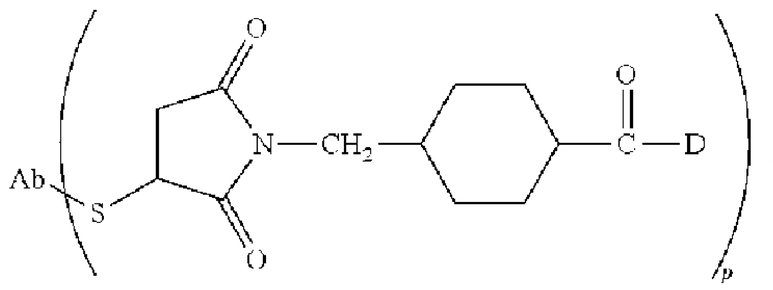
wherein n is 0 to 12. In some embodiments, n is 2 to 10. In some embodiments, n is 4 to 8.

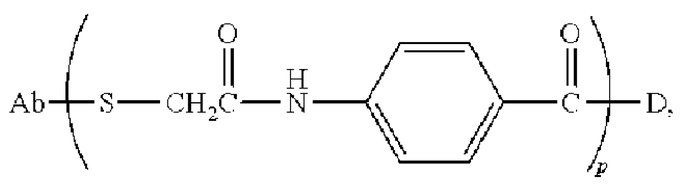
[0221] Further nonlimiting exemplary ADCs include the structures:



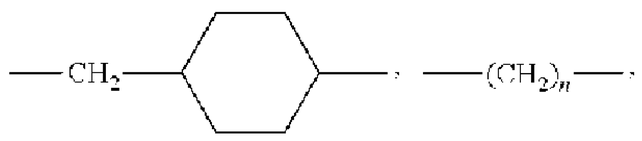


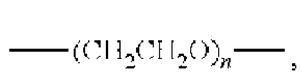


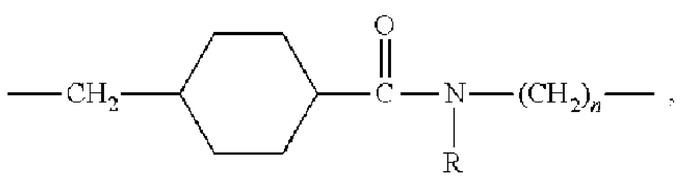


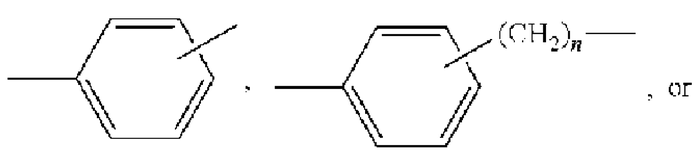


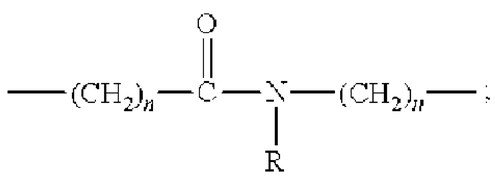
where X is:



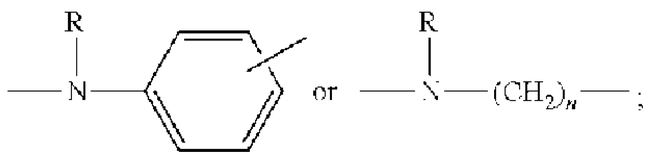








Y is:



each R is independently H or C_1-C_6 alkyl; and n is 1 to 12.

[0222] In some embodiments, a linker is substituted with groups that modulate solubility and/or reactivity. As a nonlimiting example, a charged substituent such as sulfonate (—SO_3^-) or ammonium may increase water solubility of the linker reagent and facilitate the coupling reaction of the linker reagent with the antibody and/or the drug moiety, or facilitate the coupling reaction of Ab-L (antibody-linker intermediate) with D, or D-L (drug-linker intermediate) with Ab, depending on the synthetic route employed to prepare the ADC. In some embodiments, a portion of the linker is coupled to the antibody and a portion of the linker is coupled to the drug, and then the Ab-(linker portion)^a is coupled to drug-(linker portion)^b to form the ADC of Formula I.

[0223] The compounds of the invention expressly contemplate, but are not limited to, ADCs prepared with the following linker reagents: bis-maleimido-trioxyethylene glycol (BMPEO), N-(β-maleimidopropyloxy)-N-hydroxy succinimide ester (BMPS), N-(ε-maleimidocaproyloxy) succinimide ester (EMCS), N-[γ-maleimidobutyryloxy]succinimide ester (GMBS), 1,6-hexane-bis-vinylsulfone (HBVS), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), 4-(4-N-Maleimidophenyl)butyric acid hydrazide (MPBH), succinimidyl 3-(bromoacetamido)propionate (SBAP), succinimidyl iodoacetate (SIA), succinimidyl (4-iodoacetyl)aminobenzoate (SIAB), N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), succinimidyl 6-[(beta-maleimidopropionamido)hexanoate] (SMPH), iminothiolane (IT), sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and succinimidyl-(4-vinylsulfone)benzoate (SVSB), and including bis-maleimide reagents: dithiobismaleimidoethane (DTME), 1,4-Bismaleimidobutane (BMB), 1,4 Bismaleimidyl-2,3-dihydroxybutane (BMDB), bismaleimidohexane (BMH), bismaleimidoethane (BMOE), $BM(PEG)_2$ (shown below), and $BM(PEG)_3$ (shown below); bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). In some embodiments, bis-maleimide reagents allow the attachment of the thiol group of a cysteine in the antibody to a thiol-containing drug moiety, linker, or linker-drug intermediate. Other functional groups that are reactive with thiol groups include, but are not limited to, iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.

[0224] Certain useful linker reagents can be obtained from various commercial sources, such as Pierce Biotechnology, Inc. (Rockford, Ill.), Molecular Biosciences Inc. (Boulder, Colo.), or synthesized in accordance with procedures described in the art; for example, in Toki et al., *J. Org. Chem.*, vol. 67, pp. 1866-1872, 2002; Dubowchik, et al., *Tetrahedron Letters,* vol. 38, pp. 5257-60, 1997; Walker, *J. Org. Chem.*, vol. 60, pp. 5352-5355, 1995; Frisch et al.,

Bioconjugate Chem., vol. 7, pp. 180-186, 1995; U.S. Pat. No. 6,214,345; WO 02/088172; US2003130189; US2003096743; WO 03/026577; WO 03/043583; and WO 04/032828.

[0225] Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, e.g., WO94/11026.

Exemplary Drug Moieties

1) Maytansine and Maytansinoids

[0226] In some embodiments, an immunoconjugate comprises an antibody conjugated to one or more maytansinoid molecules. Maytansinoids are derivatives of maytansine, and are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub Maytenus serrata (U.S. Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinoids are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

[0227] Maytansinoid drug moieties are attractive drug moieties in antibody-drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification or derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

[0228] Certain maytansinoids suitable for use as maytansinoid drug moieties are known in the art and can be isolated from natural sources according to known methods or produced using genetic engineering techniques (see, e.g., Yu et al., *PNAS*, vol. 99, pp. 7968-7973, 2002). Maytansinoids may also be prepared synthetically according to known methods.

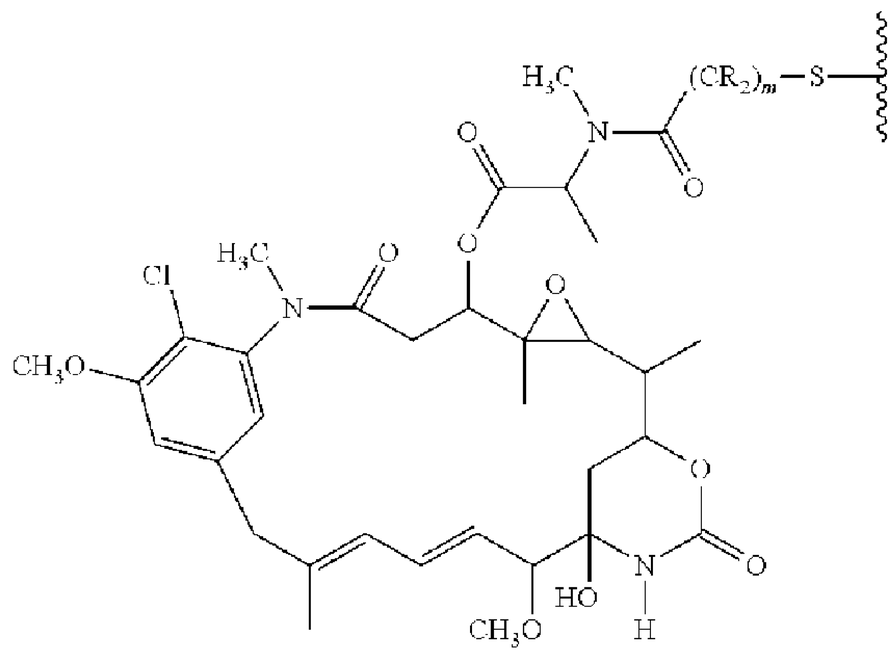
[0229] Exemplary maytansinoid drug moieties include, but are not limited to, those having a modified aromatic ring, such as: C-19-dechloro (U.S. Pat. No. 4,256,746) (prepared, for example, by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl)+/–C-19-dechloro (U.S. Pat. Nos. 4,361,650 and 4,307,016) (prepared, for example, by demethylation using *Streptomyces* or *Actinomyces* or dechlorination using LAH); and C-20-demethoxy, C-20-acyloxy (—OCOR), +/–dechloro (U.S. Pat. No.

4,294,757) (prepared, for example, by acylation using acyl chlorides), and those having modifications at other positions of the aromatic ring.

[0230] Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (U.S. Pat. No. 4,424,219) (prepared, for example, by the reaction of maytansinol with H_2S or P_2S_5); C-14-alkoxymethyl(demethoxy/CH_2OR)(U.S. Pat. No. 4,331,598); C-14-hydroxymethyl or acyloxymethyl (CH_2OH or CH_2OAc) (U.S. Pat. No. 4,450,254) (prepared, for example, from *Nocardia*); C-15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866) (prepared, for example, by the conversion of maytansinol by *Streptomyces*); C-15-methoxy (U.S. Pat. Nos. 4,313,946 and 4,315,929) (for example, isolated from *Trewia nudlflora*); C-18-N-demethyl (U.S. Pat. Nos. 4,362,663 and 4,322,348) (prepared, for example, by the demethylation of maytansinol by *Streptomyces*); and 4,5-deoxy (U.S. Pat. No. 4,371,533) (prepared, for example, by the titanium trichloride/LAH reduction of maytansinol).

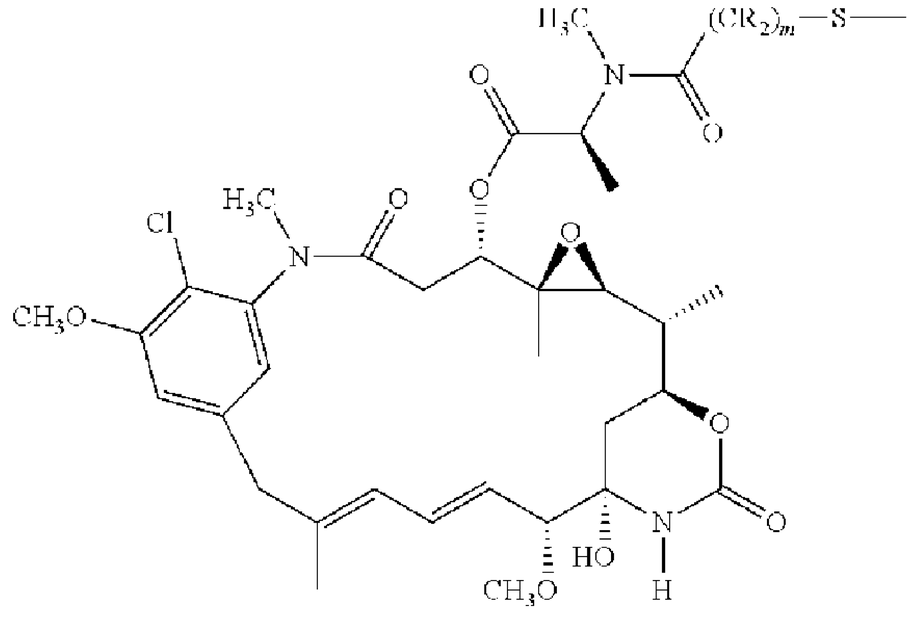
[0231] Many positions on maytansinoid compounds are useful as the linkage position. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. In some embodiments, the reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In some embodiments, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

[0232] Maytansinoid drug moieties include those having the structure:



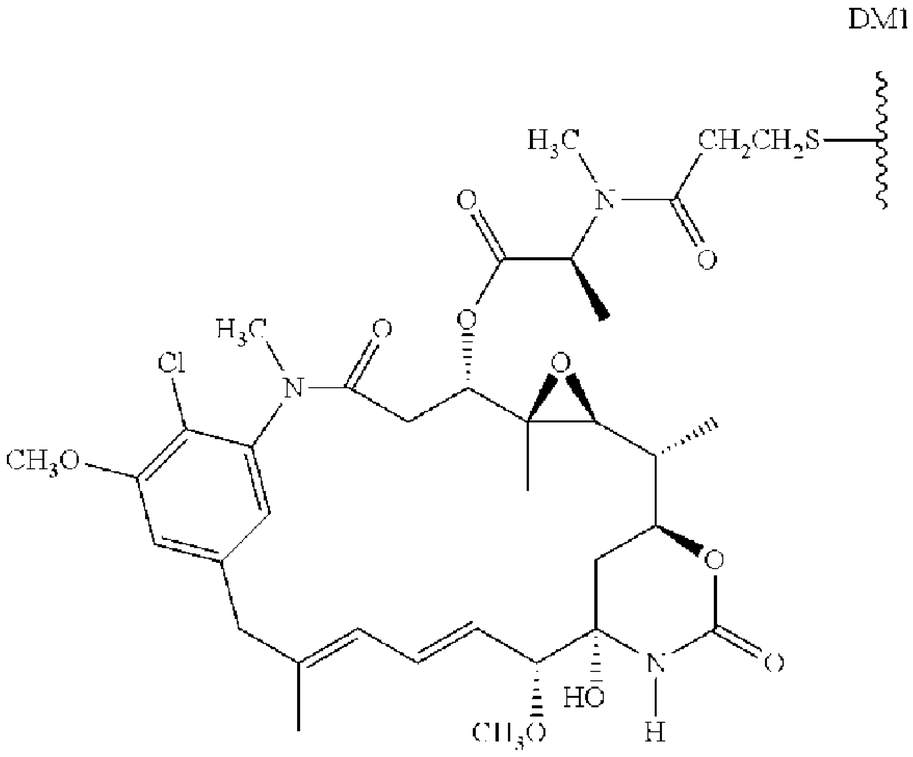
where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid drug moiety to a linker of an ADC. Each R may independently be H or a C_1-C_6 alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, i.e., m is 1, 2, or 3 (U.S. Pat. No. 633,410; U.S. Pat. No. 5,208,020; Chari et al., *Cancer Res.*, vol. 52, pp. 127-131, 1992; Liu et al., *Proc. Nall. Acad. Sci. USA*, vol. 93, pp. 8618-8623, 1996).

[0233] All stereoisomers of the maytansinoid drug moiety are contemplated for the ADC of the invention, i.e. any combination of R and S configurations at the chiral carbons (U.S. Pat. No. 7,276,497; U.S. Pat. No. 6,913,748; U.S. Pat. No. 6,441,163; U.S. Pat. No. 633,410 (RE39151); U.S. Pat. No. 5,208,020; Widdison et al (2006) J. Med. Chem. 49:4392-4408. In some embodiments, the maytansinoid drug moiety has the following stereochemistry:

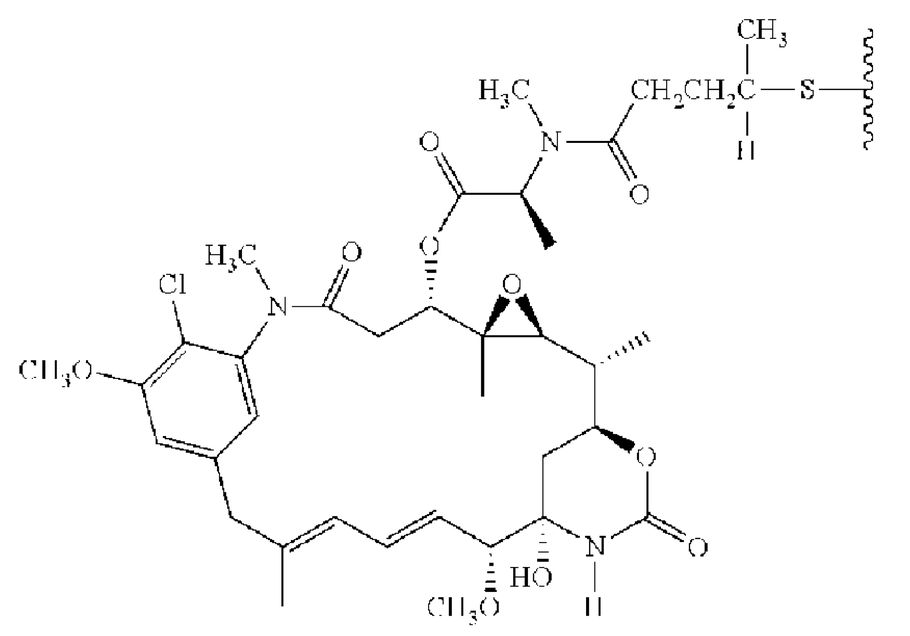


[0234] Exemplary embodiments of maytansinoid drug moieties include, but are not limited to, DM1; DM3; and DM4, having the structures:

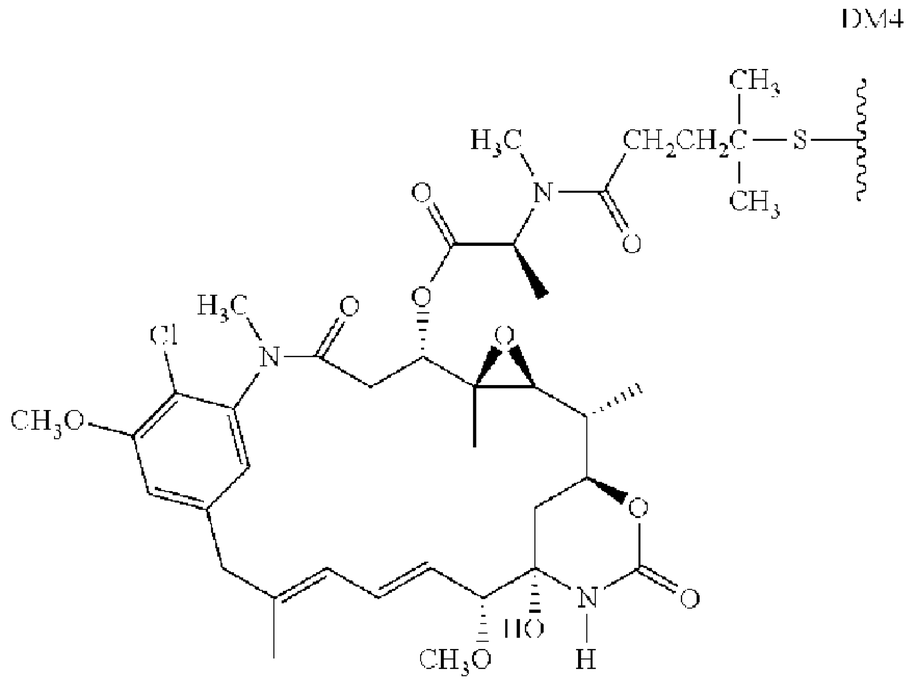
DM1



DM3

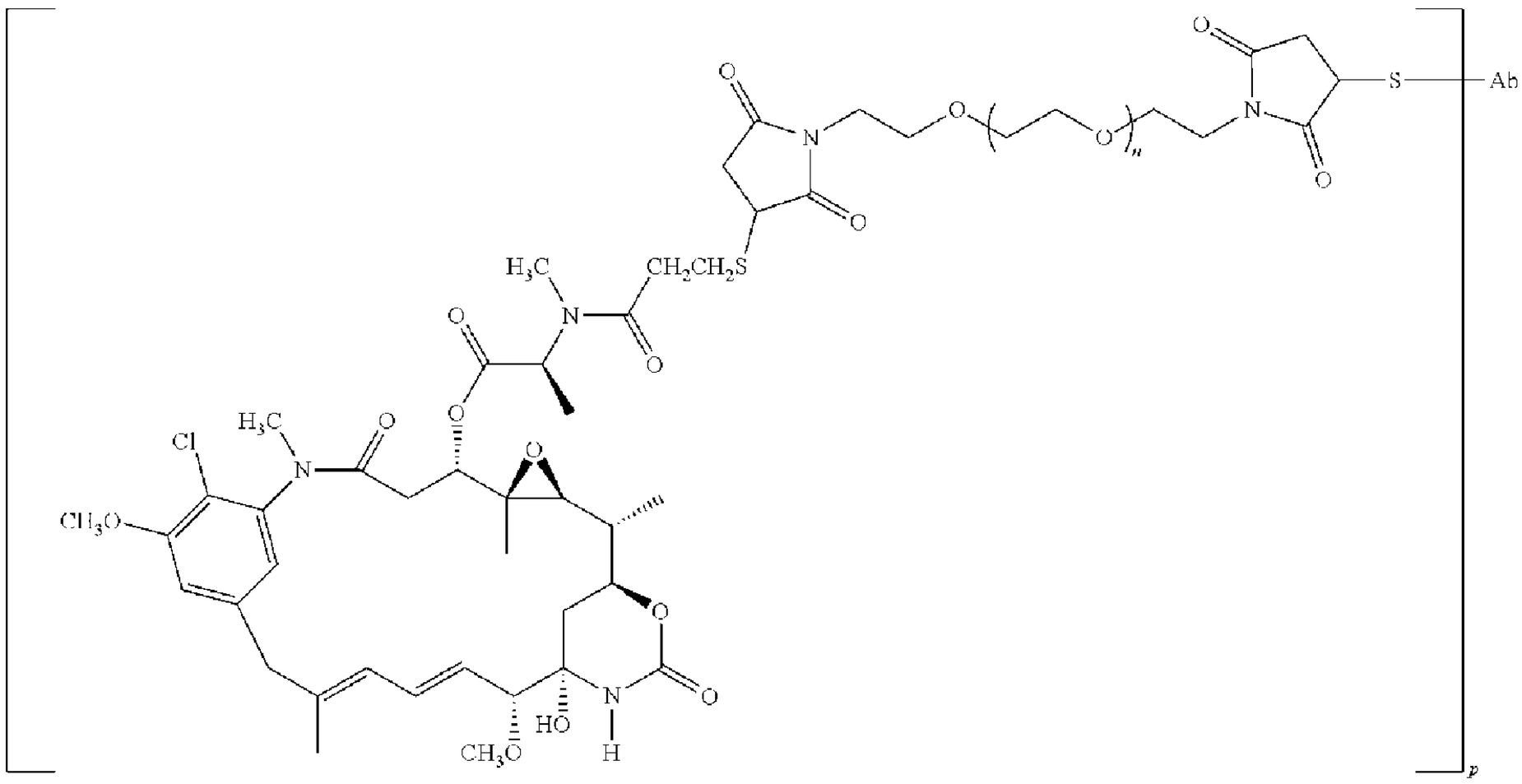


DM4



wherein the wavy line indicates the covalent attachment of the sulfur atom of the drug to a linker (L) of an antibody-drug conjugate.

[0235] Exemplary antibody-drug conjugates where DM1 is linked through a BMPEO linker to a thiol group of the antibody have the structure and abbreviation:



where Ab is antibody; n is 0, 1, or 2; and p is 1 to about 20. In some embodiments, p is 1 to 10, p is 1 to 7, p is 1 to 5, or p is 1 to 4.

[0236] Immunoconjugates containing maytansinoids, methods of making the same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020 and 5,416,064; US 2005/0276812 A1; and European Patent EP 0 425 235 B1. See also Liu et al., *Proc. Natl. Acad. Sci. USA*, vol. 93, pp. 8618-8623, 1996; and Chari et al., *Cancer Research*, vol. 52, pp. 127-131, 1992.

[0237] In some embodiments, antibody-maytansinoid conjugates may be prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. See, e.g., U.S. Pat.

No. 5,208,020. In some embodiments, ADC with an average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody. In some instances, even one molecule of toxin/antibody is expected to enhance cytotoxicity over the use of naked antibody.

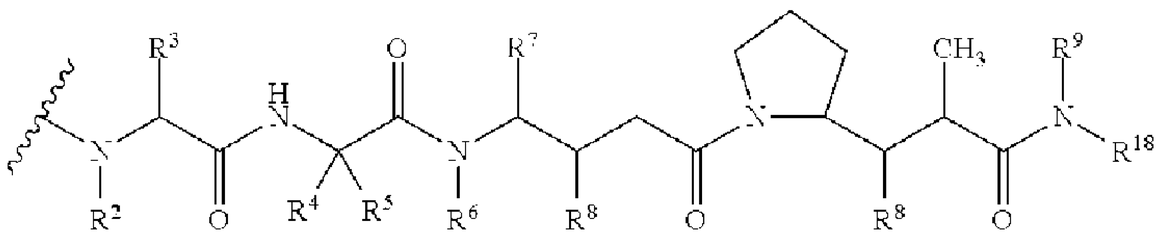
[0238] Exemplary linking groups for making antibody-maytansinoid conjugates include, for example, those described herein and those disclosed in U.S. Pat. No. 5,208,020; EP Patent 0 425 235 B1; Chari et al., *Cancer* Research, vol. 52, pp. 127-131, 1992; US 2005/0276812 A1; and US 2005/016993 A1.

(2) Auristatins and Dolastatins

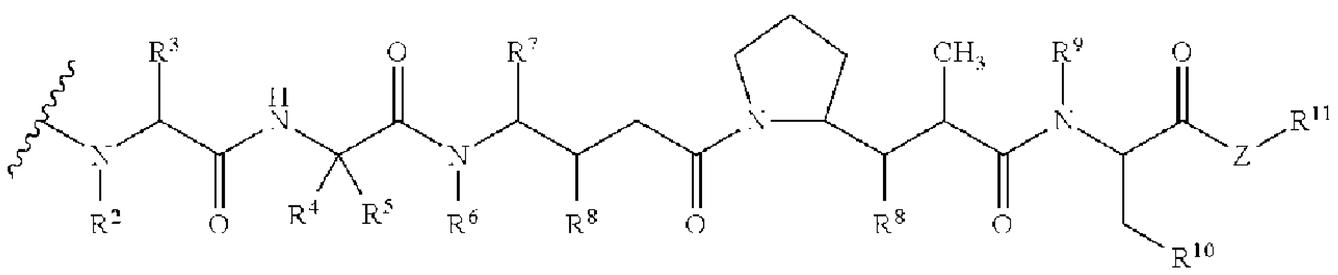
[0239] Drug moieties include dolastatins, auristatins, and analogs and derivatives thereof (U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; U.S. Pat. No. 5,767,237; U.S. Pat. No. 6,124,431). Auristatins are derivatives of the marine mollusk compound dolastatin-10. While not intending to be bound by any particular theory, dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al., *Antimicrob. Agents and Chemother.*, vol. 45, pp. 3580-3584, 2001) and have anticancer (U.S. Pat. No. 5,663,149) and antifungal activity (Pettit et al., *Antimicrob. Agents Chemother.*, vol. 42, pp. 2961-2965, 1998). The dolastatin/auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172; Doronina et al., *Nature* Biotechnology, vol. 21, pp. 778-784, 2003; Francisco et al., *Blood*, vol. 102, pp. 1458-1465, 2003).

[0240] Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties D_E and D_F, disclosed in U.S. Pat. No. 7,498,298 and U.S. Pat. No. 7,659,241:

D_E



D_F



wherein the wavy line of D_E and D_F indicates the covalent attachment site to an antibody or antibody-linker component, and independently at each location:

R^2 is selected from H and C_1-C_8 alkyl;

R^3 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^4 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

R^5 is selected from H and methyl;

or R^4 and R^5 jointly form a carbocyclic ring and have the formula —$(CR^aR^b)_n$—

wherein R^a and R^b are independently selected from H, C_1-C_8 alkyl and C_3-C_8 carbocycle and n is selected from 2, 3, 4, 5 and 6;

R^6 is selected from H and C_1-C_8 alkyl;

R^7 is selected from H, C_1-C_8 alkyl, C_3-C_8 carbocycle, aryl, C_1-C_8 alkyl-aryl, C_1-C_8 alkyl-(C_3-C_8 carbocycle), C_3-C_8heterocycle and C_1-C_8 alkyl-(C_3-C_8 heterocycle);

each R^8 is independently selected from H, OH, C_1-C_8 alkyl, C_3-C_8 carbocycle and O—(C_1-C_8 alkyl);

R^9 is selected from H and C_1-C_8 alkyl;

R^{10} is selected from aryl or C_3-C_8 heterocycle;

Z is O, S, NH, or NR^{12}, wherein R^{12} is C_1-C_8 alkyl;

R^{11} is selected from H, C_1-C_{20} alkyl, aryl, C_3-C_8 heterocycle, —$(R^{13}O)_m$—R^{14}, or —$(R^{13}O)_m$—$CH(R^{15})_2$;

m is an integer ranging from 1-1000;

R^{13} is C_2-C_8 alkyl;

R^{14} is H or C_1-C_8 alkyl;

each occurrence of e is independently H, COOH, —$(CH_2)_n$—$N(R^{16})_2$, —$(CH_2)_n$—SO_3H, or —$(CH_2)_n$—SO_3—C_1-C_8 alkyl;

each occurrence of e is independently H, C_1-C_8 alkyl, or —$(CH_2)_n$—COOH;

R^{18} is selected from —$C(R^8)_2$—$C(R^8)_2$-aryl, —$C(R^8)_2$—$C(R^8)_2$—(C_3-C_8 heterocycle), and —$C(R^8)_2$—$C(R^8)_2$—(C_3-C_8 carbocycle); and

n is an integer ranging from 0 to 6.

[0241] In one embodiment, R^3, R^4 and R^7 are independently isopropyl or sec-butyl and R^5 is —H or methyl. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is —H, and R^7 is sec-butyl.

[0242] In yet another embodiment, R^2 and R^6 are each methyl, and R^9 is —H.

[0243] In still another embodiment, each occurrence of R^8 is —OCH_3.

[0244] In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is —H, R^7 is sec-butyl, each occurrence of R^8 is —OCH_3, and R^9 is —H.

[0245] In one embodiment, Z is —O— or —NH—.

[0246] In one embodiment, R^{10} is aryl.

[0247] In an exemplary embodiment, R^{10} is -phenyl.

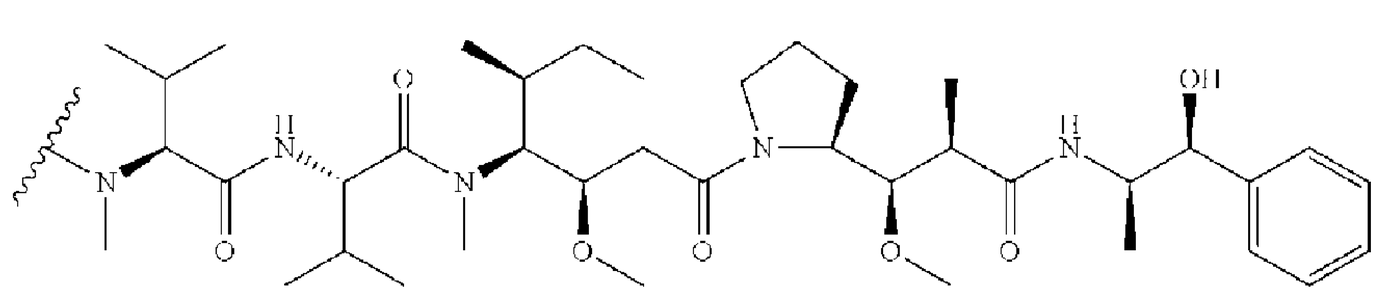
[0248] In an exemplary embodiment, when Z is —O—, R^{11} is —H, methyl or t-butyl.

[0249] In one embodiment, when Z is —NH, R^{11} is —$CH(R^{15})_2$, wherein R^{15} is —$(CH_2)_n$—$N(R^{16})_2$, and R^{16} is —C_1-C_8alkyl or —$(CH_2)_n$—COOH.

[0250] In another embodiment, when Z is —NH, R^{11} is —$CH(R^{15})_2$, wherein R^{15} is —$(CH_2)_n$—SO_3H.

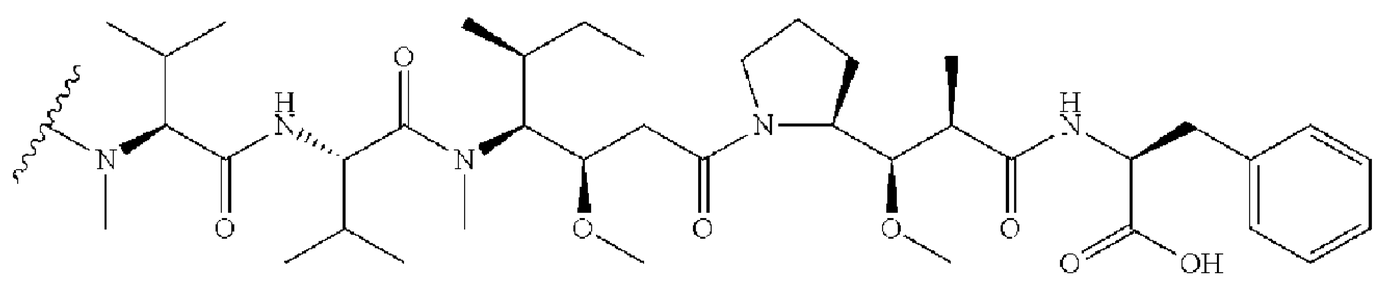
[0251] An exemplary auristatin embodiment of formula D_E is MMAE, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:

MMAE



[0252] An exemplary auristatin embodiment of formula D_E is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:

MMAF



[0253] Other exemplary embodiments include monomethylvaline compounds having phenylalanine carboxy modifications at the C-terminus of the pentapeptide auristatin drug moiety (WO 2007/008848) and monomethylvaline compounds having phenylalanine sidechain modifications at the C-terminus of the pentapeptide auristatin drug moiety (WO 2007/008603).

[0254] Nonlimiting exemplary embodiments of ADCs of Formula I comprising MMAF and various linker components further include Ab-MC-PAB-MMAF and Ab-PAB-MMAF. Immunoconjugates comprising MMAF attached to an antibody by a linker that is not proteolytically cleavable have been shown to possess activity comparable to immunoconjugates comprising MMAF attached to an antibody by a proteolytically cleavable

linker (Doronina et al., *Bioconjugate Chem.*, vol. 17, pp. 114-124, 2006). In some such embodiments, drug release is believed to be effected by antibody degradation in the cell.

[0255] Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to a liquid phase synthesis method (see, e.g., E. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press).

Auristatin/dolastatin drug moieties may, in some embodiments, be prepared according to the methods of: U.S. Pat. No. 7,498,298; U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; Pettit et al., *J. Am. Chem. Soc.*, vol. 111, pp. 5463-5465, 1998; Pettit et al., *Anti-Cancer Drug Design*, vol. 13, pp. 243-277, 1998; Pettit et al., *Synthesis,* vol. 6, pp. 719-725, 1996; Pettit et al., *J. Chem. Soc. Perkin Trans.* vol. 15, pp. 859-863, 1996; and Doronina , *Nat. Biotechnol.*, vol. 21, pp. 778-784, 2003.

[0256] In some embodiments, auristatin/dolastatin drug moieties of formulas D_E such as MMAE, and D_E, such as MMAF, and drug-linker intermediates and derivatives thereof, such as MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF, and MC-vc-PAB-MMAE, may be prepared using methods described in U.S. Pat. No. 7,498,298; Doronina et al., *Bioconjugate Chem.*, vol. 17, pp. 114-124, 2006; and Doronina et al., *Nat. Biotech.*, vol. 21, pp. 778-784, 2003 and then conjugated to an antibody of interest.

(3) Calicheamicin

[0257] In some embodiments, the immunoconjugate comprises an antibody conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics, and analogues thereof, are capable of producing double-stranded DNA breaks at sub-picomolar concentrations (Hinman et al., *Cancer Research*, vol. 53, pp. 3336-3342, 1993; Lode et al., *Cancer Research*, vol. 58, pp. 2925-2928, 1998). Calicheamicin has intracellular sites of action but, in certain instances, does not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody-mediated internalization may, in some embodiments, greatly enhances their cytotoxic effects. Nonlimiting exemplary methods of preparing antibody-drug conjugates with a calicheamicin drug moiety are described, for example, in U.S. Pat. No. 5,712,374; U.S. Pat. No. 5,714,586; U.S. Pat. No. 5,739,116; and U.S. Pat. No. 5,767,285.

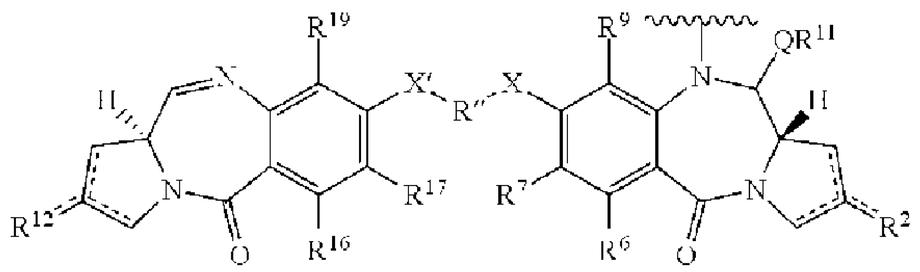
(4) Pyrrolobenzodiazepines

[0258] In some embodiments, an ADC comprises a pyrrolobenzodiazepine (PBD). In some embodiments, PDB dimers recognize and bind to specific DNA sequences. The natural product anthramycin, a PBD, was first reported in 1965 (Leimgruber et al., *J. Am. Chem. Soc.,* vol. 87, pp. 5793-5795, 1965; Leimgruber et al., *J. Am. Chem. Soc.,* vol. 87, pp. 5791-5793, 1965). Since then, a number of PBDs, both naturally-occurring and analogues, have been reported (Thurston et al., *Chem. Rev*. vol. 1994, pp. 433-465 1994, including dimers of the tricyclic PBD scaffold (U.S. Pat. No. 6,884,799; U.S. Pat. No. 7,049,311; U.S. Pat. No. 7,067,511; U.S. Pat. No. 7,265,105; U.S. Pat. No. 7,511,032; U.S. Pat. No. 7,528,126; U.S. Pat. No. 7,557,099). Without intending to be bound by any particular theory, it is believed that the dimer structure imparts the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In Antibiotics III. Springer-Verlag, New York, pp. 3-11 (1975); Hurley and Needham-VanDevanter, *Acc. Chem. Res.,* vol. 19, pp. 230-237, 1986). Dimeric PBD compounds bearing C2 aryl substituents have been shown to be useful as cytotoxic agents (Hartley et al *Cancer Res.*, vol. 70, pp. 6849-6858, 2010; Antonow, *J. Med. Chem.*vol. 53, pp. 2927-2941, 2010; Howard et al., *Bioorganic and Med. Chem. Letters*, vol. 19, pp. 6463-6466, 2009).

[0259] PBD dimers have been conjugated to antibodies and the resulting ADC shown to have anti-cancer properties. Nonlimiting exemplary linkage sites on the PBD dimer include the five-membered pyrrolo ring, the tether between the PBD units, and the N10-C11 imine group (WO 2009/016516; US 2009/304710; US 2010/047257; US 2009/036431; US 2011/0256157; WO 2011/130598).

[0260] Nonlimiting exemplary PBD dimer components of ADCs are of Formula A:

A



and salts and solvates thereof, wherein:

the wavy line indicates the covalent attachment site to the linker;

the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R^2 is independently selected from H, OH, =O, $=CH_2$, CN, R, OR, $=CH—R^D$, $=C(R^D)_2$, $O—SO_2—R$, CO_2R and COR, and optionally further selected from halo or

dihalo, wherein R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR′, NO_2, Me_3Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR′, NO_2, Me_3Sn and halo;

Q is independently selected from O, S and NH;

R^{11} is either H, or R or, where Q is O, SO_3M, where M is a metal cation;

R and R′ are each independently selected from optionally substituted C_{1-8} alkyl, C_{1-12} alkyl, C_{3-8} heterocyclyl, C_{3-20} heterocycle, and C_{5-20} aryl groups, and optionally in relation to the group NRR′, R and R′ together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

R^{12}, R^{16}, R^{19} and R^{17} are as defined for R^2, R^6, R^9 and R^7 respectively;

R″ is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, N(H), NMe and/or aromatic rings, e.g. benzene or pyridine, which rings are optionally substituted; and

X and X′ are independently selected from O, S and N(H).

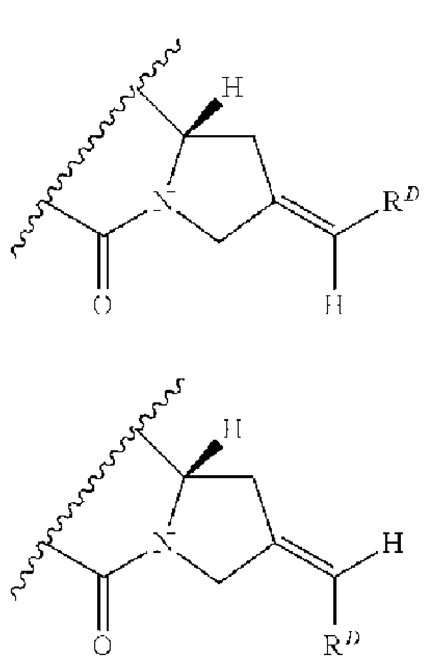
[0261] In some embodiments, R and R′ are each independently selected from optionally substituted C_{1-12} alkyl, C_{3-20}heterocycle, and C_{5-20} aryl groups, and optionally in relation to the group NRR′, R and R′ together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring. In some embodiments, R^9 and R^{19} are H. In some embodiments, R^6 and R^{16} are H.

[0262] In some embodiments, R^7 are R^{17} are both OR^{7A}, where R^{7A} is optionally substituted C_{1-4} alkyl. In some embodiments, R^{7A} is Me. In some embodiments, R^{7A} is Ch_2Ph, where Ph is a phenyl group. In some embodiments, X is O. In some embodiments, R^{11} is H. In some embodiments, there is a double bond between C2 and C3 in each monomer unit.

[0263] In some embodiments, R^2 and R^{12} are independently selected from H and R. In some embodiments, R^2 and $R^{12}$are independently R. In some embodiments, R^2 and R^{12} are independently optionally substituted C_{5-20} aryl or C_{5-7}aryl or C_{8-10} aryl. In some embodiments, R^2 and R^{12} are independently optionally substituted phenyl, thienyl, napthyl, pyridyl, quinolinyl, or isoquinolinyl. In some embodiments, R^2 and R^{12} are independently selected from =O, $=CH_2$, $=CH—R^D$, and $=C(R^D)_2$. In some embodiments, R^2 and R^{12} each $=CH_2$. In some embodiments, R^2 and R^{12} are each H. In some embodiments, R^2 and R^{12} are each =O. In

some embodiments, R^2 and R^{12} are each $=CF_2$. In some embodiments, R^2 and/or R^{12} are independently $=C(R^D)_2$. In some embodiments, R^2 and/or $R^{12}$are independently $=CH—R^D$.

[0264] In some embodiments, when R^2 and/or R^{12} is $=CH—R^D$, each group may independently have either configuration shown below:



(I)

(II)

In some embodiments, a $=CH—R^D$ is in configuration (I). In some embodiments, R″ is a C_3 alkylene group or a C_5 alkylene group.

[0265] The linkers of PBD dimer-val-cit-PAB-Ab and the PBD dimer-Phe-Lys-PAB-Ab are protease cleavable, while the linker of PBD dimer-maleimide-acetal is acid-labile.

[0266] PBD dimers and ADCs comprising PBD dimers may be prepared according to methods known in the art. See, e.g., WO 2009/016516; US 2009/304710; US 2010/047257; US 2009/036431; US 2011/0256157; WO 2011/130598.

(5) Anthracyclines

[0267] In some embodiments, an ADC may comprise anthracycline. Anthracyclines are antibiotic compounds that exhibit cytotoxic activity. While not intending to be bound by any particular theory, studies have indicated that anthracyclines may operate to kill cells by a number of different mechanisms, including: 1) intercalation of the drug molecules into the DNA of the cell thereby inhibiting DNA-dependent nucleic acid synthesis; 2) production by the drug of free radicals which then react with cellular macromolecules to cause damage to the cells, and/or 3) interactions of the drug molecules with the cell membrane (see, e.g., C. Peterson et al., "Transport And Storage Of Anthracycline In Experimental Systems And Human Leukemia" in *Anthracycline Antibiotics In Cancer Therapy*; N. R. Bachur, "Free Radical Damage" id. at pp. 97-102). Because of their cytotoxic potential anthracyclines have been used in the treatment of numerous cancers such as leukemia, breast carcinoma, lung carcinoma, ovarian adenocarcinoma and sarcomas (see e.g., P. H-Wiernik, in *Anthracycline: Current Status And New Developments* p 11).

[0268] Nonlimiting exemplary anthracyclines include doxorubicin, epirubicin, idarubicin, daunomycin, nemorubicin, and derivatives thereof. Immunoconjugates and prodrugs of daunorubicin and doxorubicin have been prepared and studied (Kratz et al., *Current Med. Chem.*, vol. 13, pp. 477-523, 2006; Jeffrey et al., *Bioorganic & Med. Chem. Letters,* vol. 16, pp. 358-362. 1996; Torgov et al., *Bioconj. Chem.,* vol. 16, pp. 717-721, 2005; Nagy et al., *Proc. Natl. Acad. Sci. USA*, vol. 97, pp. 829-834, 2000; Dubowchik et al., *Bioorg. & Med. Chem. Letters,* vol. 12, pp. 1529-1532, 2002; King et al., *J. Med. Chem.*, vol. 45, pp. 4336-4343, 2002; EP 0328147; U.S. Pat. No. 6,630,579). The antibody-drug conjugate BR96-doxorubicin reacts specifically with the tumor-associated antigen Lewis-Y and has been evaluated in phase I and II studies (Saleh et al., *J. Clin. Oncology*, vol. 18, pp. 2282-2292, 2000; Ajani et al., *Cancer Jour.,* vol. 6, pp. 78-81, 2000; Tolcher et al., *J. Clin. Oncology*, vol. 17, pp. 478-484, 1999).

[0269] PNU-159682 is a potent metabolite (or derivative) of nemorubicin (Quintieri et al., *Clinical Cancer* Research, vol. 11, pp. 1608-1617, 2005). Nemorubicin is a semisynthetic analog of doxorubicin with a 2-methoxymorpholino group on the glycoside amino of doxorubicin and has been under clinical evaluation (Grandi et al. *Cancer Treat. Rev.* vol.17, pp. 133-138, 1990; Ripamonti et al. *Brit. J. Cancer*, vol. 65, pp. 703-707, 1992), including phase II/III trials for hepatocellular carcinoma (Sun et al., *Proceedings of the American Society for Clinical Oncology*, vol. 22, Abs1448, 2003; Quintieri, *Proceedings of the American Association of Cancer Research,* vol. 44:1st Ed, Abs 4649, 2003; Pacciarini et al., *Jour. Clin. Oncology*, vol. 24, p. 14116, 2006).

[0270] Anthracyclines, including PNU-159682, may be conjugated to antibodies through several linkage sites and a variety of linkers (US 2011/0076287; WO2009/099741; US 2010/0034837; WO 2010/009124), including the linkers described herein.

[0271] The linker of PNU-159682 maleimide acetal-Ab is acid-labile, while the linkers of PNU-159682-val-cit-PAB-Ab, PNU-159682-val-cit-PAB-spacer-Ab, and PNU-159682-val-cit-PAB-spacer(R^1R^2)-Ab are protease cleavable.

[0272] (6) Other Drug Moieties

[0273] Drug moieties also include geldanamycin (Mandler et al., *J. Nat. Cancer Inst.*, vol. 92, pp. 1573-1581, 2000; Mandler et al., *Bioorganic & Med. Chem. Letters*, vol. 10, pp. 1025-1028, 2000; Mandler et al., *Bioconjugate Chem.*, vol. 13, pp. 786-791, 2002); and enzymatically active toxins and fragments thereof, including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii

proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, e.g., WO 93/21232.

[0274] Drug moieties also include compounds with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease).

[0275] In certain embodiments, an immunoconjugate may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu. In some embodiments, when an immunoconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc^{99} or 1^{123}, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, e.g., for PET imaging (WO 2011/056983).

[0276] The radio- or other labels may be incorporated in the immunoconjugate in known ways. For example, a peptide may be biosynthesized or chemically synthesized using suitable amino acid precursors comprising, for example, one or more fluorine-19 atoms in place of one or more hydrogens. In some embodiments, labels such as Tc^{99}, I^{123}, Re^{186}, Re^{188} and In^{111} can be attached via a cysteine residue in the antibody. In some embodiments, yttrium-90 can be attached via a lysine residue of the antibody. In some embodiments, the IODOGEN method (Fraker et al., *Biochem. Biophys. Res. Commun.*, vol. 80, pp. 49-57, 1978) can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes certain other methods.

[0277] In certain embodiments, an immunoconjugate may comprise an antibody conjugated to a prodrug-activating enzyme. In some such embodiments, a prodrug-activating enzyme converts a prodrug (e.g., a peptidyl chemotherapeutic agent, see WO 81/01145) to an active drug, such as an anti-cancer drug. Such immunoconjugates are useful, in some embodiments, in antibody-dependent enzyme-mediated prodrug therapy ("ADEPT"). Enzymes that may be conjugated to an antibody include, but are not limited to, alkaline phosphatases, which are useful for converting phosphate-containing prodrugs into free drugs; arylsulfatases, which are useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase, which is useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil;

proteases, such as *serratia* protease, thermolysis, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), which are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, which are useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase, which are useful for converting glycosylated prodrugs into free drugs; β-lactamase, which is useful for converting drugs derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase and penicillin G amidase, which are useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. In some embodiments, enzymes may be covalently bound to antibodies by recombinant DNA techniques well known in the art. See, e.g., Neuberger et al., *Nature*, vol. 312, pp. 604-608, 1984.

Drug Loading

[0278] Drug loading is represented by p, the average number of drug moieties per antibody in a molecule of Formula I. Drug loading may range from 1 to 20 drug moieties (D) per antibody. ADCs of Formula I include collections of antibodies conjugated with a range of drug moieties, from 1 to 20. The average number of drug moieties per antibody use in the preparation of ADCs from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of ADCs in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous ADCs where p is a certain value from ADCs with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

[0279] For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in certain exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, e.g. p>5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the average drug loading for an ADC ranges from 1 to about 8; from about 2 to about 6; or from about 3 to about 5. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5 (U.S. Pat. No. 7,498,298).

[0280] In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the drug-linker intermediate or linker reagent,

as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[0281] The loading (drug/antibody ratio) of an ADC may be controlled in different ways, and for example, by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

[0282] It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent, then the resulting product is a mixture of ADCs with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADCs may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography (see, e.g., McDonagh et al., *Prot. Engr. Design & Selection*, vol. 19, pp. 299-307, 2006; Hamblett et al., *Clin. Cancer Res.*, vol. 10, pp. 7063-7070, 2004). In certain embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

Certain Methods of Preparing Immunoconjugates

[0283] An immunoconjugate that is an ADC of Formula I may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent to form Ab-L via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with a nucleophilic group of an antibody. Exemplary methods for preparing an ADC of Formula I via the latter route are described in U.S. Pat. No. 7,498,298.

[0284] Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds

with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; and (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarbonylethylphosphine (TCEP), such that the antibody is fully or partially reduced. Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through modification of lysine residues, e.g., by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may also be introduced into an antibody by introducing one, two, three, four, or more cysteine residues (e.g., by preparing variant antibodies comprising one or more non-native cysteine amino acid residues).

[0285] Antibody-drug conjugates of the invention may also be produced by reaction between an electrophilic group on an antibody, such as an aldehyde or ketone carbonyl group, with a nucleophilic group on a linker reagent or drug. Useful nucleophilic groups on a linker reagent include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. In one embodiment, an antibody is modified to introduce electrophilic moieties that are capable of reacting with nucleophilic substituents on the linker reagent or drug. In another embodiment, the sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the antibody that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, antibodies containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, *Bioconjugate Chem.*, vol. 3, pp. 138-146, 1992; U.S. Pat. No. 5,362,852). Such an aldehyde can be reacted with a drug moiety or linker nucleophile.

[0286] Exemplary nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with

electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

[0287] Nonlimiting exemplary cross-linker reagents that may be used to prepare ADCs are described herein in the section titled "Exemplary Linkers." Methods of using such cross-linker reagents to link two moieties, including a proteinaceous moiety and a chemical moiety, are known in the art. In some embodiments, a fusion protein comprising an antibody and a cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. A recombinant DNA molecule may comprise regions encoding the antibody and cytotoxic portions of the conjugate either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

[0288] In yet another embodiment, an antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a drug or radionucleotide).

Methods and Compositions for Diagnostics and Detection

[0289] In certain embodiments, any of the anti-Axl antibodies or antibody fragments provided herein may be used for detecting the presence of Axl in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as breast, pancreas, esophagus, lung and/or brain cells or tissue.

[0290] A further aspect of the invention relates to an anti-Axl antibody of the invention for diagnosing and/or monitoring a cancer or another disease in which Axl levels are increased or decreased from a normal physiological level at least one location in the body.

[0291] In a preferred embodiment, antibodies or antibody fragments of the invention may be labelled with a detectable molecule or substance, such as a fluorescent molecule, a radioactive molecule or any other label known in the art as above described. For example, an antibody of the invention may be labelled with a radioactive molecule. For example, suitable radioactive molecules include but are not limited to radioactive atoms used for scintigraphic studies such as ^{123}I, ^{124}I, ^{111}In, ^{186}Re, and ^{188}Re. Antibodies or antibody fragments of the invention may also be labelled with a spin label for nuclear magnetic resonance (NMR) imaging, such as iodine-123, iodine-131, indium-Ill, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Following administration of the antibody, the

distribution of the radiolabeled antibody within the patient is detected. Any suitable known method can be used. Some non-limiting examples include, computed tomography (CT), position emission tomography (PET), magnetic resonance imaging (MRI), fluorescence, chemiluminescence and sonography.

[0292] Antibodies or antibody fragments of the invention may be useful for diagnosing and staging of cancer and diseases associated with Axl overexpression. Cancers associated with Axl overexpression may include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastric cancer, pancreatic cancer, glial cell tumors such as glioblastoma and neurofibromatosis, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, melanoma, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, sarcomas, hematological cancers (leukemias), astrocytomas, and various types of head and neck cancer or other Axl expressing or overexpressing hyperproliferative diseases.

[0293] Antibodies or antibody fragments of the invention may be useful for diagnosing diseases other than cancers for which Axl expression is increased or decreased. Both the (soluble or cellular Axl forms can be used for such diagnoses. Typically, such diagnostic methods involve use of a biological sample obtained from the patient. As used herein the term "biological sample" encompasses a variety of sample types obtained from a subject that can be used in a diagnostic or monitoring assay. Biological samples include but are not limited to blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or a tissue culture or cells derived therefrom, and the progeny thereof. For example, biological samples include cells obtained from a tissue sample collected from an individual suspected of having a cancer associated with Axl overexpression, and in preferred embodiments from glioma, gastric, lung, pancreatic, breast, prostate, renal, hepatic and endometrial. Biological samples encompass clinical samples, cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

[0294] In a particular embodiment, the invention is a method of diagnosing a cancer associated with Axl overexpression in a subject by detecting Axl on cells from the subject using the antibody of the invention. In particular, said method may include steps of:

(a) contacting a biological sample of a subject with an antibody or antibody fragment according to the invention under conditions suitable for the antibody or antibody fragment to form complexes with cells of the biological sample that express Axl; and

(b) detecting and/or quantifying said complexes, whereby detection of said complexes is indicative of a cancer associated with Axl overexpression.

[0295] In order to monitor the progress of a cancer, the method according to the invention may be repeated at different times, in order to determine if antibody binding to the samples increases or decreases, wherefrom it can be determined if the cancer has progressed, regressed or stabilized.

[0296] In a particular embodiment, the invention is a method of diagnosing a disease associated with the expression or overexpression of Axl or a decrease or increase of the soluble form of Axl. Examples of such diseases may include human immune disorders, thrombotic diseases (thrombosis and atherothrombosis), and cardiovascular diseases

[0297] In one embodiment, an anti-Axl antibody or antibody fragment for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of Axl in a biological sample is provided. In a further aspect, a method of quantifying the amount of Axl in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-Axl antibody or antibody fragment as described herein under conditions permissive for binding of the anti-Axl antibody or antibody fragment to Axl, and detecting whether a complex is formed between the anti-Axl antibody or antibody fragment and Axl. Such a method may be carried out *in vitro* or *in vivo*. In one embodiment, an anti-Axl antibody or antibody fragment is used to select subjects eligible for therapy. In some embodiments, the therapy will include administration of an anti-Axl antibody or antibody fragment to the subject.

[0298] In certain embodiments, labeled anti-Axl antibodies or antibody fragments are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P, ^{14}C, ^{125}I, ^{3}H, and ^{131}I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen

peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

Pharmaceutical Formulations

[0299] The anti-Axl antibodies or antibody fragments have cell killing activity. This cell killing activity extends to multiple different types of cell lines. Further, these antibodies or antibody fragments, once conjugated to a cytotoxic agent, can reduce tumor size and may exhibit reduced toxicity. See Examples 3 and 6-9 of this application. Thus, the anti-Axl antibodies, fragments or immunoconjugates thereof may be useful for treating proliferative diseases associated with Axl expression. The antibodies, fragments or immunoconjugates may be used alone or in combination with any suitable agent or other conventional treatments.

[0300] The anti-Axl antibody or antibody fragment may be used to treat hyperproliferative diseases associated with Axl and or Gas6 expression, overexpression or activation. There are no particular limitation on the types of cancer or tissue that can be treated other than the requirement for Axl expression. Examples include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastric cancer, pancreatic cancer, glial cell tumors such as glioblastoma and neurofibromatosis, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, melanoma, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, sarcomas, hematological cancers (leukemias), astrocytomas, and various types of head and neck cancer. More preferable cancers are glioma, gastric, lung, pancreatic, breast, prostate, renal, hepatic and endometrial cancer.

[0301] Anti-Axl antibodies or antibody fragments are potential activators of the innate immune response and thus may be used in the treatment of human immune disorders, such as sepsis. The anti-Axl antibody or antibody fragment of the invention may also be used as adjuvants for immunization such as for vaccines and as anti-infection agents against, for example, bacteria, viruses and parasites.

[0302] Anti-Axl antibody or antibody fragment may be used to protect against, prevent or treat thrombotic diseases such as venous and arterial thrombosis and atherothrombosis. Anti-Axl antibody or antibody fragment may also be used to protect against, prevent or treat cardiovascular diseases as well as to prevent or inhibit the entry of viruses such as Lassa and Ebola viruses and to treat viral infections.

[0303] In each of the embodiments of the treatment methods described herein, the anti-Axl monoclonal antibody, antibody fragment or anti-Axl monoclonal immunoconjugate may be delivered in a manner consistent with conventional methodologies associated with management of the disease or disorder for which treatment is sought. In accordance with the disclosure herein, an effective amount of the antibody, antibody fragment or immunoconjugate is administered to a subject in need of such treatment for a time and under conditions sufficient to prevent or treat the disease or disorder. Thus, an aspect of the invention relates to a method for treating a disease associated with the expression of Axl comprising administering to a subject in need thereof with a therapeutically effective amount of an antibody, antibody fragment or immunoconjugate of the invention.

[0304] For administration, the anti-Axl monoclonal antibody, antibody fragment or immunoconjugate may be formulated as a pharmaceutical composition. The pharmaceutical composition including anti-Axl monoclonal antibody, antibody fragment or antibody-drug conjugate can be formulated according to known methods for preparing pharmaceutical compositions. In such methods, the therapeutic molecule is typically combined with a mixture, solution or composition containing a pharmaceutically acceptable carrier.

[0305] A pharmaceutically acceptable carrier is a material that can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable pharmaceutically acceptable carriers are well-known to those in the art. (See, e.g., Gennaro (ed.), Remington's Pharmaceutical Sciences (Mack Publishing Company, 19th ed. 1995)) Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc.

[0306] The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc. These considerations can be taken into account by a skilled person to formulate suitable pharmaceutical compositions. The pharmaceutical compositions of the invention can be formulated for topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like.

[0307] Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition of, for example, sterilized water or physiological saline, permit the constitution of injectable solutions.

[0308] In some embodiments, tonicity agents, sometimes known as "stabilizers" are present to adjust or maintain the tonicity of a liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed "stabilizers" because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter- and intra-molecular interactions. Tonicity agents can be present in any amount of from 0.1% to 25% by weight, preferably 1 to 5% of the pharmaceutical composition. Preferred tonicity agents include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[0309] Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) and agents preventing denaturation or adherence to the container wall. Such excipients may include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinisitose, myoinisitol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (e.g., xylose, mannose, fructose, glucose; disaccharides (e.g., lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[0310] Non-ionic surfactants or detergents (also known as "wetting agents") may be employed to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Non-ionic surfactants may be present in a concentration range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

[0311] Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl celluose and carboxymethyl cellulose. Anionic detergents that can be used

include sodium lauryl sulfate, dioctyle sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride

[0312] The doses used for the administration can be adapted as a function of various parameters, and in particular as a function of the mode of administration used, of the relevant pathology, or alternatively of the desired duration of treatment. To prepare pharmaceutical compositions, an effective amount of the antibody or antibody fragment may be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

[0313] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0314] Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in a water suitably mixed with a surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0315] An antibody or antibody fragment can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0316] The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In

many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

[0317] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with one or more of the other ingredients enumerated above, as may be required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0318] The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of dimethyl sulfoxide (DMSO) as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area.

[0319] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

[0320] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, “Remington's Pharmaceutical Sciences” 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

[0321] The antibodies or antibody fragments may be formulated within a therapeutic mixture to deliver about 0.0001 to 10.0 milligrams, or about 0.001 to 5 milligrams, or about 0.001 to

1 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose. Multiple doses can also be administered at selected time intervals.

[0322] In addition to the compounds formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; time release capsules; and any other form currently used.

[0323] In certain embodiments, the use of liposomes and/or nanoparticles is contemplated for the introduction of antibodies or antibody fragments into host cells. The formation and use of liposomes and/or nanoparticles are known to those of skill in the art.

[0324] Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) are generally designed using polymers able to degrade *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be easily made.

[0325] Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μm. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations

[0326] Pharmaceutical formulations containing an anti-Axl antibody or antibody fragment as described herein are prepared by mixing such antibody or antibody fragment having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides,

and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0327] Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0328] Exemplary lyophilized antibody formulations are described in U.S. Pat. No. 6,267,958. Aqueous antibody formulations include those described in U.S. Pat. No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0329] The formulation herein may also contain more than one active ingredient as necessary for the particular indication being treated. Preferably, ingredients with complementary activities that do not adversely affect each other may be combined into a single formulation. For example, it may be desirable to provide an EGFR antagonist (such as erlotinib), an anti-angiogenic agent (such as a VEGF antagonist which may be an anti-VEGF antibody) or a chemotherapeutic agent (such as a taxoid or a platinum agent) in addition to the anti-Axl antibody, antibody fragment or immunoconjugate of the present invention. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0330] Active ingredients may be encapsulated in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization. For example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions may be employed. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0331] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or antibody fragment, which matrices may be in the form of shaped articles, e.g. films, or microcapsules.

[0332] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

Therapeutic Methods and Compositions

[0333] Any of the anti-Axl antibodies or antibody fragments, or immunoconjugates provided herein may be used in therapeutic methods. In one aspect, an anti-Axl antibody or antibody fragment, or an immunoconjugate for use as a medicament is provided. In further aspects, an anti-Axl antibody or antibody fragment, or immunoconjugate for use in treating cancer (e.g., breast cancer, non-small cell lung cancer, pancreatic cancer, brain cancer, cancer of pancreas, brain, kidney, ovary, stomach, leukemia, uterine endometrium, colon, prostate, thyroid, liver, osteosarcoma, and/or melanoma) is provided. In certain embodiments, an anti-Axl antibody or antibody fragment, or an immunoconjugate for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-Axl antibody or antibody fragment, or an immunoconjugate for use in a method of treating an individual having cancer comprising administering to the individual an effective amount of the anti-Axl antibody or antibody fragment, or the immunoconjugate. In certain embodiments, the invention provides an anti-Axl antibody or antibody fragment, or an immunoconjugate for use in a method of treating an individual having an immune disorder (e.g., an autoimmune disorder), a cardiovascular disorder (e.g., atherosclerosis, hypertension, thrombosis), an infectious disease (e.g., Ebola virus, Marburg virus) or diabetes, comprising administering to the individual an effective amount of the anti-Axl antibody or antibody fragment. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-Axl antibody or antibody fragment, or an immunoconjugate for use in inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function.

[0334] In certain embodiments, the invention provides an anti-Axl antibody or antibody fragment, or an immunoconjugate for use in a method of inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function in an

individual comprising administering to the individual an effective of the anti-Axl antibody or antibody fragment, or an immunoconjugate to inhibit angiogenesis, inhibit cell proliferation, inhibit immune function, inhibit inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibit tumor vasculature development (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibit tumor stromal function. An "individual" according to any of the above embodiments is preferably a human.

[0335] In a further aspect, the invention provides for the use of an anti-Axl antibody or antibody fragment, or an immunoconjugate in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cancer (in some embodiments, breast cancer, non-small cell lung cancer, pancreatic cancer, brain cancer, cancer of the pancreas, brain, kidney, ovary, stomach, leukemia, uterine endometrium, colon, prostate, thyroid, liver, osteosarcoma, and/or melanoma). In a further embodiment, the medicament is for use in a method of treating cancer comprising administering to an individual having cancer an effective amount of the medicament. In a further embodiment, the medicament is for use in a method of treating an immune disorder (e.g., an autoimmune disorder), a cardiovascular disorder (e.g., atherosclerosis, hypertension, thrombosis), an infectious disease (e.g., Ebola virus, Marburg virus) or diabetes, comprising administering to the individual an effective amount of the anti-Axl antibody or antibody fragment. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function. In a further embodiment, the medicament is for use in a method of inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function in an individual comprising administering to the individual an amount effective of the medicament to inhibit angiogenesis, inhibit cell proliferation, promote immune function, induce inflammatory cytokine section (e.g., from tumor-associated macrophages), inhibit tumor vasculature development (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibit tumor stromal function. An "individual" according to any of the above embodiments may be a human.

[0336] In a further aspect, the invention provides a method for treating a cancer. In one embodiment, the method comprises administering to an individual having such cancer an effective amount of an anti-Axl antibody or antibody fragment, or an immunoconjugate. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.

[0337] Axl is a 140 kDa cell-surface transmembrane receptor protein tyrosine kinase that belongs to a subfamily of closely related receptors including TYRO3, Axl, and MER (TAM; Lai 1991; O'Bryan 1991). TAM activation and signaling has been implicated in multiple cellular responses including cell survival, proliferation, migration and adhesion (Hafizi 2006). Axl was originally identified as an oncogene from patients with chronic myelogenous leukemia and, when overexpressed, it exhibits transforming potential (Janssen 1991; O'Bryan 1991). Axl overexpression has been reported in a variety of human cancers (Craven 1995; Ito 1999; Berclaz 2001; Sun 2004; Shieh 2005), and is associated with invasiveness and metastasis in lung (Shieh 2005), prostate (Sainaghi 2005), breast (Meric 2002), and gastric cancers (Wu 2002) as well as in renal cell carcinoma (Chung 2003) and glioblastoma (Hutterer 2008).

[0338] A recent study showed that Axl overexpression via a 'tyrosine kinase switch' leads to resistance to imatinib in gastrointestinal stromal tumors (Mahadevan 2007). Axl expression is induced by chemotherapy drugs and overexpression of Axl confers drug resistance in acute myeloid leukemia (Hong 2008). Axl has also been shown to regulate endothelial cell migration and tube formation (Holland 2005). These findings suggest that Axl may be involved in the regulation of multiple aspects of tumorigenesis.

[0339] The Axl-expressing solid tumor types are of interest for several reasons. There is a high unmet need for new treatment options in each of these diseases. Preclinical data suggest targeting Axl may result in antitumor activities in various tumor types, such as NSCLC, and melanoma. Taken together with the proposed mechanism of Axl-ADC and underlying biology, antitumor activity in these malignancies is anticipated. Axl is highly expressed and activated in numerous human sarcomas, including aggressive subtypes of leiomyosarcoma, Ewing's sarcoma and liposarcoma, for examples (May 2015; Fleuren 2014; Dantas-Barbosa 2017). Leiomyosarcoma (LMS) are 15% of adult sarcomas and remain difficult to treat in the metastatic phase. The TAM receptors, including TYRO3 and Axl, and their ligands are overexpressed or activated in multiple malignancies, including LMS. LMS patients, especially those who develop metastasis, express higher levels of TYRO3 and GAS6.

Crizotinib and foretinib showed effective antitumour activity in LMS through TYRO3 and Axl deactivation, indicating that clinical trials using TYRO3 and Axl inhibitors are warranted in advanced LMS. These data suggest that Axl is a potential novel, therapeutic target in Axl-expressing sarcomas.

[0340] Immunotherapy, particularly in the form of PD-1 blockade, has emerged as a revolutionary oncologic therapy during the last decade. In the recent SARC028 study (Petitprez 2020), most (75%) of the patients with undifferentiated pleiomorphic sarcoma who responded to the investigational PD-1 inhibitor had PD-L1–positive tumors suggesting combination treatment with a PD-1 inhibitor. The expression of immune biomarkers differs by sarcoma subtype and may be associated with response to immunotherapy (Petitprez 2020). Immune cell dedifferentiated liposarcoma and undifferentiated pleomorphic sarcoma. It has been shown that sarcomas with lymphocyte infiltration and tertiary lymphoid structures respond to the checkpoint inhibitor nivolumab (Petitprez 2020). Together, this suggests that combining the use of PD-1 inhibitors may provide better outcomes in patients with inflamed soft-tissue sarcomas. For pediatric sarcoma patients, treatment with BA3011 is not expected to cause developmental, skeletal, or gonadal defects. Mice homozygous for the Axl knockout allele exhibit an overtly normal phenotype (Lu 1999).

[0341] In this aspect of the present invention, methods of treating Axl-expressing tumors are provided. In some embodiments, the methods comprise administering an anti-Axl antibody or antibody fragment, or immunoconjugate that includes the anti-Axl antibody or antibody fragment of the invention.

[0342] In one embodiment, the methods of treating an Axl expressing tumors comprise administering an immunoconjugate that includes the antibody or antibody fragments of the invention, optionally conjugated to an agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent, and a cytotoxic agent. The immunoconjugate is an antibody-drug conjugate (ADC) in which a Conditionally Active Biologic (CAB) anti-Axl antibody is conjugated to one or more drug moiety via a cleavable linker (CAB-Axl-ADC). For example, the CAB-Axl-ADC is mAbBA3011-cleavable linker-$MMAE_{(n)}$, in which the drug moiety is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0343] In some embodiments, the present invention provides a therapeutic regimen with a CAB-Axl-ADC such as mAbBA3011-cleavable linker-$MMAE_{(n)}$, in which the drug moiety is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive, at doses of about 0.3 mg/kg to about 2.0 mg/kg administered either once or twice every 21 days, on

days 1 and 8 every 21 days, or on days 1 and 8 every 14 days. Such therapeutic regimen is surprisingly efficacious, as the antibody-drug conjugate of the present invention administered at such doses and at such intervals, provides a surprisingly high response rate and an acceptable toxicity or tolerability profile. Accordingly, the present methods provides a dosing regimen for administering a CAB-Axl-ADC antibody-drug conjugate to a subject. In some embodiments, the dosing regimen increases the subject's probability of responding to the therapy as compared to other dosing regimens. In some embodiments, the dosing regimen does not increase the subject's probability of suffering from an adverse event (including a dose limiting toxicity) as compared to other dosing regimens. The present invention also provides maintenance therapy following the dosing regimen.

[0344] In certain embodiments, mAbBA3011-cleavable linker-$MMAE_{(n)}$ is administered at doses of about 0.3 mg/kg to about 1.8mg/kg either once or twice every 21 days, on days 1 and 8 every 21 day period, or on days 1 and 8 every 14 day period. Preferably, mAbBA3011-cleavable linker-$MMAE_{(n)}$ is administered at doses of about 0.8 mg/kg to about 1.8 mg/kg either once or twice every 21 days, on days 1 and 8 every 21 day period, or on days 1 and 8 every 14 day period.

[0345] CAB-Axl-ADCs such as mAbBA3011-cleavable linker-$MMAE_{(n)}$ of the present invention, preferentially bind under defined physiological conditions associated with different diseases and tissues. For example, in cancer, the unique cell metabolism described by Warburg (Warburg 1924; Warburg 1956) contributes to a characteristic microenvironment such as, low pH and high lactate. The mAbBA3011-cleavable linker-$MMAE_{(n)}$ of the present invention takes advantage of the unique TME and selectively binds to its target when in close proximity to a tumor expressing Axl. The activated binding property of mAbBA3011-cleavable linker-$MMAE_{(n)}$ is reversible, such that there are no permanent changes as it transitions from diseased normal to diseased tissue microenvironments. In particular, mAbBA3011-cleavable linker-$MMAE_{(n)}$ includes a humanized mAb (BA3011) without the addition of non-antibody sequences to achieve these properties.

[0346] In a specific aspect, the methods of treating Axl expressing tumor comprises administering to a human subject in need of such treatment, a mAbBA301-cleavable linker-$MMAE_{(n)}$, where the mAbBA301 is an antibody or antibody fragment having a heavy chain variable region that includes a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region that includes a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18, and a lcCDR3 of SEQ ID NO. 19; MMAE is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0347] In another embodiment, the polypeptide, the antibody or antibody fragment, or the immunoconjugate useful in the methods of the present invention, is in a pharmaceutical composition together with a pharmaceutically acceptable carrier.

[0348] In another embodiment, the polypeptide, the antibody or antibody fragment, or the immunoconjugate useful in the methods of the present invention, is included in a kit with instructions for use to diagnose or treat Axl expressing tumors.

[0349] In yet another embodiment, the methods of treating an Axl expressing tumor comprises administering to a human subject in need of such treatment, a pharmaceutical composition including a mAbBA301-cleavable linker-$MMAE_{(n)}$ and a pharmaceutically acceptable carrier, in which the pharmaceutical composition is administered at a dose of 1.8 mg/kg of the human subject weight on days 1 and 8 every 21 days by intravenous infusion. The mAbBA301 is an antibody or antibody fragment having a heavy chain variable region that includes a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region that includes a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18, and a lcCDR3 of SEQ ID NO. 19; and (n) is an integer between 1 and 4, inclusive, preferably, (n) equals 4.

[0350] In certain embodiments, the heavy chain variable region includes SEQ ID NO. 20 and the light chain variable region of the mAbBA301 includes SEQ ID NO. 21.

[0351] In another embodiment, the cleavable linker is mc-vc-PAB.

[0352] In certain embodiments, the Axl expressing tumor is a sarcoma, an adenocarcinoma, or a non-small lung cell cancer. Preferably, the Axl expressing tumor is a sarcoma.

[0353] In certain embodiments, the methods further include administering a programmed death receptor-1 (PD-1) blocking antibody.

[0354] In yet another embodiment, the Axl-expressing tumor has a tumor membrane P score of at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, or at least 95. Preferably, the Axl expressing tumor has a tumor membrane P score of at least 70, at least 75, at least 80, at least 85, at least 90, or at least 95.

[0355] In certain embodiments, the methods further include administering a granulocyte colony stimulating factor or an analog thereof.

[0356] In yet another embodiment, the pharmaceutically acceptable carrier has a pH of 6.0 and comprises 20 mM histidine-HCl, 70 mg/mL sucrose and 0.5 mg/mL polysorbate 80.

[0357] In a further aspect, the invention provides a method for treating an immune disorder (e.g., an autoimmune disorder), a cardiovascular disorder (e.g., atherosclerosis, hypertension, thrombosis), an infectious disease (e.g., Ebola virus, Marburg virus) or diabetes. In one such

embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.

[0358] In a further aspect, the invention provides a method for inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-Axl antibody or antibody fragment to inhibit angiogenesis, inhibit cell proliferation, promote immune function, induce inflammatory cytokine section (e.g., from tumor-associated macrophages), inhibit tumor vasculature development (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibit tumor stromal function. In one embodiment, an "individual" is a human.

[0359] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-Axl antibodies or antibody fragments provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-Axl antibodies or antibody fragments provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-Axl antibodies or antibody fragments provided herein and at least one additional therapeutic agent, e.g., as described below.

[0360] In each and every treatment described above, the antibodies or antibody fragments of the invention can be used alone, as immunoconjugates or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is an anti-angiogenic agent. In certain embodiments, an additional therapeutic agent is a VEGF antagonist (in some embodiments, an anti-VEGF antibody, for example bevacizumab). In certain embodiments, an additional therapeutic agent is an EGFR antagonist (in some embodiment, erlotinib). In certain embodiments, an additional therapeutic agent is a chemotherapeutic agent and/or a cytostatic agent. In certain embodiments, an additional therapeutic agent is a taxoid (e.g., paclitaxel) and/or a platinum agent (e.g., carboplatinum). In certain embodiments the additional therapeutic agent is an agents that enhances the patient's immunity or immune system.

[0361] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and

separate administration, in which case, administration of the antibody or antibody fragment can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or antibody fragments can also be used in combination with radiation therapy.

[0362] Antibodies or antibody fragments may be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody or antibody fragment need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody or antibody fragment present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0363] For the prevention or treatment of disease, the appropriate dosage of an antibody or antibody fragment (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody or antibody fragment, the severity and course of the disease, whether the antibody or antibody fragment is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody or antibody fragment, and the discretion of the attending physician. The antibody or antibody fragment is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 μg/kg to 40 mg/kg of antibody or antibody fragment can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody or antibody fragment). An initial higher loading dose, followed by one or more

lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0364] It is understood that any of the above formulations or therapeutic methods may be carried out using an antibody fragment or an immunoconjugate of the invention in place of or in addition to an anti-Axl antibody.

[0365] Enhancing the host's immune function to combat tumors is the subject of increasing interest. Conventional methods include (i) APC enhancement, such as (a) injection into the tumor of DNA encoding foreign MHC alloantigens, or (b) transfecting biopsied tumor cells with genes that increase the probability of immune antigen recognition (e.g., immune stimulatory cytokines, GM-CSF, co-stimulatory molecules B7.1, B7.2) of the tumor, (iii) adoptive cellular immunotherapy, or treatment with activated tumor-specific T-cells. Adoptive cellular immunotherapy includes isolating tumor-infiltrating host T-lymphocytes, expanding the population in vitro, such as through stimulation by IL-2 or tumor or both. Additionally, isolated T-cells that are dysfunctional may be also be activated by in vitro application of the anti-PD-L1 antibodies. T-cells that are so-activated may then be readministered to the host. One or more of these methods may be used in combination with administration of the antibody, antibody fragment or immunoconjugate of the present invention.

[0366] Traditional therapies for cancer include the following: (i) radiation therapy (e.g., radiotherapy, X-ray therapy, irradiation) or the use of ionizing radiation to kill cancer cells and shrink tumors. Radiation therapy can be administered either externally via external beam radiotherapy (EBRT) or internally via brachytherapy; (ii) chemotherapy, or the application of cytotoxic drug which generally affect rapidly dividing cells; (iii) targeted therapies, or agents which specifically affect the deregulated proteins of cancer cells (e.g., tyrosine kinase inhibitors imatinib, gefitinib; monoclonal antibodies, photodynamic therapy); (iv) immunotherapy, or enhancement of the host's immune response (e.g., vaccine); (v) hormonal therapy, or blockade of hormone (e.g., when tumor is hormone sensitive), (vi) angiogenesis inhibitor, or blockade of blood vessel formation and growth, and (vii) palliative care, or treatment directed to improving the quality of care to reduce pain, nausea, vomiting, diarrhea and hemorrhage. Pain medication such as morphine and oxycodone, anti-emetics such as ondansetron and aprepitant, can permit more aggressive treatment regimens.

[0367] In the treatment of cancer, any of the previously described conventional treatments for the treatment of cancer immunity may be conducted, prior, subsequent or simultaneous with the administration of the anti-Axl antibodies or antibody fragments. Additionally, the anti-

Axl antibodies or antibody fragments may be administered prior, subsequent or simultaneous with conventional cancer treatments, such as the administration of tumor-binding antibodies (e.g., monoclonal antibodies, toxin-conjugated monoclonal antibodies) and/or the administration of chemotherapeutic agents.

Dosing Regimen

[0368] The present invention provides a dosing regimen for the treatment of Axl-expressing tumors. The dosing regimen comprises a dose of an anti-Axl antibody or antibody fragment or immunoconjugate that includes the anti-Axl antibody or antibody fragment of the invention, as described herein of from about 0.3 mg/kg body weight to about 2.0 mg/kg body weight, 0.4 mg/kg body weight to about 1.8 mg/kg body weight, 0.5 mg/kg body weight to about 1.8 mg/kg body weight, 0.6 mg/kg body weight to about 1.8 mg/kg body weight, 0.7 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.9 mg/kg body weight to about 1.8 mg/kg body weight, 1.0 mg/kg body weight to about 1.8 mg/kg body weight, 1.1 mg/kg body weight to about 1.8 mg/kg body weight, 1.2 mg/kg body weight to about 1.3 mg/kg body weight, 0.7 mg/kg body weight to about 1.8 mg/kg body weight, 1.4 mg/kg body weight to about 1.8 mg/kg body weight, 1.5 mg/kg body weight to about 1.8 mg/kg body weight, 1.6 mg/kg body weight to about 1.8 mg/kg body weight, or 1.7 mg/kg body weight to about 1.8 mg/kg body weight, for at least a two week (e.g., 14 day period) or three week period (e.g., 21 day period). More preferably from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight for at least a two week (e.g., 14 day period) or a three week period (e.g., 21 day period). The weekly dose can either be administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week).

[0369] In certain embodiments, the dosing regimen comprises a dose of a polypeptide, an antibody or antibody fragment, or an immunoconjugate of the invention as described herein of from 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.6 mg/kg body weight, 0.8 mg/kg body weight to about 1.4 mg/kg body weight, 0.8 mg/kg body weight to about 1.2 mg/kg body weight or 0.8 mg/kg body weight to about 1.0 mg/kg body weight, for at least a two week (e.g., 14 day period) or three week period (e.g., 21 day period). The weekly dose can either be administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week).

[0370] In certain embodiments, the dosing regimen comprises a dose of an antibody-drug conjugate as described herein of from about 0.3 mg/kg body weight to about 2.0 mg/kg body

weight, 0.4 mg/kg body weight to about 1.8 mg/kg body weight, 0.5 mg/kg body weight to about 1.8 mg/kg body weight, 0.6 mg/kg body weight to about 1.8 mg/kg body weight, 0.7 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.9 mg/kg body weight to about 1.8 mg/kg body weight, 1.0 mg/kg body weight to about 1.8 mg/kg body weight, 1.1 mg/kg body weight to about 1.8 mg/kg body weight, 1.2 mg/kg body weight to about 1.3 mg/kg body weight, 0.7 mg/kg body weight to about 1.8 mg/kg body weight, 1.4 mg/kg body weight to about 1.8 mg/kg body weight, 1.5 mg/kg body weight to about 1.8 mg/kg body weight, 1.6 mg/kg body weight to about 1.8 mg/kg body weight, or 1.7 mg/kg body weight to about 1.8 mg/kg body weight, for at least a two week (e.g., 14 day period) or a three week period (e.g., 21 day period). More preferably from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight for at least a two week (e.g., 14 day period) or a three week period (e.g., 21 day period). The weekly dose can either be administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week).

[0371] In some embodiments, the dosing regimen comprises a dose of an antibody-drug conjugate as described herein of from 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.6 mg/kg body weight, 0.8 mg/kg body weight to about 1.4 mg/kg body weight, 0.8 mg/kg body weight to about 1.2 mg/kg body weight or 0.8 mg/kg body weight to about 1.0 mg/kg body weight, for at least a two week (e.g., 14 day period) or three week period (e.g., 21 day period). The weekly dose can either be administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week).

[0372] In certain embodiments, the weekly dose is administered, as a split delivery or as a single weekly dose, for at least one two week (e.g., 14 day) treatment cycle or for at least one three week (e.g., 21 day) treatment cycle. In some embodiments, the dose will be administered as a single weekly dose on days 1 and 8 of a 14 day treatment cycle. Preferably, the weekly dose, as a split delivery or as a single weekly dose, is administered for two or more 14 day treatment cycles, even more preferably for three or more, four or more, five, or even six or more treatment cycles. In some embodiments, the weekly dose is administered for no more than 3, no more than 4, no more than 5, or no more than 6 treatment cycles. In some embodiments, the dose will be administered as a single weekly dose on days 1 and 8 of a 21 day treatment cycle. Preferably, the weekly dose, as a split delivery or as a single weekly dose, is administered for two or more 21 day treatment cycles, even more preferably for three or more, four or more, five, or even six or more treatment cycles. In some

embodiments, the weekly dose is administered for no more than 3, no more than 4, no more than 5, or no more than 6 treatment cycles. Preferably, there will a period of rest between treatment cycles.

[0373] For example, in some preferred embodiments, the dosing regimen will be a total weekly dose of the antibody-drug conjugate of from about 0.3 mg/kg body weight to about 2.0 mg/kg body weight, 0.4 mg/kg body weight to about 1.8 mg/kg body weight, 0.5 mg/kg body weight to about 1.8 mg/kg body weight, 0.6 mg/kg body weight to about 1.8 mg/kg body weight, 0.7 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.9 mg/kg body weight to about 1.8 mg/kg body weight, 1.0 mg/kg body weight to about 1.8 mg/kg body weight, 1.1 mg/kg body weight to about 1.8 mg/kg body weight, 1.2 mg/kg body weight to about 1.3 mg/kg body weight, 0.7 mg/kg body weight to about 1.8 mg/kg body weight, 1.4 mg/kg body weight to about 1.8 mg/kg body weight, 1.5 mg/kg body weight to about 1.8 mg/kg body weight, 1.6 mg/kg body weight to about 1.8 mg/kg body weight, or 1.7 mg/kg body weight to about 1.8 mg/kg body weight, for at least two treatment cycles with a one week period of rest between each of the treatment cycles. In some embodiments, the treatment cycle will be greater than 14 days. In some embodiments, the treatment will be greater than 21 days. The weekly dose can be administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week).

[0374] For example, in some preferred embodiments, the dosing regimen will be a total weekly dose of the antibody-drug conjugate of from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.6 mg/kg body weight, 0.8 mg/kg body weight to about 1.4 mg/kg body weight, 0.8 mg/kg body weight to about 1.2 mg/kg body weight or 0.8 mg/kg body weight to about 1.0 mg/kg body weight for at least two treatment cycles with a one week period of rest between each of the treatment cycles (e.g., six single weekly doses during an eight week time period). In some embodiments, the treatment cycle will be greater than 14 days. In some embodiments, the treatment cycle will be greater than 21 days. The weekly dose can be administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week).

[0375] In some embodiments, the weekly dose of the antibody drug conjugate will be about 0.8 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be about 0.9 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some

embodiments, the weekly dose of the antibody drug conjugate will be about 1.0 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be about 1.1 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be about 1.2 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be about 1.3 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be about 1.4 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be about 1.5 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be about 1.6 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be about 1.7 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be about 1.8 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate will be 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7 or 1.8 mg/kg of the subject's body weight.

[0376] The two week (14 day period) treatment cycle with a one week period of rest between treatment cycles, can also be referred to as a 3 week (21 day) treatment cycle where the antibody-drug conjugate is delivered 2 out of 3 weeks in the 3 week treatment cycle. Likewise, the three week (21 day period) treatment cycle with a one week period of rest between treatment cycles, can also be referred to as a 4 week (28 day) treatment cycle where the antibody-drug conjugate is delivered 3 out of 4 weeks in the 4 week treatment cycle. Accordingly, in some embodiments, the dose is administered weekly, as a split delivery or as a single weekly dose, 2 out of 3 weeks in a 3 week treatment cycle, or the dose is administered weekly, as a split delivery or as a single weekly dose, 3 out of 4 weeks in a 4 week treatment cycle. In some embodiments, the dose will be administered as a single

weekly dose on days 1 and 8 of a 21 day treatment cycle, or the dose will be administered as a single weekly dose on days 1 and 8 of a 28 day treatment cycle. Preferably, the weekly dose, as a split delivery or as a single weekly dose, is administered for two or more four week treatment cycles, even more preferably for three or more, four or more, five or more, or even six or more four week treatment cycles (e.g., 2, 3, 4, 5, or 6 consecutive treatment cycles). In some embodiments, the weekly dose is administered for no more than 3, no more than 4, no more than 5, or no more than 6 treatment cycles. For example, in some preferred embodiments, the dosing regimen will be a weekly dose, as a split delivery or as a single weekly dose, for a total weekly dose of from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.6 mg/kg body weight, 0.8 mg/kg body weight to about 1.4 mg/kg body weight, 0.8 mg/kg body weight to about 1.2 mg/kg body weight or 0.8 mg/kg body weight to about 1.0 mg/kg body weight of the antibody-drug conjugate, 2 out of 3 weeks, for at least two three week treatment cycles. For example, in some preferred embodiments, the dosing regimen will be a weekly dose, as a split delivery or as a single weekly dose, for a total weekly dose of from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.6 mg/kg body weight, 0.8 mg/kg body weight to about 1.4 mg/kg body weight, 0.8 mg/kg body weight to about 1.2 mg/kg body weight or 0.8 mg/kg body weight to about 1.0 mg/kg body weight of the antibody-drug conjugate, 3 out of 4 weeks, for at least two four week treatment cycles.

[0377] In some preferred embodiments, the dosing regimen will be a weekly dose, as a split delivery or as a single weekly dose, for a total weekly dose of from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.6 mg/kg body weight, 0.8 mg/kg body weight to about 1.4 mg/kg body weight, 0.8 mg/kg body weight to about 1.2 mg/kg body weight or 0.8 mg/kg body weight to about 1.0 mg/kg body weight of the antibody-drug conjugate, 2 out of 3 weeks, for one, two, three, four, or five four week treatment cycles (e.g., four single weekly doses in an six week time period, six single weekly doses in a nine week time period, eight single weekly doses in a twelve week time period).

[0378] In some preferred embodiments, the dosing regimen will be a weekly dose, as a split delivery or as a single weekly dose, for a total weekly dose of from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, 0.8 mg/kg body weight to about 1.6 mg/kg body weight, 0.8 mg/kg body weight to about 1.4 mg/kg body weight, 0.8 mg/kg body weight to about 1.2 mg/kg body weight or 0.8 mg/kg body weight to about 1.0 mg/kg body weight of the antibody-drug conjugate, 3 out of 4 weeks, for one, two, three, four, or five four week treatment cycles (e.g., six single weekly doses in an eight week time period, nine single

weekly doses in a twelve week time period, twelve single weekly doses in a sixteen week time period).

[0379] Following or during one or more treatment cycles (e.g., during days 14-21 of the second treatment cycle or during days 21-28 of the second treatment cycle), the subject can be evaluated (e.g., through clinical or diagnostic testing) to determine whether the subject should remain on the treatment schedule. For example, following or during one or more 28 day treatment cycles (e.g., 1, 2, 3, 4, 5, or 6 28 day treatment cycles), the subject can be evaluated (e.g., a clinical and/or diagnostic evaluation). Depending on the evaluation, the subject will discontinue treatment, continue on treatment with additional treatment cycles, or commence maintenance therapy. If the subject continues treatment, the subject can be further evaluated following one or more additional treatment cycles. Depending on each successive evaluation, the subject will discontinue treatment, continue on treatment with additional treatment cycles, or commence maintenance therapy.

[0380] The present invention encompasses embodiments in which the subject remains on the weekly treatment cycle (e.g., the two week treatment cycle or the three week treatment cycle) following an evaluation indicating that the subject has no detectable cancer, for example, following a diagnostic test that is negative for the CD30 expressing cancer (i.e., the diagnostic test is unable to detect any cancer in the subject). For example, in some embodiments, the subject will remain on the weekly treatment cycle for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more treatment cycles following such an evaluation. In some embodiments, the subject will remain on the weekly treatment cycle for at least two but no more than 3, no more than 4, no more than 5, or no more than 6 treatment cycles. One example of a diagnostic test used for determining the presence and severity of cancers is positron emission tomography (PET).

[0381] In some embodiments, the subject will commence maintenance therapy following one or more, preferably two or more, (e.g, following 1, 2, 3, 4, 5, or 6) treatment cycles (e.g., the four week treatment cycle). In some embodiments, the subject will commence maintenance therapy following an evaluation indicating that the subject has little or no detectable cancer, e.g., following an evaluation indicating that the subject has had a complete response. As used herein, maintenance therapy refers to therapy with the antibody-drug conjugate but at a reduced administration schedule at either the same or different dosages. During maintenance therapy, the antibody-drug conjugate is preferably administered at least once every two week treatment period, once every three week treatment period, on days 1 and 8 of every two week treatment period, or on days 1 and 8 of every three week treatment period. Following these

maintenance therapy cycles, the subject can be further evaluated (e.g., through clinical or diagnostic testing) to determine whether the subject should remain on the maintenance therapy, continue with regular treatment or discontinue treatment. In some embodiments, maintenance therapy will be once every two weeks to four weeks, or every three weeks to six weeks. The dosage of the antibody drug conjugate administered during maintenance therapy can range, for example, from about 0.3 mg/kg body weight to about 2.0 mg/kg body weight, preferably from about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, preferably from about 1.2 mg/kg body weight to about 2.0 mg/kg body weight, more preferably from about 1 mg/kg body weight to about 1.8 mg/kg body weight per dose, with 1.8 mg/kg being an exemplary dose.

[0382] In some embodiments, following conclusion of the weekly treatment at a dosage of the antibody drug conjugate of from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, more preferably a dosage of from about 0.8 mg/kg body weight to about 1.2 mg/kg body weight and evaluation, the subject will begin a maintenance therapy which comprises administration of the antibody-drug conjugate once every two to four weeks or once every three to six weeks, at a dosage of from about 0.3 mg/kg body weight to about 2 mg/kg body weight, preferably from about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, preferably from about 1.2 mg/kg body weight to about 2.0 mg/kg body weight, more preferably from about 1 mg/kg body weight to about 1.8 mg/kg body weight with about 1.8 mg/kg being preferred. In some embodiments, following conclusion of the weekly treatment (e.g., for one, two, three, four or five treatment cycles), the subject will begin a once every two week administration schedule (e.g., treatment on day 1 of a two week maintenance therapy cycle) of the antibody drug conjugate at a dosage of from about 0.4 mg/kg body weight to about 2 mg/kg body weight, from about 0.6 mg/kg body weight to about 2.0 mg/kg body weight, or from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight with about 1.8 mg/kg being preferred. In some embodiments, following conclusion of the weekly treatment (e.g., for one, two, three, four or five treatment cycles), the subject will begin a once every three week administration schedule (e.g., treatment on day 1 of a three week maintenance therapy cycle) of the antibody drug conjugate at a dosage of from about 0.4 mg/kg body weight to about 2 mg/kg body weight, from about 0.6 mg/kg body weight to about 2.0 mg/kg body weight, or from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight with about 1.8 mg/kg being preferred.

[0383] The present invention encompasses embodiments wherein a subject will be administered a weekly dose, as a split delivery or as a single weekly dose, of the antibody

drug conjugate for a total weekly dose of from about 0.8 mg/kg of the subject's body weight to about 1.8 mg/kg of the subject's body weight, about 0.8 mg/kg body weight to about 1.6 mg/kg body weight, about 0.8 mg/kg body weight to about 1.4 mg/kg body weight, about 0.8 mg/kg body weight to about 1.2 mg/kg body weight or about 0.8 mg/kg body weight to about 1.0 mg/kg body weight, 2 out of 3 weeks, for one, two, three, four, five, or six 21 day treatment cycles followed by administration of an every two to four week dose, preferably every two week dose, of antibody drug conjugate at a dose of from about 0.4 mg/kg body weight to about 2 mg/kg body weight, from about 0.6 mg/kg body weight to about 2.0 mg/kg body weight, or from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight per dose for 2 or more maintenance therapy cycles. In some embodiments, the weekly administration cycle will be for 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more treatment cycles and the every two week administration schedule will be for 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more maintenance therapy cycles. In some embodiments, the weekly administration cycle will be for no more than 2, 3, 4, 5, or 6 treatment cycles.

[0384] The present invention encompasses embodiments wherein a subject will be administered a weekly dose, as a split delivery or as a single weekly dose, of the antibody drug conjugate for a total weekly dose of from about 0.8 mg/kg of the subject's body weight to about 1.8 mg/kg of the subject's body weight, about 0.8 mg/kg body weight to about 1.6 mg/kg body weight, about 0.8 mg/kg body weight to about 1.4 mg/kg body weight, about 0.8 mg/kg body weight to about 1.2 mg/kg body weight or about 0.8 mg/kg body weight to about 1.0 mg/kg body weight, 3 out of 4 weeks, for one, two, three, four, five, or six 28 day treatment cycles followed by administration of an every three to six week dose, preferably every three week dose, of antibody drug conjugate at a dose of from about 0.4 mg/kg body weight to about 2 mg/kg body weight, from about 0.6 mg/kg body weight to about 2.0 mg/kg body weight, or from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight per dose for 2 or more maintenance therapy cycles. In some embodiments, the weekly administration cycle will be for 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more treatment cycles and the every three week administration schedule will be for 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more maintenance therapy cycles. In some embodiments, the weekly administration cycle will be for no more than 2, 3, 4, 5, or 6 treatment cycles.

[0385] The present invention encompasses embodiments wherein a subject will be administered a weekly dose, as a split delivery or as a single weekly dose, of the antibody drug conjugate at a total weekly dose of dose of from about 0.8 mg/kg of the subject's body weight to about 1.8 mg/kg 2 out of 3 weeks (e.g., on days 1 and 8 of a 21 day treatment

cycle) for one, two, three, four, five, or six 21 day treatment cycles followed by administration of an every two to four week dose. Preferably, every two week dose, of antibody drug conjugate at a dose of about 1.8 mg/kg per body weight for 2 or more maintenance therapy cycles (e.g., a dose of about 1.8 mg/kg per body weight every two weeks for two or more two week maintenance therapy cycles). Preferably, every two week dose, of antibody drug conjugate at a dose of about 0.8 mg/kg per body weight for 2 or more maintenance therapy cycles (e.g., a dose of about 0.8 mg/kg per body weight every two weeks for two or more two week maintenance therapy cycles).

[0386] The present invention encompasses embodiments wherein a subject will be administered a weekly dose, as a split delivery or as a single weekly dose, of the antibody drug conjugate at a total weekly dose of dose of from about 0.8 mg/kg of the subject's body weight to about 1.8 mg/kg 3 out of 4 weeks (e.g., on days 1 and 8 of a 28 day treatment cycle) for one, two, three, four, five, or six 28 day treatment cycles followed by administration of an every three to six week dose. Preferably, every three week dose, of antibody drug conjugate at a dose of about 1.8 mg/kg per body weight for 2 or more maintenance therapy cycles (e.g., a dose of about 1.8 mg/kg per body weight every three weeks for two or more three week maintenance therapy cycles). Preferably, every three week dose, of antibody drug conjugate at a dose of about 0.8 mg/kg per body weight for 2 or more maintenance therapy cycles (e.g., a dose of about 0.8 mg/kg per body weight every three weeks for two or more three week maintenance therapy cycles).

[0387] The present invention encompasses embodiments wherein the subject to be treated by the present methods is being treated with mAbBA301-cleavable linker-$MMAE_{(n)}$ antibody-drug conjugate of the present invention but at a schedule other than the weekly dosing regimen (e.g., administration of the antibody drug conjugate at a dose of about 1.8 mg/kg body weight every two weeks for one or more two week therapy cycles, or every three weeks for one or more three week therapy cycles) and is switched to a weekly dosing regimen as described herein for no more than 1, 2, 3, 4, 5, or 6 treatment cycles. Following the weekly dosing regimen, the patient can optionally commence maintenance therapy as described herein.

[0388] The antibody-drug conjugate is preferably administered as a monotherapy. By the term "monotherapy" it is meant that the antibody drug conjugate is the only anti-cancer agent administered to the subject during the treatment cycle. Other therapeutic agents, however, can be administered to the subject as described herein. For example, a programmed death receptor-1 (PD-1) blocking antibody or a granulocyte colony stimulating factor or analog

thereof. Additionally, anti-inflammatory agents or other agents administered to a subject with cancer to treat symptoms associated with cancer, but not the underlying cancer itself, including, for example inflammation, pain, weight loss, and general malaise can be administered during the period of monotherapy. A subject being treated by the present methods will preferably have completed any prior treatment with anti-cancer agents before administration of the antibody drug conjugate. In some embodiments, the subject will have completed any prior treatment with anti-cancer agents at least 1 week (preferably 2, 3, 4, 5, 6, 7, or 8 weeks) prior to treatment with the antibody drug conjugate. The subject will also, preferably, not be treated with any additional anti-cancer agents for at least 2 weeks (preferably at least 3, 4, 5, 6, 7, or 8 weeks) following completion of the first treatment cycle with the antibody drug conjugate and preferably for at least 2 weeks (preferably at least 3, 4, 5, 6, 7, or 8 weeks) following completion of the last dose of the antibody drug conjugate.The methods of the present invention encompass administering an anti-Axl antibody or antibody fragment or immunoconjugate comprising an anti-Axl antibody or antibody fragment of the present invention, to a subject for the treatment of Axl-expressing tumors.

[0389] In some embodiments, after administration of immunoconjugate comprising an anti-Axl antibody or antibody fragment of the present invention, to a subject and binding of the anti-Axl antibody to an Axl-expressing tumor cell, the antibody-drug conjugate internalizes into the cell, and the drug is released. For example, the methods of the present invention encompass administering an mAbBA3011-cleavable linker-$MMAE_{(n)}$ antibody-drug conjugate, to a subject for the treatment of a Axl-expressing tumors. Following binding, the mAbBA3011-cleavable linker-$MMAE_{(n)}$ antibody-drug conjugate is internalized into the tumor cell where the peptide linker is cleaved by proteases to release MMAE. Delivery of the MMAE specifically to tumor cells expressing Axl is expected to prevent further tumor cell proliferation and result in tumor shrinkage.

[0390] The subjects to be treated with the methods of the present invention are those that have been diagnosed with an Axl-expressing cancer or are suspected of having an Axl-expressing cancer. Diagnosis can be by methods known in the art, including, for example, tissue biopsy.

Articles of Manufacture and Kits

[0391] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on

or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody or antibody fragment of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody or antibody fragment; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0392] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-Axl antibody

[0393] Finally, the invention also provides kits comprising at least one antibody or antibody fragment of the invention. Kits containing polypeptide, antibodies or antibody fragments, or antibody drug conjugate of the invention find use in detecting Axl expression (increase or decrease), or in therapeutic or diagnostic assays. Kits of the invention can contain an antibody coupled to a solid support, e.g., a tissue culture plate or beads (e.g., sepharose beads). Kits can be provided which contain antibodies for detection and quantification of Axl in vitro, e.g. in an ELISA or a Western blot. Such antibody useful for detection may be provided with a label such as a fluorescent or radiolabel.

[0394] The kits further contain instructions on the use thereof. In some embodiments, the instructions comprise instructions required by the U.S. Food and Drug Administration for in vitro diagnostic kits. In some embodiments, the kits further comprise instructions for diagnosing the presence or absence of cerebrospinal fluid in a sample based on the presence or absence of Axl in said sample. In some embodiments, the kits comprise one or more

antibodies or antibody fragments. In other embodiments, the kits further comprise one or more enzymes, enzyme inhibitors or enzyme activators. In still other embodiments, the kits further comprise one or more chromatographic compounds. In yet other embodiments, the kits further comprise one or more compounds used to prepare the sample for spectroscopic assay. In further embodiments, the kits further comprise comparative reference material to interpret the presence or absence of Axl according to intensity, color spectrum, or other physical attribute of an indicator.

Plasma Membrane Scoring of Axl in Tumor (Tumor Membrane P Score)

[0395] Axl is reactive in a subset of tumor cells and macrophages. In tumor cells, Axl is primarily localized to the plasma membrane but can also be observed in the cytoplasm. Macrophages that express Axl are often present among tumor cells/nests and within the stroma adjacent to tumor (tumor-associated stroma or tumor-stroma). Axl macrophage staining is localized to the plasma membrane or the cytoplasm, although not all macrophages label with Axl.

[0396] CD68 is expressed in the cytoplasm of macrophages and is a standard biomarker for identification of this immune cell type. Macrophages can be present throughout tissue samples but are often of most interest when present among tumor cells (within the tumor mass) and at the tumor/stroma interface (tumor-associated stroma).

[0397] Because Axl is expressed in both tumor cells and macrophages, the present invention uses a scoring approach to compare Axl and CD68 staining in serial sections of each sample. In this way, the CD68 biomarker is used to identify macrophages in the tumors stained for Axl. That is, the CD68 serial section is used to distinguish Axl reactivity in tumor cells versus macrophages. Using this approach, Axl plasma membrane staining is scored only in tumor cells. CD68 staining is "subtracted out" of the assessment for Axl to provide Axl tumor scoring exclusive of macrophages (hereinafter "tumor membrane P score").

[0398] The approaches used for scoring Axl and CD68 may be detected by methods, including but not limited to, immunohistochemistry (IHC) in formain-fixed, paraffin-embeded (FFPE) tumor samples as described below. All samples are also stained with hematoxylin and eosin (H&E) for morphological assessment to assist in scoring.

[0399] Axl plasma membrane expression in tumor is scored semi-quantitatively. The main components to scoring are percentage of cells staining at appropriate differential intensities.

[0400] Percentage scores are assigned to describe the penetrance of plasma membrane staining per sample. Percentages are estimated and reported as an increment, including but

not limited to, the one of the following increments: 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or 100%.

[0401] In certain embodiments of the present invention, the Axl-expressing tumor has a tumor membrane P score of at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, or at least 95%. Preferably, the Axl expressing tumor has a tumor membrane P score of at least 70, at least 75, at least 80, at least 85, at least 90, or at least 95%.

[0402] Differential intensities for plasma membrane staining are recorded using a four-point scale semi-quantitatively (0, 1+, 2+, 3+). On this scale, 0 = null, negative, or non-specific staining, 1+ = low or weak staining, 2+ = medium or moderate staining, and 3+ = high or strong staining.

[0403] When assessing Axl reactivity only in tumor cells (Axl tumor staining exclusive of macrophages), stained slides for both Axl and for CD68 (macrophage biomarker) are required in serial. Staining with CD68 is closely compared to staining with Axl in regions of a tumor. Cells that stain with both Axl and CD68 are considered Axl-reactive macrophages (not tumor cells) and are excluded from the Axl score.

[0404] Axl staining in macrophage makes it difficult to score plasma membrane staining in tumor cells when there is a mixed population of positive cell types. To gain a full understanding of Axl expression on the plasma membrane in tumors exclusive of macrophages across cancer indication, both standard Percent Score and H-Score approaches are used to capture the pattern of reactivity observed.

Percent Score Method

[0405] Percent Scores are calculated by summing the percentages of intensities at either ≥1+, ≥2+ or ≥3+. Thus, scores range from 0 to 100.

Percent Score ≥1+ = (% at 1+) **+** (% at 2+) **+** (% at 3+)

Percent Score ≥2+ = (% at 2+) **+** (% at 3+)

Percent Score ≥3+ = (% at 3+)

H-Score Method

[0406] The H-Score is calculated by summing the percentage of cells with intensity of expression (brown staining) multiplied by their corresponding differential intensity on a four-point semi quantitative scale (0, 1+, 2+, 3+). Thus, scores range from 0 to 300.

H-Score = [(% at <1) x 0 **] + [** (% at 1+) x 1 **] + [** (% at 2+) x 2 **] + [** (% at 3+) x 3**]**

Scoring of Macrophage Staining

• As stated, CD68 is a standard biomarker for macrophages and Axl can be expressed in this cell type.
• Slides stained with CD68 and Axl are used to assess the relative abundance of CD68-positive and Axl-positive macrophages in each tumor tissue.
• Macrophage estimates are made for CD68 and Axl positivity in the following regions: within the tumor mass (called "tumor") and within tumor-associated stroma or stroma that interacts with tumor (called "tumor-stroma"). Tumor-stroma represents the parenchymal response to a tumor. It is the stromal response, outside or adjacent to the outer edge or "face" of the tumor mass.
• In a tumor, scoring represents the percentage (0-100%) of cells in the tumor mass or tumor nests that are comprised of CD68 or Axl-positive macrophages.
• In tumor-stroma, macrophage abundance for Axl and CD68 is scored using a semi-quantitative scale from 0-3. On this scale, 0 represents no positive macrophages, 1 indicates low density of positive macrophages, 2 indicates moderate density of positive macrophages, and 3 indicates high density of positive macrophages.

Scoring of Axl in Tumor Cytoplasm

[0407] • The percentage of a tumor showing diffuse Axl cytoplasmic staining (% Positive Cells) is estimated from 0-100%. The average intensity of such staining is estimated using a scale from 0-3. On this scale, 0 represents no cytoplasmic staining, 1 represents weak cytoplasmic staining, 2 represents moderate cytoplasmic staining, and 3 represents strong or intense cytoplasmic staining.

[0408] • Strong Axl staining in the cytoplasm can make it difficult to discern potential membrane signal.

[0409] The following examples are illustrative, but not limiting, of the soft gelatin capsules of the present disclosure. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in the field, and which are obvious to those skilled in the art, are within the scope of the disclosure.

EXAMPLES

Example 1: Conditionally active antibody to Axl

[0410] Axl is a cell-surface transmembrane tyrosine kinase with an extracellular domain accessible by the conditionally active antibody. This cell surface protein is highly expressed in thyroid carcinoma tissues and overexpressed in many other cancers such as sarcoma,

myeloproliferative disorders, prostatic carcinoma cells, or breast cancer. Increased AXL expression has been associated with tumor resistance to chemotherapy, programmed death-1 (PD-1) inhibitors, molecular targeted therapy, and radiation therapy. A conditionally active antibody to the extracellular domain of the Axl protein was developed herein.

[0411] A wild-type antibody to Axl was selected as the template antibody (with a heavy chain variable region of 063-hum10F10-HC in FIG. 1A and a light chain variable region of 063-hum10F10-HC in FIG. 1B). The DNA encoding the wild-type antibody was evolved to generate a mutant antibody library using Comprehensive Positional Evolution (CPE), a method by which each position in the template antibody is randomized one at a time. Each mutant antibody in the library has only one single point mutation. The mutant antibodies in the library were generated by simultaneously screening for selective binding affinity to Axl at pH 6.0 over pH 7.4 as determined by ELISA.

[0412] Simultaneously, the expression level of the mutant antibodies was also optimized for the purpose of higher fields in a manufacturing process. The screening was done in serum using a FLAG tag because there were human antibodies in the serum which might cause false positives for the screening. The screening buffer was a carbonate buffer (Krebs buffer with ringer – standard buffer but different from PBS). The generated conditionally active antibodies were found to have a higher affinity to the Axl at pH 6.0 but lower affinity to the Axl at pH 7.4, both in comparison with the wild-type antibody. Some of the selected mutant antibodies (scFv) were represented in Figure 2 with their higher activity at pH 6.0 than at pH 7.4, while their activity ratios between pH 6.0 to pH 7.4 were at least 11 fold (Figure 3).

[0413] Further, these conditionally active antibodies all have high expression levels as shown in Table 4 below, with column “Clone” showing the antibodies and the expression level “mg/ml” being shown in the second column.

[0414] The clones of these antibodies were sent to a service provider with a requested expression level (“amount ordered”, expected expression levels). However, the actual expression levels of these antibodies (“amount delivered”) were very high and exceeded the expected expression levels.

Table 4. Conditionally active antibodies with high expression levels

Clone	[mg/mL]	estimated yield	actual yield
BAP063.6-01-05	6.6	150	238
BAP063.2-01-10	7	150	294
BAP063.8-46-04	7	200	333
BAP063.8-62-02	5.8	200	220
BAP063.9-13-01	5.3	50	123
BAP063.9-29-02	4.9	50	102
BAP063.9-45-02	5.4	50	129
BAP063.9-13-03	5.9	50	130
BAP063.9-21-03	5.3	50	117
BAP063.9-21-04	7	50	176
BAP063.9-29-04	8.2	50	196
BAP063.9-48-03	7	50	125
BAP063.9-49-04	5.3	50	126
BAP063.9-61-01	5.1	50	97
BAP063.9-61-02	5	50	92

[0415] The conditionally active antibodies did not show aggregation in a buffer as demonstrated in FIG. 4, using BAP063.9-13-1 antibody as an example. The BAP063.9-13-1 antibody was analyzed by size exclusion chromatography. In FIG. 4, only one peak was detected, demonstrating little or no aggregation of the antibody.

[0416] The conditionally active antibodies were also assayed using surface plasmon resonance (SPR) to measure their on and off rates to the Axl. The SPR assay has been known to measure on and off rates for the conditionally active antibodies. The SPR assay was performed in the presence of bicarbonate. The *in vivo* on and off rate (in animals and humans) of the conditionally active antibodies is a very important feature for the conditionally active antibodies.

[0417] It was observed that the conditionally active antibodies have higher binding affinity at pH 6.0 and lower binding affinity at pH 7.4, in comparison with the negative control (BAP063 10F10 which has similar binding affinity at both pH 6.0 and pH 7.4) (FIG. 5). In addition, raising the temperature from room temperature to 60 °C does not significantly alter the ELISA assay results (FIG. 5). The ELISA assay also showed that these conditionally active antibodies were highly selective at pH 6.0 as compared to pH 7.4 (FIGS. 6A-6B show one antibody as an example).

[0418] The conditionally active biological antibodies are summarized in Table 5. Two of the antibodies were expressed as scFv (BAP063.9-13.3 and BAP063.9-48.3). Incubating the antibodies at 60 °C for one hour did not change the affinities of most of the antibodies ("Thermostability").

[0419] The conditionally active antibody may be used to detect Axl protein on the surface of CTCs according to the present invention.

Table 5 - Summary of the conditionally active anti-Axl antibodies

Clone	mg/ml	estimated yield	actual yield	Aggregation (PBS,pH7.4)	Thermostability (1 h 60 °C)	Ka [M·s]	Kd[s^{-1}]	KD[M] pH6.0
BAP063.1-01-10	7	150	294	No	100%	5.14E+06	8.38E-04	1.63E-10
BAP063.6-01-05	6.6	150	238	N.D.		2.41E+06	5.12E-03	2.12E-09
BAP063.9-13-01	5.3	50	123	No	100%	1.98E+06	2.88E-03	1.46E-09
BAP063.9-29-02	4.9	50	102	No	100%	1.19E+06	2.14E-03	1.79E-09
BAP063.9-45-02	5.4	50	129	No	reduced	1.53E+06	2.31E-03	1.51E-09
BAP063.9-13-03	5.9	50	130	No	100%	1.42E+06	1.82E-03	1.28E-09
BAP063.9-21-03	5.3	50	117	No	100%	1.53E+06	4.13E-03	2.69E-09
BAP063.9-21-04	7	50	**176**	No	100%	1.03E+06	3.26E-03	3.16E-09
BAP063.9-29-04	8.2	50	**196**	No	100%	1.40E+06	2.21E-03	1.58E-09
BAP063.9-48-03	7	50	125	<5%	reduced	8.92E+05	2.33E-03	2.61E-09
BAP063.9-49-04	5.3	50	126	No	100%	2.35E+06	3.42E-03	1.45E-09
BAP063.9-61-01	5.1	50	97	<10%	100%	n.d.	n.d.	
BAP063.9-61-02	5	50	92	<10%	100%	1.72E+06	2.85E-03	1.66E-09

Example 2: pH-Dependent Binding Affinity of the Anti-Axl Antibodies

[0420] Some of the anti-Axl antibodies of the present invention were tested in buffers at different pH levels. One type of buffer was a KREBS buffer with 1% bovine serum albumin (BSA) present. The KREBS buffer was titrated to have a pH in the range of 5-7.4. The binding affinity of the antibodies with Axl was measured using an ELISA assay (OD_{450}) and the results are presented in FIG. 7. The two control antibodies (BAP063-3831 and BAP063-3818) were not conditionally active as they have a binding affinity that was not significantly affected by the change in pHs. On the other hand, the anti-Axl antibodies of the present invention are conditionally active since their binding affinity with Axl was dependent on the pH (FIG. 7).

Example 3: Cell Killing by the Anti-Axl Antibodies

[0421] The cell killing activities of the anti-Axl antibodies of the present invention were tested using A549 cells. The results are shown in FIGS. 8A-8E. The cell killing activities were measured at two pH levels: 6.0 and 7.4, representing a pH in tumor microenvironment and a normal physiological pH, respectively. The percentage of cell killing at various antibody concentrations iss shown in FIGS. 8A-8E.

[0422] The two tests gave consistent cell killing results. The negative control (anti-Axl humanized WT) showed a similar cell killing activity at pH 6.0 and pH 7.4 for the A549 cells (FIG. 8A). In contrast, the anti-Axl antibodies of the present invention showed significantly higher cell killing activity at pH 6.0 in comparison with cell killing activity at pH 7.4, especially at low antibody concentrations at which the antibodies did not saturate the A549 cells (FIGS. 8B-8E).

Example 4: Binding affinity to cyno-Axl by the anti-Axl antibodies

[0423] The binding affinity to cyno-Axl by the anti-Axl antibodies of the present invention was measured and compared with the binding affinity of human Axl (hAxl) in two different buffers at pHs of 6.0 and 7.4. The results are shown in FIGS. 9A-9D. The cyno-Axl is an Axl protein from a non-human primate, namely, the *cynomolgus macaques* monkey.

[0424] The control (BA-3831-WT) showed similar binding affinity to both human Axl (hAxl) and *cynomolgus* Axl (cyno-Axl) at both pH 6.0 and 7.4 in the two buffers (FIG. 9A). The anti-Axl antibodies of the present invention showed similar binding affinity profiles for hAxl and cyno-Axl in one of the two buffers, i.e., lower binding affinity to cyno-Axl at pH

7.4, in comparison with the binding affinity to cyno-Axl at pH 6.0 (FIGS. 9B-9D). The difference between the binding affinities at pH 6.0 and pH 7.0 in the other buffer was not significant.

Example 5: Cytotoxicity of anti-Axl antibodies conjugated to duomycin

[0425] Duomycin is cytotoxic since it inhibits cell growth by stopping protein synthesis. One of the anti-Axl antibodies of the present invention, BAP063.9 4007, was conjugated to duomycin. Two controls were used in this test, BAP063 hum WT and B12 (an anti-B12 antibody), both were also conjugated duomycin.

[0426] Several cell lines were treated with the three duomycin-conjugated antibodies (BAP063.9 4007, BAP063 hum WT, and B12) at pHs 6.0 and 7.4 (FIGS. 10A-10H). The duomycin-conjugated antibody BAP063.9 4007 of the present invention showed significantly higher cytotoxicity to cell lines DU145 (prostate cancer cells), MDA-MD-231 (breast cancer cells), PL45 (pancreatic cancer cells), and A549 (adenocarcinoma cells) at pH 6.0 in comparison with the cytotoxicity to the same cells at pH 7.4.

Example 6: Anti-Axl antibodies conjugated to model toxin

[0427] The anti-Axl antibody of the present invention was conjugated to a model toxin (e.g., gemcitabine) to produce a conditionally active antibody-drug conjugate (CAB-Axl-ADC). The CAB-Axl-ADC was first tested to confirm that the conditional cell killing activity was not altered by the drug conjugation process. This test showed that the CAB-Axl-ADC killed significantly more cells at pH 6.0 than at pH 7.4 (FIG. 11).

[0428] The CAB-Axl-ADC was then injected into mice bearing MiaPaCa2 xenograft tumors at a dose of 1 mg/kg twice weekly for 3 weeks. Several controls were used in this study, including naked CAB (anti-Axl antibody with no conjugation), vehicle, the toxin alone (unconjugated gemcitable), control ADC, an affinity matching anti-Axl ADC (AM ADC). The study showed that the CAB-Axl-ADC (CAB ADC) and AM ADC provided a significantly greater reduction in the size of the tumor, in comparison with the controls (FIG. 12). The unconjugated anti-Axl antibody did not reduce the size of tumors. This study showed that the anti-Axl antibody conjugated with toxin is as effective in reducing tumor size as an affinity matching antibody.

Example 7: Serum concentrations of anti-Axl antibody drug conjugates in *cynomolgus macaque* monkeys

[0429] A drug (duomycin) conjugate of the anti-Axl antibody (CAB-ADC) of the present invention was injected into male and female *cynomolgus macaques* monkeys at three doses: 0.1, 1, and 10 mg/kg. Naked anti-Axl antibody was used as a control. The affinity matching antibody drug conjugate (AM-ADC) was also used as a control. The serum concentrations of the antibody were measured over a period of one week (168 hours, see FIGS. 13A-13B). The CAB-ADC persisted in the monkey serum longer than the AM-ADC control (FIG. 13B). There was no significant difference between the male and female monkeys (FIGS. 13A-13B).

Example 8: Toxicity of anti-Axl antibody drug conjugates in *cynomolgus macaque* monkeys

[0430] The toxicity of the CAB-ADC of the present invention tested in *cynomolgus macaque* monkeys. Aspartate transaminase (AST) and alanine transaminase (ALT) have been used as indicators of liver toxicity of drugs by the Food and Drug Administration (FDA). The serum AST and ALT levels were measured in both the male and female monkey (FIGS. 14A-14B). The vehicle (PBS) caused no AST or ALT level alteration in the serum, while the matching antibody drug conjugate (AM) showed very high AST and ALT levels at 3 days post 10 mg/kg dose. The CAB-ADC showed significantly reduced AST and ALT levels, in comparison with the AM control. This indicated that the anti-Axl antibody drug conjugates of the present invention had significantly reduced liver toxicity relative to the matching antibody drug conjugate AM.

[0431] The anti-Axl antibody drug conjugate of the present invention was also found to cause less inflammation in the monkeys (FIG. 15). The counts of lymphocytes in the blood of the monkeys after injections of CAB, AM and PBS were collected. In comparison with AM which caused significant inflammation, the anti-Axl antibody drug conjugate of the present invention (CAB-ADC) caused only mild inflammation in the monkeys.

Example 9: *In vivo* experiments in mice

[0432] Mice were implanted with one of 2 tumor cell lines (LCLC103H or DU145) that would develop into tumors. The tumor size after treatment with the antitumor drug monomethyl auristatin E (MMAE) was measured. For the mice receiving LCLC103H, the mice were treated with a single dose of vehicle (as negative control), CAB anti-Axl antibody conjugated MMAE ADC (CAB Axl-MMAE), or non-CAB anti-Axl antibody conjugated

MMAE ADCC (non-CAB Axl-MMAE), FIG. 16A. Tumors in mice treated with ADC shrank while the tumors in mice treated with vehicle continued growing.

[0433] Further, mice receiving DU145 were treated with vehicle (negative control) or CAB anti-Axl antibody conjugated MMAE ADC (CAB Axl-MMAE) at two different concentration (6 mg/kg and 10 mg/kg). Tumor volume was measured over time. The tumors continued growing in the negative control group (vehicle) while the tumor growth slowed in the mice treated with the ADCs (FIG. 16B).

Example 10: Tumor Membrane P score

Procedure

[0434] Immunohistochemical (IHC) staining for Axl (a cell surface receptor tyrosine kinase) used monoclonal Mouse IgG clone 7E10 from Lifespan Biosciences (Cat # LS-B6124) for detection of Axl in formalin-fixed, paraffin-embedded (FFPE) tissues.

[0435] IHC staining for CD68 used a standard mouse monoclonal antibody (clone KP1) from Dako (Cat # M0814) for detection of macrophages (Table 13). The IHC procedure used the protocol are described below on TechMate staining platform.

[0436] • **Step 1**: FFPE tissue blocks were cut at 4-5 μm thickness and sections mounted onto positively-charged, capillary gap glass slides. Slides were baked (60°C, dry heat) prior to use.

Slide Preparation

[0437] a. Microtomy was performed. Four to five-micron (4-5 μm) sections were mounted onto Fisher Biotech 22-230-900 Probe-On Plus microscope slides.

[0438] b. Slides baked for at least 1 hour at 60°C (dry-heat) and allowed to cool a minimum of 15 min at room temperature prior to initiation of Step 2.

[0439] • **Step 2**: Tissue sections are de-waxed using organic solvents (xylene, 100%, four changes) and an alcohol series (100%, 70%, 30% ethanol) descending to distilled water to sufficiently hydrate the tissues and allow proper binding of the primary antibody and other detection reagents.

De-wax/Pre-Antigen Retrieval

a. Four (4) changes of room temperature (25°C) absolute xylene for 5min each [no agitation]

b. Two (2) changes of room temperature (25°C) absolute alcohol for 2min each [no agitation]

c. Two (2) changes of room temperature (25°C) 70% alcohol for 2min each [no agitation]

d. Two (2) changes of room temperature (25°C) 30% alcohol for 2min each [no agitation]

e. Two (2) changes of room temperature (25°C) distilled water for rinsing [min. 16 dips in-out]

f. Slides immersed in room temperature (25°C) distilled water (transfer to antigen retrieval)

[0440] • Step 3: Antigen retrieval was performed after tissue sections were dewaxed. This step used a steam heat induced epitope recovery (SHIER) solution that was drawn into the capillary gap formed between paired microscope slides with a commercial steamer (20 minutes above 97°C) as a heat source (for description please see Ladner *et al*, Cancer Res.; vol. 60, pp 3493-3503, 2000).

Steam Heat Antigen Retrieval

a. Commercial steamer pre-heated above 98°C

b. Heat induced antigen/epitope retrieval using SHIER 2 (Dako S1700, citrate-based, pH 6.0-6.2) for Axl and SHIER 1 (QualTek formulation, citrate-based, pH5.6-6.1) for CD68 above 98°C for 20min (Black & Decker HS1000 model steamer or equivalent). Up to ten (10) slides were face-paired with clean blank slides in TechMate reagent trays containing exactly 10mL antigen/epitope retrieval solution to capillary draw reagent up and over the tissues.

c. Post-retrieval cool for 5min, slide pairs firmly inserted into a TechMate slide holder and drained of SHIER 1/SHIER 2 with an absorbent wick pad.

d. Wash two (2) times manually using capillary action (drain-draw) with Tris-buffered saline containing 0.02% v/v Tween-20 detergent (TBST, formulated as 20X stock solution by QualTek according to SOP MFB003 & used as 1X solution following dilution with distilled/deionized water [stored 4°C]).

[0441] • Step 4: Samples were tested by IHC according to the general procedure outlined in Table 6 using the TechMate instrumentation platform and the MIP program (which does not include enzymatic digestion) or the MIPE program (which includes digestion with Proteinase K at a 1:40 dilution). Sequential detection of antibodies is employed during IHC with a high level of specificity for the antigen or for the primary antibody. The location of the primary antibody is ultimately visualized by the application of a colorimetric chromogen (DAB) that precipitates a discrete insoluble reaction product at the site of antigen in the presence horseradish peroxidase (HRP). Nuclei are counterstained using hematoxylin (blue stain) to assess cell and tissue morphology.

Table 6

TechMate Sequence	Reagent
1	Proteinase K – 10 min (MIPE protocol only)
2	Blocking Reagent – 15 min
3	Primary Antibody – 1 hour
4	Secondary Antibody – 25 min
5	Hydrogen Peroxide Block – 3 x 2.5 min
6	Conjugated Horseradish Peroxidase (HRP) – 25 min
7	DAB Chromogen – 3 x 5 min
8	Hematoxylin Counterstain – 1 min

Immunohistochemistry

[0442] *Mouse Polink2+ HRP reagents (Golden Bridge International [GBI]; Cat #: D37-110) are stored ready-to-use at 2-8°C, with all procedures below automated at room temperature (25°C) on the TechMate running QualTek MIPE Procedure. Reagent changes (washes, incubations) take place by capillary action (drain-draw) using absorbent wick pads (drain) and TechMate reagent trays (draw)*

a. Wash three (3) times with TBST.

b. TBST for Axl and Proteinase K digestion (1:40 dilution, Dako, Cat #: S3020), 10 min for CD68.

c. Wash three (3) times with TBST.

d. Goat Blocking Reagent (QML), 15min.

e. Wash one (1) time with TBST.

f. Axl (1:1500) antibody clone 7E10 (LifeSpan BioSciences Cat #: LS-B6124) or CD68 (1:7500) antibody clone KP1 (Dako Cat #: M0814), freshly diluted in QualTek reagent manufacturing buffer (RMB: 0.01M Phosphate, 0.151M NaCl, 1% w/v BSA, 0.1% v/v ProClin 300, 0.2% v/v Tween-20, 1% v/v normal goat serum, pH 7.2, as formulated by QualTek according to SOP MFB002) from 1:10 working stock (held 4°C, also in RMB) for an hour.

g. Wash five (5) times with TBST.

h. Mouse Polink2+ secondary (part of GBI Kit Cat #: D37-110), 25min.

i. Wash two (2) times with TBST wash buffer.

j. Peroxidase block (3% USP H2O2, with ~0.02% v/v Tween-20 added), 3X 2.5min (7.5min total) with intervening reagent drain.

k. Wash three (3) times with TBST.

l. Mouse Polink2+ HRP conjugated polymer (part of GBI Kit Cat #: D37-110), 25min

m. Wash five (5) times with TBST.

n. GBI (Cat #: C09-12) DAB Chromogen (reagent made freshly at conclusion of polymer incubation, using 40µl DAB chromogen concentrate per 1mL supplied substrate buffer), 3X 5min (15min total) with intervening reagent drain and one (1) wash in TBST.

o. Wash four (4) times with TBST

p. Hematoxylin counterstain (1:5), 1min

q. Wash six (6) times with TBST.

r. Slides immersed in room temperature (25°C) distilled water (transfer to coverslip area).

[0443] • **Step 5**: Slides were unpaired, rinsed in distilled water, dehydrated in an alcohol series (70%, 95%, 100% ethanol) and in organic solvent (xylene, 100%, four changes), then permanently coverslipped, using CytoSeal (or equivalent), for interpretation and storage. Slides were examined under a microscope to assess staining.

[0444] SHIER 2 (Citrate-based, pH 6.0-6.2) solution was used for unmasking the epitopes of Axl in FFPE tissues. SHIER 1 (Citrate-based, pH 5.6-6.1) solution was used for unmasking the epitopes of CD68. After heat induced epitope retrieval, the process steps were automated with TechMate Instrument (Roche Diagnostics) running QML workmate software v3.96. This automated platform uses a capillary gap process for all reagent changes, up to and including counterstaining, and intervening buffer washes. All steps were carried out at room temperature (25°C).

[0445] Reagent Manufacturing Buffer [RMB; made by QualTek's Santa Barbara lab (QML-SB)] with Goat Serum was used to prepare working dilutions of primary antibodies and negative control antibodies. Target recognition for Axl and CD68 at the site of antigen-primary antibody interaction in FFPE sections used reagents from Polink-2 Plus HRP kits from GBI Labs designed for detection of Mouse primary antibodies. Refer to Table 7 for antibody specifications and optimized IHC assay conditions for Axl and CD68.

Dehydration/Coverslipping

a. Two (2) changes of room temperature (25°C) 95% alcohol for rinsing [min. 16 dips in-out].

b. Six (6) changes of room temperature (25°C) absolute alcohol for rinsing [min. 16 dips in-out].

c. Four (4) changes of room temperature (25°C) absolute xylene for rinsing [min. 16 dips in-out].

d. Coverslip with Thermo Scientific 8312 Cytoseal XYL or equivalent non-aqueous semi-permanent mounting media.

Table 7

Antibody	**AXL**	**CD68**
Source	Lifespan Biosciences	Dako
Catalog No.	LS-B6124	M0814
Vendor Lot No.	50923	20058801
QualTek Lot No.	NT2751	NT2771
Species/Isotype	Mouse IgG1	Mouse IgG1
Clone	Monoclonal/7E10	Monoclonal/KP1
Immunogen	Extracellular fragment of human AXL	Human CD68
Suggested Dilution	1:1500	1:7500
Incubation Time	1 hour	1 hour
Pre-treatment	SHIER , No Enzyme	SHIER 1 + Proteinase K (1:40)
TechMate Protocol	MIP	MIPE
Detection System	Polink-2 Plus HRP Mouse	Polink-2 Plus HRP Mouse
Chromogen	DAB	DAB

Internal Process Controls

[0446] Species-match positive controls (standard antibodies) with established signal strength in control tissues were used in each run to confirm proper detection reagent performance. The positive control used throughout the duration of this project was LCA (derived in Mouse)

run on formalin-fixed, paraffin-embedded (FFPE) control tonsil tissues or CK (cytokeratin) (derived in Mouse) run on FFPE colon cancer control tissues.

[0447] Lung cancer multi-tissue blocks (QMTB246, QMTB395) from a tissue bank that include a range of Axl expression levels were used as positive controls for Axl and CD68 in each IHC run. Mouse IgG1 isotype-match negative controls for the corresponding biomarker assay conditions were used to determine any nonspecific staining inherent in the detection reagents or tissues and to define any potential background reactivity from these sources.

Test Tissues

[0448] For assay concordance between laboratories, a total of 13 different formalin-fixed, paraffin embedded (FFPE) Lung Cancer (Non-Small Cell Lung Carcinoma or NSCLC) tissues from a tissue bank. For CLIA sensitivity testing, Axl and CD68 testing was evaluated in FFPE tissue samples for the following cancer indications: Melanoma (31 untreated, 16 previously-treated), Ovarian Cancer (52 untreated), Pancreatic Cancer (31 untreated, 2 previously-treated), Lung Cancer (43 untreated, 1 previously-treated), and Prostate Cancer (51 untreated).

[0449] All untreated samples were from a tissue bank. All previously-treated samples were supplied by BioAtla. Detailed information on each sample is included in the sensitivity scoring table in the Results section. A subset of these cancer samples was used for validation testing of the Axl and CD68 IHC assays in the Melanoma, Ovarian Cancer, Pancreatic Cancer, and Lung Cancer indications.

[0450] For Axl and CD68 specificity testing, FDA multi-normal human tissue microarray (TMA) slides from Pantomics, Inc (Cat # MNO961) were obtained. The TMA (designated as P1478Q0035) contained 96 different samples derived from 35 different organs or sites.

Scoring Scheme

[0451] Comparison scoring of Axl and CD68 staining in serial sections of each sample was performed as described in the Plasma Membrane Scoring of Axl in Tumor (Tumor Membrane P Score) section above.

Results

[0452] Axl and CD68 IHC Assay Concordance (Part A)

[0453] A total of 13 different FFPE lung cancer samples of non-small cell lung carcinoma (NSCLC) samples were used for concordance testing between laboratories. The NSCLC samples represented a range of Axl plasma membrane tumor cell staining.

[0454] The tissues for concordance testing were stained using the non-GLP Axl and CD68 IHC assays at a facility. They were also stained as serial sections (one section of tissue per

slide with minimal loss of material between preparations) at a second facilty by a different operator using the assays described in Table 7 and above. All assay testing was performed using a TechMate automated staining platform.

[0455] For Axl, scoring was performed by recording the percentage of tumor cells with plasma membrane staining at differential intensities from 0-3+ as described herein. Comparisons between the same samples run at different laboratories were made based on H-Scores [(% at <1) x 0] + [(% at 1+) x 1] + [(% at 2+) x 2] + [(% at 3+) x 3]. For CD68, scoring was performed by recording the percentage of tumor cells (0-100%) that are comprised of CD68-positive macrophages (% in Tumor).

[0456] When comparing Axl H-Scores and CD68 scores for Percent in Tumor between samples stained at different laboratories, the following concordance parameters were set: scores within +/- 20% of each other are deemed concordant; and 85% of the samples tested must be concordant for approved assay transfer. Under these conditions, acceptable concordance was noted throughout the set of NSCLC tissues when run at the two different laboratories.

[0457] Scoring data that compared the NSCLC samples stained at each facility were obtained (Table 4). The percent change (if any) between Axl H-Scores and CD68 scores for Percent in Tumor for between each facility sample were obtained. A total of 13 samples were run at both laboratories. Of these 13 samples, all were considered concordant (within 20% change) for Axl, and all but 2 were concordant for CD68. This yielded 100% sample set concordance between laboratories for Axl and 85% for CD68.

[0458] Any non-concordant results for CD68 were explainable by low scores for percentage of tumor that is macrophages. When percentage scores are low, differences between scores in comparison slides translate into higher percent change values by nature of the test. For example, while a scoring difference of 5% to 2% between samples of the two facilities is small, it represents a large percent change using a 0-100 scale. Such samples were considered acceptable.

[0459] According to the assay transfer scoring data obtained and the established criteria for concordance, the Axl and CD68 assays were deemed successfully transferred.

[0460] Part B: Sensitivity Screening for Axl and CD68 Cancers

[0461] The optimized IHC assays for Axl and CD68 in Table 7 along with the general IHC procedure in Table 6 and reagents in Table 8 to screen serial sections (one section of tissue per slide with minimal loss of material between preparations) of human tumor tissues from different cancer indications.

Table 8

Reagent	Source or Vendor	Part # or Catalog #
Probe-On Plus Microscope Slides	Fisher	22-230-900
Ethanol	Fisher	A962P-4
Xylene	Fisher	X5-4
SHIER 1	QML-SB	310055
SHIER 2	Dako	S1699, S1700
Proteinase K	Dako	S3020
Goat RMB	QML-SB	400001
TBS Buffer	QML-SB	300010
Normal Goat Block	QML-SB	300003
Polink-2 Plus HRP Detection Kit Mouse	GBI Labs	D37-110
Hydrogen Peroxide	Supervalu (OTC)	Equaline
DAB	GBI Labs	C09-100
Hematoxylin	QML-SB	100005
Cytoseal 60	Thermo/Fisher Scientific	8310-4, 23-244256

[0462] The cancer indications and the number of samples analyzed in each for CLIA sensitivity screening were as follows:

[0463] Melanoma (31 untreated, 16 previously-treated), Ovarian Cancer (52 untreated), Pancreatic Cancer (31 untreated, 2 previously-treated), Lung Cancer (43 untreated, 1 previously-treated), and Prostate Cancer (51 untreated). All tissues for sensitivity testing were formalin-fixed, paraffin-embedded (FFPE) human cancer specimens. Treatment-naïve samples were from the QualTek tissue bank and previously-treated samples were supplied by BioAtla via OHSU (Table 9 below).

[0464] The sensitivities of the Axl and CD68 IHC assays were evaluated in the following indications: Melanoma (31 untreated, 16 previously-treated), Ovarian Cancer (52 untreated), Pancreatic Cancer (31 untreated, 2 previously-treated), Lung Cancer (43 untreated, 1 previously-treated), Prostate Cancer (51 untreated). All tissues were formalin-fixed, paraffin-embedded (FFPE) human cancer specimens. Treatment-naïve samples were from the QualTek tissue bank and previously-treated samples as described in Table 9 below.

Table 9

	Cancer	Prior Therapy
1	Bladder	Ipl/Nivo
2	Blader	Ipl/Nivo
3	Gastric	Ipl//Nivo
4	SCC NSCLC	Lenvatnib + pembrolizumab
5	Melanoma	Lenvatnib + pembrolizumab
6	Melanoma	Lenvatnib + pembrolizumab
7	Melanoma	Ipl/Nivo
8	Melanoma	Dabrafenib/trametnib, Ipl/Nivo
9	Melanoma	Dabrafenib/trametnib, Anti-PD-1
10	Melanoma	BRAF/MEK inhibitors, PD-1 inhibitor
11	Melanoma	Dabrafenib/trametnib
12	Melanoma	BRAF/MEK inhibitors, PD-1 inhibitor
13	Melanoma	Anti-PD-1
14	Melanoma	Nivolumab
15	Melanoma	Ipl/Nivo
16	Melanoma	Pembrolizumab
17	Melanoma	Pembrolizumab
18	Melanoma	Ipl/Nivo
19	Melanoma	Pembrolizumab, BRAF/MEK inhibitors
20	Melanoma	Dabrafenib/trametinib

[0465] Lung cancer tissues that showed a range of Axl reactivity in prior testing served as a positive control/quality control (QC) to demonstrate appropriate reactivity during the current tumor screen. Tonsil tissues served as a control for the CD68 macrophage biomarker. Standard species-match positive controls (Mouse CK) and isotype-match negative controls (Mouse IgG1) were included during testing and reacted as expected. Samples were also stained with hematoxylin and eosin (H&E) for morphological assessment to assist in scoring.

[0466] Axl is reactive in a subset of tumor cells and macrophages. In tumor cells, Axl reactivity is primarily localized to the plasma membrane but can also be present in the cytoplasm. Macrophages that express Axl can be present among tumor cells and within the stroma that interacts with tumor (tumor-associated stroma or tumor-stroma). Not all macrophages label with Axl. CD68 is expressed in all macrophages (within tumor and in the tumor-stroma) and is a standard biomarker for identification of this immune cell type.

[0467] All tissues in the tumor screen were evaluated. Axl plasma membrane staining in tumor was evaluated using Percent Scores [sum of percentages of intensities ≥1+, ≥2+, and ≥3+ with values ranging from 0 to 100] and H-Scores [sum of each percentage score (0-100%) multiplied by its corresponding intensity score (0, 1+, 2+, 3+) with values ranging from 0 to 300]. Because Axl is expressed in both tumor cells and macrophages, the scoring approach (as described in the Plasma Membrane Scoring of Axl in Tumor (Tumor Membrane P Score) section above) compared Axl reactivity to CD68 staining in serial sections. The CD68 biomarker was used to identify and "subtract out" macrophage staining in the Axl slides to obtain a "tumor-only" score for Axl that is exclusive of macrophages.

[0468] The scoring approach described herein also included recording values for the percentage of tumor with Axl cytoplasmic staining at an average intensity. Macrophages were evaluated for CD68 and Axl positivity within the tumor mass by estimating the percentage of tumor that was comprised of CD68 or Axl-positive macrophages. Macrophages were also evaluated within the tumor-stroma for the relative abundance of Axl and CD68 staining.

[0469] The Axl tumor screen was performed to understand the range of staining intensities and abundance (penetrance) of reactivity across a representative sample set from different cancer indications.

[0470] Scoring results for Axl and CD68 in evaluable cancer samples were obtained for Melanoma, Previously-Treated Melanoma, Ovarian Cancer, Pancreatic Cancer, Lung Cancer, and Prostate Cancer.

[0471] Many of samples tested in each cancer indication were negative for Axl plasma membrane tumor staining (100% of tumor cells at 0 staining intensity). However, for the Melanoma, Ovarian Cancer, Pancreatic Cancer, and Lung Cancer indications, examples of low, moderate, and high Axl plasma membrane staining were also observed among the samples screened.

[0472] Representative images of high or moderate Axl staining alongside CD68 staining in the corresponding tissue region were observed for Melanoma, for Previously-Treated Melanoma, for Ovarian Cancer, for Pancreatic Cancer, and for Lung Cancer.

[0473] For the Prostate Cancer indication, 51 samples were evaluated and only 1 of these showed Axl plasma membrane staining above zero. A representative image of negative staining for Axl in Prostate Cancer alongside the CD68 biomarker was observed. Because the Prostate Cancer indication was highly nonreactive, it was not included in subsequent precision and reproducibility testing for Axl validation.

[0474] Mouse IgG1 isotype-match negative controls were included on each tissue sample tested in the sensitivity screen. Staining with these controls was nonreactive.

H-Score and Percent Scroe Analysis for Sensitivity Screen

[0475] The sensitivity screen for Axl (in conjunction with CD68) is intended to assiste in determining a cut-off for Axl positivity for use in clinical testing. To aid in comparative evaluation of Axl plasma membrane expression in tumor (exclusive of macrophages), the scoring data was divided according to different theoretical thresholds of positivity.

[0476] For this analysis, the evaluable Previously-Treated Melanoma samples (n=16) were grouped and analyzed separately from the Treatment-Naïve Melanoma samples from the QualTek tissue bank (n=31). The Previously-Treated Pancreatic Cancer (n=2) and Lung Cancer (n=1) samples were excluded from the sensitivity summary because there were too few samples to comprise their own groups. They were not included among the QualTek tissues for these indications because they were not treatment-naïve.

[0477] Table 10 presents the number and percent of cases in each cancer indication that met the following H-Score cut-offs for Axl plasma membrane tumor staining: ≥1, ≥50, ≥100, ≥150, ≥200, ≥250. Table 10 also includes average H-Scores across the samples tested in each indication. These average H-Scores for Axl reactivity were also compared in the bar graph shown in FIG. 21.

Table 10

Axl Plasma Membrane Reactivity Grouped by H-Score Thresholds

Breakdown of AXL Plasma Membrane Tumor Reactivity Exclusive of Macrophages by H-Score														
Tumor Type	Evaluable Cases	H-Score ≥1		H-Score ≥ 50		H-Score ≥ 100		H-Score ≥ 150		H-Score ≥ 200		H-Score ≥ 250		Average H-Score
		No. Cases	Pct. of Indication	No. Cases	Pct. of Indication	No. Cases	Pct. of Indication	No. Cases	Pct. of Indication	No. Cases	Pct. of Indication	No. Cases	Pct. of Indication	
Melanoma	31	4	13%	1	3%	1	3%	1	3%	1	3%	0	0%	7.4
Melanoma-Treated	16	5	31%	3	19%	2	13%	1	6%	0	0%	0	0%	22.3
Ovarian Cancer	52	10	19%	3	6%	2	4%	1	2%	1	2%	0	0%	9.0
Pancreatic Cancer	31	6	19%	4	13%	2	6%	0	0%	0	0%	0	0%	13.2
Lung Cancer	43	12	28%	3	7%	3	7%	1	2%	1	2%	0	0%	12.8
Prostate Cancer	51	1	2%	1	2%	0	0%	0	0%	0	0%	0	0%	1.8

[0478] According to the H-Score analysis, all indications were very low for Axl plasma membrane staining in tumor (average indication H-Scores <25). However, the highest overall reactivity was observed in the Previously-Treated Melanoma cases (including chemo, IO, and TKI therapies) versus Treatment-Naïve Melanoma and all other untreated indications.

[0479] Axl plasma membrane expression in tumor by Percent Score was also evaluated. Summary tables for Percent Score analyses are provided as follows:

[0480] Table 11 presents the number and percent of positive cases in each cancer indication when considering intensity of 1+, 2+ or 3+ (≥1+) in various proportions of tumor.

Table 11

Axl Reactivity Grouped by Thresholds ≥1+ Staining

Breakdown of AXL Plasma Membrane Reactivity Exclusive of Macrophages at Intensities of ≥ 1+													
Tumor Type	Evaluable Cases	≥ 1+ Staining in ≥1% Tumor Cells		≥ 1+ Staining in ≥ 10% Tumor Cells		≥ 1+ Staining in ≥ 25% Tumor Cells		≥1+ Staining in ≥50% Tumor Cells		≥ 1+ Staining in ≥ 75% Tumor Cells		≥ 1+ Staining in ≥90% Tumor Cells	
		No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication
Melanoma	31	4	13%	2	6%	1	3%	1	3%	1	3%	1	3%
Melanoma-Treated	16	5	31%	4	25%	2	13%	2	13%	0	0%	0	0%
Ovarian Cancer	52	10	19%	5	10%	2	4%	2	4%	1	2%	0	0%
Pancreatic Cancer	31	6	19%	6	19%	4	13%	3	10%	0	0%	0	0%
Lung Cancer	43	12	28%	6	14%	3	7%	3	7%	1	2%	1	2%
Prostate Cancer	51	1	2%	1	2%	1	2%	1	2%	0	0%	0	0%

[0481] Table 12 presents the number and percent of positive cases in each cancer indication when considering intensity of 2+ or 3+ (≥2+) in various proportions of tumor.

Table 12

Axl Reactivity Grouped by Thresholds ≥2+ Staining

Breakdown of AXL Plasma Membrane Reactivity Exclusive of Macrophages at Intensities of ≥ 2+													
Tumor Type	Evaluable Cases	≥ 2+ Staining in ≥1% Tumor Cells		≥ 2+ Staining in ≥ 10% Tumor Cells		≥ 2+ Staining in ≥ 25% Tumor Cells		≥2+ Staining in ≥50% Tumor Cells		≥ 2+ Staining in ≥ 75% Tumor Cells		≥ 2+ Staining in ≥90% Tumor Cells	
		No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication
Melanoma	31	4	13%	2	6%	1	3%	1	3%	1	3%	1	3%
Melanoma-Treated	16	5	31%	4	25%	2	13%	1	6%	0	0%	0	0%
Ovarian Cancer	52	10	19%	5	10%	2	4%	2	4%	0	0%	0	0%
Pancreatic Cancer	31	5	16%	5	16%	2	6%	1	3%	0	0%	0	0%
Lung Cancer	43	9	21%	5	12%	3	7%	1	2%	1	2%	1	2%
Prostate Cancer	51	1	2%	1	2%	1	2%	0	0%	0	0%	0	0%

[0482] Table 13 presents the number and percent of positive cases in each cancer indication when considering intensity of 3+ (≥3+) in various proportions of tumor.

Table 13

Axl Reactivity Grouped by Thresholds ≥3+ Staining

Breakdown of AXL Plasma Membrane Reactivity Exclusive of Macrophages at Intensities of ≥ 3+												
Tumor Type	Evaluable Cases	≥ 3+ Staining in ≥1% Tumor Cells		≥ 3+ Staining in ≥ 10% Tumor Cells		≥ 3+ Staining in ≥ 25% Tumor Cells		≥3+ Staining in ≥50% Tumor Cells		≥ 3+ Staining in ≥ 75% Tumor Cells		≥ 3+ Staining in ≥90% Tumor Cells	
		No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication
Melanoma	31	1	3%	0	0%	0	0%	0	0%	0	0%	0	0%
Melanoma-Treated	16	4	25%	3	19%	1	6%	0	0%	0	0%	0	0%
Ovarian Cancer	52	8	15%	4	8%	1	2%	1	2%	0	0%	0	0%
Pancreatic Cancer	31	2	6%	2	6%	1	3%	0	0%	0	0%	0	0%
Lung Cancer	43	6	14%	4	9%	2	5%	0	0%	0	0%	0	0%
Prostate Cancer	51	1	2%	1	2%	0	0%	0	0%	0	0%	0	0%

[0483] If using the criteria of ≥1+ Axl staining in ≥1% of tumor cells as a cut-off for positivity, the following number and percentage of cases for each indication would be considered positive: Melanoma- 4/31 (13%), Previously-Treated Melanoma- 5/16 (31%), Ovarian Cancer- 10/52 (19%), Pancreatic Cancer- 6/31 (19%), Lung Cancer- 12/43 (28%), and Prostate Cancer- 1/51 (2%). Other positivity thresholds/cut-offs were considered using Tables 10-13.

[0484] Although Axl is primarily expressed in tumor cells on the plasma membrane, it can also localize to the cytoplasm. In many cases, cytoplasmic Axl reactivity in tumor was absent or weak (0 or 1+ intensity). However some samples with strong (2+ or 3+ intensity) Axl expression in tumor cytoplasm were observed. Such staining may be a significant measure of Axl expression.

[0485] The tumor screen samples that showed Axl plasma membrane *or* cytoplasmic expression above zero were observed. These samples included samples without Axl plasma membrane staining (100% at 0), but with cytoplasmic staining of ≥10% at ≥2+. These samples represented cases without Axl plasma membrane reactivity but with significant Axl cytoplasmic staining. Such cases could be considered for inclusion criteria when determining Axl positivity.

Specificity Testing of Axl and CD68 in Normal Tissues (Part C)

[0486] the specificity Axl and CD68 to their targets using the IHC methods described in Table 6 and Table 7. Specificity testing was performed using 96 different tissues from an FDA-recommended multi-normal human tissue microarray (TMA). The TMA (P1478Q0035) was purchased from Pantomics, Inc (Cat # MNO961, multi-normal human tissues, FDA, 96 samples, 35 organs/sites from 3 individuals, 1.5mm) and as fully described in Table 9. Sections of all normal tissue samples were histology-stained with hematoxylin and eosin (H&E) and IHC-stained with Axl, CD68, and Mouse IgG1.

[0487] All stained normal tissues were assessed using the H-Score method for Axl plasma membrane staining described in the Tumor Membrane P Score section above to evaluate normal tissue components (as opposed to tumor). Scoring data for this specificity test also included estimated abundance (0-3) of macrophages throughout each normal tissue using the CD68 biomarker. Specificity data for Axl and CD68 in normal tissues was obtained.

[0488] Axl showed nonreactive plasma membrane staining (100% at 0) in all normal human tissues tested; except for reactivity in Sertoli cells in normal testis samples. As such, this specificity testing suggested that the Axl antibody and IHC test was specific to targets in tumor cells and in a subset normal cells of testis.

[0489] CD68 staining represented a range of macrophage abundance throughout the normal tissues tested. Axl and CD68 expression in normal tissues was observed. The Mouse IgG1 isotype-match negative control was nonreactive in all normal tissues tested.

Axl and CD68 Precision and Reproducibility Testing (Part D)

[0490] The results from the sensitivity screen helped identify appropriate tissues for testing the precision and reproducibility of the Axl and CD68 IHC assays in the Melanoma, Ovarian Cancer, Pancreatic Cancer, and Lung Cancer indications. Prostate Cancer was not included for validation because the indication was almost completely negative for Axl plasma membrane reactivity in tumor cells (50/51 samples showing 100% tumor staining at 0 intensity).

[0491] For the validation in Melanoma, Ovarian Cancer, Pancreatic Cancer, and Lung Cancer, 4 tumor samples per indication that showed showing a range of Axl plasma membrane staining in tumor cells were selected for use. Samples were chosen based on Axl expression and were run in serial with the CD68 biomarker to validate the combined test. Because the samples run with CD68 had to be the same as those selected for Axl, a range of CD68 staining was not necessarily observed within each indication but was reflected throughout the entire set.

[0492] Each sample for each indication was run in triplicate for both Axl and CD68 according to the IHC assays in Table 7 and the protocol described above in a single run (precision). In two separate runs, performed on non-consecutive days, the same samples were run in triplicate with Axl and CD68 by both the same and a different operator (reproducibility). All replicate slides were prepared as serial sections (one section of tissue per slide with minimal loss of material between slide preparations).

[0493] In other words, intra-assay (precision) and inter-assay (reproducibility) was determined using a 3-run series with 3 replicate sections (per run) of each of the 4 selected tumor samples for Axl and CD68, resulting in a set of 9 replicates for each sample. Two operators ran the assays using different TechMate instruments (Operator 1, Run 1; Operator 1, Run 2; Operator 2, Run 3). Positive, standard, and negative controls included in each run reacted as expected.

[0494] All replicates stained during validation were reviewed and scored. Axl plasma membrane staining in tumor was evaluated using Percent Scores ≥1+. For the precision and reproducibility test, a sample with 10% or more tumor cells staining at ≥1+ intensity (Percent Score ≥1+ that is ≥10) was called positive (POS). A sample was negative (NEG) if staining was present at ≥1+ in 0-9% of tumor cells or if only <1+ staining was observed. CD68 was

evaluated for the estimated percentages of macrophages in tumor as described in the Tumor Membrane P Score section above.

[0495] The replicates for precision and reproducibly testing were deemed acceptable by the pathologist as compared to the sections stained with Axl and CD68 during sensitivity screening for each sample. Full validation scoring results, including statistical analysis and for replicates, were obtained for Melanoma, for Ovarian Cancer, for Pancreatic Cancer, and or Lung Cancer.

[0496] Similar cellular patterns of Axl reactivity were observed in all replicates in the pattern, percent, and intensity of tumor staining. The same percentage of CD68 staining in tumor was also estimated in each replicate. This consistency was observed for Axl and CD68 in for Melanoma, for Ovarian Cancer, for Pancreatic Cancer, and for Lung Cancer. Corresponding Mouse IgG1 negative controls were provided for each of the indications.

Statistical Analysis and Confidence Interval Assessment

[0497] Acceptance/rejection of IHC assay validation was determined through evaluation of consistency in staining patterns, statistical analysis of semi-quantitative scores, and the percent of agreement/concordant estimates. Acceptance of the precision and reproducibility testing requirds that the lower bounds of the selected 95% confidence interval (CI), computed by percent agreement, meets or exceeds 85%. The general criteria also stated that the standard error of the mean (SEM) among replicates does not exceed 5, and the coefficient of variation (CV) does not exceed 20% (for samples with Percent Score values ≥10).

[0498] Summarized validation scoring results for Axl Percent Scores ≥1+ and CD68 percentage scores in tumor; including the mean, standard deviation (Std Dev), standard error of the mean (SEM), and coefficient of variation (CV) for each replicate set are presented in Table 14**.** A positivity cut-off for Axl of ≥10% tumor cells staining at ≥1+ intensity was applied to the evaluation of these samples for precision and reproducibly analysis.

Table 14

Validation Statistical Summary for Axl and CD68 in Cancer Indications

Precision & Reproducibility Statistics Summary									
Tissue Type	Q/MTB	AXL				CD68			
		% Score Mean	% Score Std Dev	% Score SEM	% Score CV	% Score Mean	% Score Std Dev	% Score SEM	% Score CV
Melanoma	Q2832	20.0	0.0	0.0	0%	5.0	0.0	0.0	0%
Melanoma	Q2834-01	0.0	0.0	0.0	0%	1.0	0.0	0.0	0%
Melanoma	Q2837	5.0	0.0	0.0	0%	20.0	0.0	0.0	0%
Melanoma-treated	P1478Q0005	70.0	0.0	0.0	0%	5.0	0.0	0.0	0%
Ovarian Cancer	QMTB310-2	0.0	0.0	0.0	0%	0.0	0.0	0.0	0%
Ovarian Cancer	QMTB310-4	70.0	0.0	0.0	0%	0.0	0.0	0.0	0%
Ovarian Cancer	QMTB310-5	10.0	0.0	0.0	0%	0.0	0.0	0.0	0%
Ovarian Cancer	Q3214-01	50.0	0.0	0.0	0%	0.0	0.0	0.0	0%
Pancreatic Cancer	Q9517 A	50.0	0.0	0.0	0%	0.0	0.0	0.0	0%
Pancreatic Cancer	Q9530 A	0.0	0.0	0.0	0%	0.0	0.0	0.0	0%
Pancreatic Cancer	Q9534 A	26.3	5.2	1.8	20%	0.0	0.0	0.0	0%
Pancreatic Cancer	Q9549 A	18.3	3.5	1.2	19%	0.0	0.0	0.0	0%
Lung Cancer	QMTB249-1	1.0	0.0	0.0	0%	10.0	0.0	0.0	0%
Lung Cancer	QMTB249-3	2.1	1.7	0.6	80%	0.0	0.0	0.0	0%
Lung Cancer	QMTB357-1	100.0	0.0	0.0	0%	50.0	0.0	0.0	0%
Lung Cancer	QMTB357-4	0.0	0.0	0.0	0%	0.0	0.0	0.0	0%

[0499] The SEM for each set of 9 replicates from the 4 cases tested for each biomarker did not exceed 1.8 and the CV did not exceed 20% (for cases with a Percent Score ≥1+ >5). The Axl Percent Score for Lung Cancer sample QMTB249-3 ranged from 1 to 5 among the replicates tested. This variation was caused by reduction in the cluster of reactive cells as the FFPE tissue block was serially sectioned. It resulted in a CV value of 80% but with a very low SEM of 0.6 and a Standard Deviation of only 1.7.

[0500] When Percent Scores are low, differences between replicates translate into higher CV values by nature of the test. In cases where Percent Scores are <10, the sample can be

considered acceptable. Sample QMTB249-3 was deemed acceptable and all other samples were within the defined limits.

[0501] Confidence interval assessment for Axl is shown in Table 15 and includes positive/negative staining agreement and mean, standard deviation, standard error of the mean (SEM), and pre-defined Z-value for the 95% confidence interval (CI). For this evaluation, the reference point used to calculate the CI was based on the staining result (positive or negative) for the majority of the Axl replicates. For example, 9/9 replicates for Melanoma sample Q2832, were positive for Axl. If a Q2832 replicate had been negative, it would have been against the majority and would have lowered the CI. However, no discordant results were found.

Table 15

Confidence Interval Validation Analysis for Axl in Cancer Indications

Precision & Reproducibility Confidence Interval Analysis								
Tissue Type	Q/MTB	AXL Pos/Neg Agreement	95% Confidence Interval					
			Total N	Mean	Std. Dev.	SEM	Z-Value	95% CI
Melanoma	Q2832	9/9	36	100%	0	0	1.96	0
Melanoma	Q2834-01	9/9						
Melanoma	Q2837	9/9						
Melanoma-Treated	P1478Q0005	9/9						
Ovarian Cancer	QMTB310-2	9/9	36	100%	0	0	1.96	0
Ovarian Cancer	QMTB310-4	9/9						
Ovarian Cancer	QMTB310-5	9/9						
Ovarian Cancer	Q3214-01	9/9						
Pancreatic Cancer	Q9517 A	9/9	35	100%	0	0	1.96	0
Pancreatic Cancer	Q9530 A	9/9						
Pancreatic Cancer	Q9534 A	8/8						
Pancreatic Cancer	Q9549 A	9/9						
Lung Cancer	QMTB249-1	9/9	36	100%	0	0	1.96	0
Lung Cancer	QMTB249-3	9/9						
Lung Cancer	QMTB357-1	9/9						
Lung Cancer	QMTB357-4	9/9						

[0502] In total, pair-wise comparisons from the Axl precision and reproducibility study resulted in 36 concordant and 0 discordant outcomes in Melanoma, Ovarian Cancer, and Lung Cancer. In Pancreatic Cancer, 35 concordant and 0 discordant outcomes were observed (one stained sample was not evaluable) (Table 15). As such, 107/107 tests for Axl agreed with the appropriate majority. Acceptance criteria were met for the Axl IHC assay with a 95% CI.

[0503] The Axl and CD68 IHC assays for detection in human Melanoma, Ovarian Cancer, Pancreatic Cancer, and Lung Cancer indications were considered successfully validated for use in clinical testing.

Example 11: ***In Vitro And In Vivo Activity of BA3011***

[0504] BA3011 binds to both human Axl and cynomolgus monkey Axl, but not mouse or rat Axl with high affinity and specificity in conditions mimicking the TME (pH 6.0–7.0) but had reduced binding in conditions mimicking the normal tissue environment (pH 7.3–7.4) (Stubbs 2000; Gillies 1994; van Sluis 1999; Estrella 2013; Anderson 2016). In *in vitro* functional assays, BA3011 demonstrated the ability to induce cellular toxicity of Axl expressing human cancer cell lines in the tumor conditions but had reduced cytotoxicity in normal conditions.

[0505] Anti-tumor efficacy of BA3011 was also demonstrated *in vivo* using human tumor cell line derived xenograft tumors expressing Axl in immune-deficient animals. Tumor cell lines representing NSCLC (LCLC103H), prostate (DU145), and pancreatic tumor (MIAPaCa2) were tested in this *in vivo* mouse model system. Once tumors were established at a size of about 150 mm^3, animals were treated with BA3011 at the indicated dose levels following the indicated schedule. The tumor growth inhibition (TGI) relative to control demonstrated for all tested models is shown in FIGS. 17A-17D. BA3011 induced greater than 70% TGI in 4 out of 7 models tested, as shown on FIG. 17D. BA3011 did not induce body weight loss at the dose and schedules tested, and no other clinical signs of toxicity were noted.

Example 12: ***In vivo*** **Toxicology and Pharmacokinetics**

[0506] The nonclinical intravenous (IV) toxicity of BA3011 was evaluated in the monkey in an initial dose-range finding study followed by a definitive Good Laboratory Practice repeat dose toxicology study. The cynomolgus monkey was chosen as the toxicology species, as BA3011 cross-reacted to the cynomolgus Axl protein, while BA3011 lacked binding to the

rodent enzyme. Many of the toxicities observed with BA3011 are similar to other ADCs conjugated to MMAE.

[0507] Administration of BA3011 to cynomolgus monkeys for a total of 2 doses on Days 1 and 22 by IV slow bolus injection at dosages of 1, 5, or 10 mg/kg/dose resulted in early deaths in females at 10 mg/kg/dose, adverse hematological changes at 5 and 10 mg/kg/dose in both sexes, and adverse pathological changes in lymphoid organs at 10 mg/kg/dose in males and 5 mg/kg/dose in females. No test article-related changes in body weight, electrocardiogram (ECG), ophthalmology, or urinalysis were observed at any of the dose levels tested in this study. Based on the results at the 5 and 10 mg/kg/dose, the no-observed-adverse-effect-level (NOAEL) of BA3011 was considered to be 1 mg/kg/dose for both males and females. At 1 mg/kg/dose, the group mean maximum plasma exposure (C_0) of BA3011 on Day 22 was 20.0 μg/mL and area under the concentration versus time curve $(AUC)_{0\text{-inf}}$ was 376 μg·h/mL. The highest non-severely toxic dose (HNSTD) was 5 mg/kg/dose, with C_0 on Day 22 at 111 μg/mL and $AUC_{0\text{-inf}}$ at 2370 μg·h/mL. Based on the projected exposures of the clinical starting dose (0.3 mg), the HNSTD in the monkey is 9.4-fold above the projected human $AUC_{\tau\tau}$ (253 μg·h/mL) at 0.3 mg, and 14-fold above the projected human maximum observed concentration (C_{max}), 7.87 μg/mL).

Example 13: Phase I/II study

[0508] This is a multi-center, open-label, Phase 1/2 study designed to evaluate the safety, tolerability, PK, immunogenicity, and antitumor activity of BA3011 alone and in combination with nivolumab, in adult and adolescent patients 12 years and older with advanced solid tumors.

[0509] Phase 1 will comprise 2 sequential parts—dose escalation and dose expansion—and is designed to evaluate the safety and tolerability of BA3011 in adult patients with advanced solid tumors and to identify the MTD and/or RP2D for BA3011.

[0510] Phase 2 is an open-label study to evaluate the efficacy and safety of BA3011 alone and in combination with nivolumab in adult and adolescent patients with advanced, refractory sarcoma. The study consists of a Screening Period (up to 28 days prior to first dose), a Treatment Period, an End-of-Treatment (EOT) Visit (at IP discontinuation or within 28 days after last dose of IP), and a Follow-Up Period (Follow-Up Visit 1 [3 months from the last dose of IP] and Follow-Up Visit 2 and beyond [every 3 months after Follow-Up Visit 1]).

Phase I:

Primary:

• To define the safety profile, including dose limiting toxicity (DLT), and determine the maximum tolerated dose (MTD) and/or recommended Phase 2 dose (RP2D) and other safety parameters for BA3011 in patients with advanced solid tumors.

Secondary:

• To assess the pharmacokinetics (PK) of BA3011.

• To assess the antitumor activity of BA3011.

• To evaluate the immunogenicity of BA3011.

Phase II Study

[0511] Primary:

• To assess antitumor activity of BA3011 alone and in combination with nivolumab.

• To assess the safety of BA3011 alone and in combination with nivolumab.

Secondary:

• To further characterize the clinical activity of BA3011 alone and in combination with nivolumab.

[0512] Exploratory:

• To assess the PK of BA3011 alone and in combination with nivolumab.

• To evaluate the immunogenicity of BA3011 alone and in combination with nivolumab.

• To explore the relationship between tumor Axl status and clinical response to BA3011.

• To evaluate potential candidate tumor and blood-based biomarkers for patient selection or correlation with antitumor activity of BA3011.

Primary Endpoints

Phase 1:

• Safety: DLTs, MTD and/or RP2D, adverse events (AEs), serious adverse events (SAEs), and changes from baseline in laboratory parameters and vital signs.

Phase 2:

• Efficacy: overall response rate (ORR).

• Safety: AEs, SAEs, and changes from baseline in laboratory parameters and vital signs.

Secondary Endpoints

Phase 1:

• PK of BA3011: plasma concentrations of ADC, total antibody and MMAE, and PK parameters, including C_{max} and AUC.

• Efficacy (expansion cohorts only): ORR, disease control rate (DCR), time to response (TTR), duration of response (DOR), best objective response (OR), progression-free survival (PFS), overall survival (OS), and percent change from baseline in tumor size.

• Immunogenicity of BA3011: the number and percentage of patients who develop detectable anti-drug antibodies (ADAs).

Phase 2:

• Efficacy:

- DOR
- PFS
- Best OR
- DCR
- TTR
- Progression-free rate (PFR) at 12 weeks
- OS
- Percent change from baseline in tumor size

Exploratory Endpoints

Phase 2:

• PK of BA3011: plasma concentrations of ADC, total antibody and MMAE, and PK parameters, including C_{max} and AUC.

• Immunogenicity of BA3011: the number and percentage of patients who develop detectable ADAs.

• Relationship between tumor Axl status and clinical response to BA3011.

• Potential candidate tumor and blood-based biomarkers for patient selection or correlation with antitumor activity of BA3011.

Inclusion Criteria:

[0513] 1. Phase 1: Patients must have histologically or cytologically confirmed locally advanced unresectable or metastatic solid tumor and have failed available standard of care (SoC) therapy and for whom no curative therapy is available or who are not eligible, intolerant to or refuse standard therapy.

[0514] Phase 2: Patients must:

- Have histologically or cytologically confirmed locally advanced unresectable or metastatic sarcoma.
- Have documented progression according to Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1 criteria within the 6 months prior to enrollment.
- Be ineligible for chemotherapy or have received at least 1 regimen containing anthracycline and a maximum of 3 previous lines of systemic therapy for metastatic disease (no more than 2 lines of combination regimens), including pazopanib, trabectedin, eribulin mesylate, or tazemetostat, if applicable per regional prescribing information. The requirements for prior treatments do not apply to sarcoma subtypes for which no treatment is approved.

2. Patients must have measurable disease according to RECIST v1.1. Previously radiated tumor lesion should not be considered a target lesion.

3. Phase 1: Patients must be age ≥ 18 years.

Phase 2: Patients must be age ≥ 12 years.

4. Patients must have an ECOG performance status of 0 or 1.

5. Patients must have a life expectancy of at least 3 months.

6. Archived tumor tissue or tissue amenable to biopsy must be available to the Sponsor for Axl and other gene expression testing. All patients must consent to provide a pretreatment tumor specimen for biomarker studies. If archival tissue is unavailable, patients must consent to undergo a tumor biopsy during screening. Core needle (a minimum of 3 core samples are required) or excisional biopsies or resected tissue specimens are required.

7. In Phase 1 dose expansion and Phase 2, patients must have Axl-positive disease determined by BioAtla Axl IHC assay based on archival tissue or biopsy; a minimum of 3 core samples are required to ensure a sufficient quantity of cells are obtained. For Phase 1 dose expansion, the Axl expression cutoff is ≥ 1+ in ≥ 10% tumor cells. For Phase 2, a percent score greater than or equal to 70 (consisting of 1+, 2+, and 3+ intensities) is considered positive.

8. Patients must have:

- Completed (and recovered from treatment-related toxicities) any prior treatment with radiotherapy, chemotherapy, and/or treatment with other investigational anticancer agents at least 5 half-lives or 2 weeks, prior to first study dose, or biologics (such as a monoclonal antibody [mAb]) at least 4 weeks prior to first study dose. Exceptions are bisphosphonates, denosumab and gonadotropin-releasing hormone agonist or antagonist.

- Completed any prior treatment with nitrogen mustard agents, melphalan, or carmustine (BCNU) therapy at least 6 weeks prior to first study dose.
- Received any prior autologous hematopoietic stem cell infusion at least 8 weeks prior to first study dose.

9. Patients must have adequate organ functions. The following are required baseline laboratory values:

- Absolute neutrophil count $\geq$ 1,500/μL or 1.5 x 10^9/L.
- Platelets $\geq$ 100,000/μL or 100 x 10^9/L.
- Hemoglobin $\geq$ 9.0 g/dL.
- Bilirubin $\leq$ 1.5 x upper limit of normal (ULN).
- Serum creatinine $\leq$ 1.5 x ULN.
- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq$ 2.5 x ULN; ALT and AST $\leq$ 5 x ULN if metastasis in liver.

Phase 2 only:

- INR < 1.7 or prothrombin time < 4 seconds above control.
- Albumin > 3.5 g/dL.

10. Patients must be available for periodic blood sampling, study-related assessments and management of toxicity at the treating institution and be willing to comply with the expected drug administration schedule.

11. Females of childbearing potential must have a negative serum or urine pregnancy test result prior to the first dose of BA3011 and must agree to use an effective contraceptive method, either a barrier/intrauterine method or a hormonal method, during the course of the study.

- Females of non-child-bearing potential are those who are postmenopausal greater than 1 year or who have had a bilateral tubal ligation or hysterectomy.
- Both females and males, of childbearing/reproductive potential must agree to use effective contraception while included in the study and for 6 months after the last infusion of BA3011.

12. Patients or their legally acceptable representative or legal guardian(s) must provide written informed consent. Patient assent must be obtained for patients < 18 years old.

Exclusion Criteria:

1. Patients must not have clinically significant cardiac disease, in the judgment of the Investigator.

2. Patients must not have known congestive heart failure (New York Heart Association classes II-IV) or serious cardiac arrhythmia requiring treatment; patients with stable condition and medication for ≥ 3 months can be enrolled.

3. Patients with moderate (Child-Pugh B) or severe (Child-Pugh C) hepatic impairment. For Phase 1 only, patients with mild (Child-Pugh A) hepatic impairment, the initial BA3011 dose may not be greater than 1.2 mg/kg 1Q3W (Day 1) or 1.2 mg/kg 2Q3W (Days 1 and 8).

4. Patients with severe renal impairment (CrCL less than 30 mL/min).

5. Patients must not have known non-controlled central nervous system (CNS) metastasis.

6. Phase 1 only: Patients must not have received granulocyte colony stimulating factor (G-CSF) or granulocyte/macrophage colony stimulating factor support 2 weeks prior to first BA3011 administration.

7. Patients must not have a history of ≥ Grade 3 allergic reactions to mAb therapy as well as known or suspected allergy or intolerance to any agent given during this study.

8. Patients must not have had major surgery within 4 weeks before first BA3011 administration.

9. Patients must not have any history of intracerebral arteriovenous malformation, cerebral aneurysm, or stroke.

10. Patients must not have had prior therapy with a conjugated or unconjugated auristatin derivative/vinca-binding site targeting payload.

11. Patients must not have known additional malignancy that is active and/or progressive requiring treatment; patients with other malignancies that have been definitively treated and who have been rendered disease free will be eligible.

12. Patients must not have Grade 2 or higher peripheral neuropathy.

13. Patients must not have clinically significant (in the judgment of the Investigator) active viral, bacterial or fungal infection requiring systemic antibiotics/antivirals/antifungals.

14. Patients must not have known HIV, active hepatitis B, and/or hepatitis C.

15. Patients must not be women who are pregnant or breast feeding.

16. Patients must not be using concurrent therapy with other anti-neoplastic or experimental agents.

17. Phase 1 only: Patients must not be using concurrent therapy with corticosteroids at greater than or equal to 12 mg/day prednisone equivalent.

18. Patients must not be using moderate or strong CYP3A inducers or inhibitors, including cannabidiol.

19. Patients must not be using P-glycoprotein (P-gp) inhibitors.

20. Patients must not have any serious underlying medical condition that, in the opinion of the
Investigator or Medical Monitor, would impair their ability to receive or tolerate the planned treatment. In such cases, the Sponsor-designated Medical Monitor must review each case prior to patient enrollment.
21. Patients must not have any clinically significant pleural, pericardial, and/or peritoneal effusion (e.g., effusion affecting normal organ function and/or requiring percutaneous drainage or diuretic control).
22. Patients must not have any history of hepatic encephalopathy; any current clinically significant ascites, as measured by physical examination; or active drug or alcohol abuse.
23. Phase 2 only: To be eligible for the combination arm with nivolumab, patients must not have a history of interstitial lung disease, non-infectious pneumonitis, or uncontrolled diseases, including pulmonary fibrosis, acute lung diseases, etc.
24. Phase 2 only: To be eligible for the combination arm with nivolumab, patients must not have been administered a live vaccine ≤ 4 weeks before enrollment.
Note: Seasonal vaccines for influenza are generally inactivated vaccines and are allowed. Intranasal vaccines are live vaccines and are not allowed.
25. Phase 2 only: To be eligible for the combination arm with nivolumab, patients with an active, known, or suspected autoimmune disease are excluded. Patients with type 1 diabetes mellitus, hypothyroidism only requiring hormone replacement, skin disorders (such as vitiligo, psoriasis, or alopecia) not requiring systemic treatment, or conditions not expected to recur in the absence of an external trigger may be permitted to enroll.
26. Phase 2 only: To be eligible for the combination arm with nivolumab, patients must not have any condition that required systemic treatment with either corticosteroids (> 10 mg daily of prednisone or equivalent) or other immunosuppressive medication ≤ 14 days before enrollment.
Note: Patients who are currently or have previously been on any of the following steroid regimens are not excluded:
- Adrenal replacement steroid (dose ≤ 10 mg daily of prednisone or equivalent).
- Topical, ocular, intra-articular, intranasal, or inhaled corticosteroid with minimal systemic absorption.
- Short course (≤ 7 days) of corticosteroid prescribed prophylactically (e.g., for contrast dye allergy) or for the treatment of a non-autoimmune condition (e.g., delayed-type hypersensitivity reaction caused by contact allergen).

27. Phase 2 only: To be eligible for the combination arm with nivolumab, patients must not have had prior allogeneic stem cell transplantation or organ transplantation.

Phase 1 Dose Escalation (BA3011 alone; 21-day cycles)

[0515] During Phase 1 dose escalation, adult patients with advanced solid tumors are enrolled sequentially to receive BA3011 on 21-day cycles via IV infusion at the planned dose levels listed in Table 16. BA3011 is administered once (1Q3W) or twice (2Q3W) every 3 weeks, on Day 1 only or Days 1 and 8 of each 21-day cycle, respectively, via intravenous (IV) infusion according to the dose level assigned to each cohort. The starting dose of BA3011 is 0.3 mg/kg 1Q3W. In each cohort, the administration of the first dose of BA3011 is staggered by a minimum of 24 hours between the first and second patients treated.

Investigational products, dosage and mode of administration:

[0516] BA3011, dose escalation starting at 0.3 mg/kg 1Q3W (Phase 1), RP2D (Phase 2), IV infusion.

[0517] Nivolumab (Phase 2 only): For patients 18 years old and above: 240 mg Q2W; for patients 12-17 years old: 3 mg/kg Q2W IV infusion.

Duration of treatment:

[0518] Patients will be treated until disease progression, unacceptable toxicity, or other reason for treatment discontinuation.

Statistical methods:

Safety Analyses

[0519] MTD evaluation will be based on the DLT-evaluable Population. The DLT-evaluable Population includes all patients enrolled in Phase 1 dose escalation who receive at least 1 full assigned dose of BA3011 and complete the safety follow-up through the DLT-evaluation period (defined as the time period from the first dose of BA3011 through 21 days post Dose 1) or experience any DLT during the DLT-evaluation period. Non-DLT-evaluable patients will be replaced. The safety evaluation will be based on the As-Treated Population. Adverse events (AEs) will be coded by Medical Dictionary for Regulatory Activities (MedDRA) and graded according to the NCI CTCAE v4.03, and the type, incidence, severity, and relationship to each IP will be summarized. If any associations of interest between AEs and baseline characteristics are observed, additional stratified results may be presented. All treatment-emergent AEs will be summarized by MedDRA system organ class and preferred term. Laboratory abnormalities with toxicity grades according to the NCI CTCAE will be summarized.

Efficacy Analyses

[0520] The primary efficacy endpoint in Phase 2 is objective response (OR) per RECIST v1.1 using blinded independent central review (BICR). Objective response rate (ORR) is defined as the proportion of subjects with a best overall response of confirmed CR or confirmed PR that occur prior to the initiation of subsequent anticancer treatment. ORR will be estimated with a 95% CI using the exact probability method for each sarcoma subtype and overall in each of the 2 treatment groups (BA3011 alone and in combination with nivolumab). Other efficacy endpoints are duration of response (DOR), progression-free survival (PFS), best overall response (OR), disease control rate (DCR), time to response (TTR), progression-free rate (PFR) at 12 weeks, overall survival (OS), and percent change from baseline in target lesion sum of diameters. All efficacy endpoints except PFS and OS will be summarized primarily based on the full analysis set (FAS). PFS and OS will be summarized based on the As-Treated Population. Time-to-event data will be summarized using Kaplan-Meier estimates. Response rates and their confidence intervals will be estimated using the exact probability method. Graphical analyses will include spider and waterfall plots for the change and best change from baseline in tumor size, respectively.

[0521] During Phase 1 dose escalation, approximately 35 to 78 DLT-evaluable patients with advanced solid tumors are treated, depending on cohort expansion and tolerability of BA3011. Each cohort will require a minimum of 3 and up to 6 patients, except for the single-patient cohorts. A minimum of 6 patients are enrolled at the MTD.

Table 16

Modified-Fibonacci Method for BA3011 Dose Escalation

Cohort	Dose Level (mg/kg)	N	Increments
1	0.3, 1Q3W	3 up to 6	--
2	0.6, 1Q3W	1 up to 6	100%
3	1.2, 1Q3W	1 up to 6	100%
4	1.8, 1Q3W	3 up to 6	50%
5	2.4, 1Q3W	3 up to 6	33%
5A	1.2, 2Q3W	3 up to 6	--
5-int	2.7, 1Q3W	3 up to 6	10%
6[a]	3.0, 1Q3W	3 up to 6	25%
6A	1.5, 2Q3W	3 up to 6	--
6-int[b]	3.3, 1Q3W	3 up to 6	10%
7[c]	3.6, 1Q3W	3 up to 6	30%
7A	1.8, 2Q3W	3 up to 6	--
8A	2.0, 2Q3W	3 up to 6	--

Abbreviations: 1Q3W, once every 3 weeks; 2Q3W, twice every 3 weeks; int; intermediate.

[a] indicates MTD at the 2.4 mg/kg 1Q3W dose level. No additional patients will be dosed at and/or above 3 mg/kg, irrespective of neutropenia prophylaxis.

[b] and grayscale shading indicates no patients have been or will be dosed in Cohorts 6-int or 7 based on confirmation of the MTD.

[0522] Rules for dose escalation are provided in Table 17 and Figure 18 (dose escalation flow chart). At least 3 patients in a 3+3 cohort or 1 patient in a single-patient cohort must have completed 21 days of safety assessments in Cycle 1 (i.e., during the DLT evaluation period) before the next cohort initiates accrual. In the single-patient cohorts, 1 patient is enrolled only until Grade 2 or greater toxicity is observed, after which a total of at least 3 patients are evaluated at that dose and at all subsequent dose levels. In the 3+3 cohorts, if no DLTs are observed in the first 3 patients during the 21-day DLT-evaluation period, escalation continues to the next dose-level cohort. The criteria for DLTs are presented below. All available safety data from these patients, including toxicities occurring beyond Cycle 1, are reviewed prior to advancing to the next dose level. Escalation to the next specified dose level continues until the MTD is identified.

[0523] Enrollment in Phase 1 dose escalation cohorts proceed sequentially as shown in Figure 18 based on the dose escalation rules in Table 17. To define the MTD, patients are evaluated according to the actual starting dose of BA3011 during the first treatment cycle. The maximum administered dose (MAD) and the MTD are determined based on the incidence of DLTs. Maximum tolerated doses without and with co-administration of pegfilgrastim are explored. If ≥ 2 out of 6 patients in a cohort experience a DLT during the DLT-evaluation period, the MTD will be exceeded and no further patients will be enrolled into that cohort. The preceding cohort is then evaluated for the MTD and at least 6 patients will be treated at the preceding dose level. If ≤ 1 of 6 patients experiences a DLT at the preceding dose level, then this dose level is considered the MTD. A minimum of 6 patients are enrolled at the MTD. A dosing cohort may be discontinued and/or continued with a lower dose level, including lower doses not stated in the protocol, in the context of evolving safety and efficacy data. The MTD and/or RP2D are determined based on the totality of the data.

Table 17

Dose Escalation Rules for 3-Plus-3 Cohorts

0 in 3 patients	Proceed with enrollment in next cohort
1 of 3 patients	Enroll 3 additional patients in current cohort
≥ 2 of 3 patients	Do not escalate; enroll 3 additional patients in previous cohort and intermediate dose level cohort
1 of 6 patients	Proceed with enrollment in next cohort
≥ 2 of 6 patients	Do not escalate; enroll 3 additional patients in previous cohort and intermediate dose level cohort

[0524] Individual patients continue dosing with BA3011 until disease progression, unacceptable toxicity, or other reason for treatment discontinuation. Following the observation of dose-limiting neutropenia at higher BA3011 dose levels, administration of pegfilgrastim (or biosimilar) is required for patients in Phase 1 and must occur between 48 to 72 hours after every Day 1 infusion of BA3011.

Treatment Assignment

[0525] During Phase 1 dose escalation, patients are assigned to cohorts of escalating BA3011 dose levels as outlined in Table 17 and Figure 19. BA3011 is administered via IV infusion once (1Q3W) or twice (2Q3W) every 3 weeks, on Day 1 only or Days 1 and 8 of each 21-day cycle, respectively, according to the dose level assigned to each cohort. The starting BA3011 dose level is 0.3 mg/kg 1Q3W. Escalation to the next specified dose level continues until the MTD is identified. A dosing cohort may be discontinued and/or continued with a lower dose level, including lower doses not stated in the protocol, in the context of evolving safety and efficacy data. The Phase 1 dose expansion determines the BA3011 dose and treatment schedule patients receive in Phase 2 (e.g., the RP2D) and is conducted in patients with Axl-expressing, advanced solid tumors. The RP2D is determined based on the totality of the data and not to exceed the MTD. Based on data from the Phase 1 part of the study, the dose of BA3011 for Phase 2 is 1.8 mg/kg Q2W. Rationale for the Q2W dosing regimen is based on evaluation of BA3011 alone and in combination with nivolumab using an every 2-week dosing schedule (28-day cycles; 2 doses per cycle). Based on the data available for BA3011 in the Q3W dosing cohorts, 1Q2W dosing offers several advantages. The 1Q2W dosing schedule may improve patient safety with a lower C_{max} over time. In addition, the increased duration between individual doses compared to the 2Q3W dosing on Days 1 and 8 provides more recovery time between doses and, as a result, may reduce the incidence of adverse events, particularly for those associated with decreased neutrophil counts and elevated liver enzymes. The 1Q2W dosing schedule may also demonstrate better efficacy by maintaining

higher levels of BA3011 throughout the cycle (i.e., higher C_{min}). Finally, the 1Q2W schedule reduces the number of visits required for patients in the combination therapy cohorts since it aligns with the standard Q2W (240 mg) dosing schedule for nivolumab.

[0526] For Phase 2, patients who meet enrollment criteria are assigned to receive BA3011 alone or in combination with nivolumab. Patients with tumors showing B cell infiltration (per CD20 IHC assay) are preferentially assigned to receive BA3011 in combination with nivolumab.

[0527] In case of allergy to pegfilgrastim, filgrastim (or biosimilar) may be substituted. Starting at Cycle 3, pegfilgrastim or filgrastim can be self-administered.

Dose-limiting Toxicity:

A DLT is defined as meeting 1 of the following criteria during the 21-day DLT evaluation period or a treatment-related toxicity of any grade requiring dose delay of 1 week or more between the first and second cycles:

• Non-hematologic Toxicity

- For non-hematologic toxicities, DLT will be defined as any National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v4.03 (published 14 Jun 2010) Grade 3 or greater toxicity at least possibly related to treatment, except for:
    - Grade 3 fatigue.
    - Grade 3 nausea and vomiting lasting less than 24 hours.
    - Grade 3 non-hematologic laboratory abnormalities that are asymptomatic and resolve to Grade 1 or baseline (if the patient entered the study with existing toxicity) within 14 days.
    - Grade 3 or 4 allergic or hypersensitivity reaction at first occurrence in the absence of prophylactic steroid that resolves in less than 6 hours with appropriate clinical intervention

• Hematologic Toxicity

- DLT for hematologic toxicity, using NCI CTCAE v4.03, will be defined as:
    - Grade 4 neutropenia lasting greater than 7 days.
    - Grade 3 or 4 febrile neutropenia.
    - Grade 4 platelet count (< 25,000/mm3) at any time.

In addition, clinically important or persistent toxicities that are not included above may also be considered a DLT following review by the PSC. In view of BA3011's potential for myelosuppression, it is particularly important that patients are not infused until any

neutrophil toxicity has resolved to at least Grade 1, or Grade 2 for patients who have received prophylactic pegfilgrastim.*Maximum Tolerated Dose*

[0528] Enrollment in Phase 1 dose escalation cohorts will proceed sequentially as shown in Figure 18 based on the dose escalation rules in Table 17. To define the MTD, patients will be evaluated according to the actual starting dose of BA3011 during the first treatment cycle. The maximum administered dose (MAD) and the MTD will be determined based on the incidence of DLTs. Maximum tolerated doses without and with co-administration of pegfilgrastim will be explored. If ≥ 2 out of 6 patients in a cohort experience a DLT during the DLT-evaluation period, the MTD will be exceeded and no further patients will be enrolled into that cohort. The preceding cohort will then be evaluated for the MTD, and at least 6 patients will be treated at the preceding dose level. If ≤ 1 of 6 patients experiences a DLT at the preceding dose level, then this dose level will be considered the MTD.

Phase 1 Dose Expansion (BA3011 alone; 21-day cycles)

The Phase 1 dose expansion assists in determining the RP2D (Section 3.1.2.1) and is conducted in approximately 30 adult patients with Axl-expressing, advanced solid tumors enrolled to receive BA3011 at a dose and regimen determined to be appropriate and that will not exceed the MTD. Intrapatient dosing may be modified discretionally. The number of patients with a specific tumor type can be limited to ensure sufficient representation of Axl-expressing solid tumors. Individual patients will continue dosing with BA3011 until disease progression, unacceptable toxicity, or other reason for treatment discontinuation.

Recommended Phase 2 Dose

[0529] The RP2D will be determined based on the totality of the data, and not to exceed the MTD. Based on data from the Phase 1 part of the study, the dose of BA3011 for Phase 2 is 1.8 mg/kg Q2W. The rationale for the Q2W dosing regimen is as follows.

[0530] BA3011 will be evaluated alone and in combination with nivolumab using an every 2-week dosing schedule (28-day cycles; 2 doses per cycle). Based on the data available for BA3011 in the Q3W dosing cohorts, 1Q2W dosing may provide several advantages. The 1Q2W dosing schedule may improve patient safety with a lower C_{max} over time. In addition, the increased duration between individual doses compared to the 2Q3W dosing on Days 1 and 8 provides more recovery time between doses and, as a result, may reduce the incidence of adverse events, particularly for those associated with decreased neutrophil counts and elevated liver enzymes. The 1Q2W dosing schedule may also demonstrate better efficacy by

maintaining higher levels of BA3011 throughout the cycle (i.e., higher C_{min}). Finally, the 1Q2W schedule reduces the number of visits required for patients in the combination therapy cohorts since it aligns with the standard Q2W (240 mg) dosing schedule for nivolumab.

Phase 2 (BA3011 alone or in combination with nivolumab; 28-day cycles)

[0531] Phase 2 is an open-label study to evaluate the efficacy and safety of BA3011 alone and in combination with nivolumab in adult and adolescent patients with Axl-expressing tumor membrane percent score (TmPS) $\geq$ 70, advanced, refractory sarcoma who have measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.1 criteria and have documented progression according to RECIST v1.1 criteria within the 6 months prior to enrollment.

[0532] Enrolled patients must either be ineligible for chemotherapy or have received at least 1 regimen containing anthracycline and a maximum of 3 previous lines of systemic therapy for metastatic disease (no more than 2 lines of combination regimens), including pazopanib, trabectedin, eribulin mesylate, or tazemetostat, if applicable per regional prescribing information. Patients who meet enrollment criteria will be assigned to receive either BA3011 alone or in combination with nivolumab (for patients 18 years old and above: 240 mg every 2 weeks [Q2W]; for patients 12-17 years old: 3 mg/kg Q2W IV infusion). Patients with tumors showing B cell infiltration (per CD20 immunohistochemistry [IHC] assay) will be preferentially assigned to receive BA3011 in combination with nivolumab. Based on data from the Phase 1 part of the study, the dose of BA3011 for Phase 2 is 1.8 mg/kg Q2W. Enrollment will be staged, beginning with approximately 10 patients per sarcoma subtype in the monotherapy arm. Up to 7 sarcoma subtype groups may be enrolled:

[0533] Soft tissue sarcoma:

- leiomyosarcoma
- synovial sarcoma
- liposarcoma
- all other soft tissue sarcomas, except GISTs, dermatofibrosarcoma protuberans, inflammatory myofibroblastic tumor, and malignant mesothelioma

[0534] Bone sarcoma:

- osteosarcoma
- Ewing sarcoma
- other bone sarcomas, including undifferentiated pleomorphic sarcoma, malignant fibrous histiocytoma, and chondrosarcoma

[0535] In the combination arm (BA3011 with nivolumab) of the study, 20 patients of any sarcoma subtype will be enrolled. Among these 20 patients, approximately 10 patients will have tumor showing Bcell infiltration (CD20 positive) as determined by a CD20 IHC assay and 10 patients with no CD20 expression (CD20 negative). Tumor assessment will occur approximately every 6 weeks from C1D1 until 12 weeks, and every 8 weeks thereafter. Pharmacokinetic, pharmacodynamic (PD), immunogenicity, and biomarker assessments will be performed at the time points described in Table 19. Patient safety monitoring will begin at enrollment and will continue following administration of the final dose of investigational product(s) (IP).

[0536] Sample collection timepoints for BA3011 PK, PD, and immunogenicity testing are presented in Table 18 (Phase 1) and Table 19 (Phase 2).

Table 18
BA3011 Pharmacokinetic, Pharmacodynamic, and Immunogenicity Assessment Timepoints for Phase 1

Cycle Number	Study Day Visit	Collection Time
Pharmacokinetics and Pharmacodynamics		
Cycle 1	Day 1	Pre-dose (PK and PD)
		0.5 h [± 5 min] (PK only)
		4 h [± 15 min] (PK only)
	Day 2 (dose escalation only)	24 h [± 2 h] (PK only)
	Day 3	48 h [± 4 h] (PK only)
	Day 8	168 h [± 4 h] (PK only)
	Day 8 post infusion, if cohort 5A, 6A, 7A, or 8A (2Q3W) or expansion cohort (2Q3W)	0.5 h [± 5 min] End of Infusion C1D8 (PK only)
	Day 15	336 h [± 4 h] (PK only)
Cycle 2	Day 1	Pre-dose (PK and PD)
		0.5 h [± 5 min] (PK only)
	Day 3	48 h [± 4 h] (PK only)
	Day 8	168 h [± 4 h] (PK only)
	Day 8 post infusion, if cohort 5A, 6A, 7A, or 8A (2Q3W) or expansion cohort (2Q3W)	0.5 h [± 5 min] End of Infusion C2D8 (PK only)
	Day 15 (dose escalation only)	336 [± 4 h] (PK only)
Subsequent cycles (every other cycle C3, C5, C7...)	Day 1	Pre-dose (PK and PD)
		0.5 h [± 5 min] (PK only)
EOT visit	EOT	May be collected at any timepoint (PK and PD)
Biomarkers and ADA		
Cycle Number	Study Day Visit	Collection Time
Cycle 1	Day 1	<24 hours pre-dose
Cycle 2	Day 1	Pre-dose (ADA only)
	Day 8	May be collected at any timepoint (Biomarkers only)
Subsequent cycles (every other cycle C3, C5, C7...)	Day 1	Pre-dose (ADA only)
EOT visit	EOT	May be collected at any timepoint.

Abbrevations: ADA, anti-drug antibodies; C, cycle; D, day; EOT, end of treatment; PD, pharmacodynamic; PK, pharmacokinetic. Serum collection for ADA assessment occurs < 24 hours predose.

Table 19

BA3011 Pharmacokinetic, Pharmacodynamic, and Immunogenicity Assessment Timepoints for Phase 2

Cycle Number	Study Day Visit	Collection Time
Pharmacokinetics and Pharmacodynamics		
Cycle 1 (28 days)	Day 1	Pre-dose (PK and PD)
		0.5 h [± 5 min] (PK only)
	Day 3	48 h [± 4h] (PK only)
	Day 8	168 h [± 4h] (PK only)
	Day 15	Pre-dose (PK only)
		0.5 h [± 5 min] (PK only)
Cycle 2 (28 days)	Day 1	Pre-dose (PK and PD)
		0.5 h [± 5 min] (PK only)
	Day 15	Pre-dose (PK only)
		0.5 h [± 5 min] (PK only)
Subsequent cycles (Cycle 3 and every cycle thereafter)	Day 1	Pre-dose (PK and PD)
All cycles	Unscheduled visits with an adverse event	May be collected at any timepoint (PK)
EOT visit	EOT	May be collected at any timepoint (PK and PD)
Biomarkers and ADA		
Cycle Number	Study Day Visit	Collection Time
Cycle 1 (28 days)	Day 1	<24 hours pre-dose
	Day 15	Pre-dose (ADA only)
Cycle 2 (28 days)	Day 1	Pre-dose (ADA only)
	Day 15	Pre-dose
Subsequent cycles (Cycle 3 and every cycle thereafter)	Day 1	Pre-dose (ADA only)
All cycles	Unscheduled visits with an adverse event	May be collected at any timepoint (ADA only)
EOT visit	EOT	May be collected at any timepoint

Abbrevations: ADA, anti-drug antibodies; C, cycle; D, day; EOT, end of treatment; PD, pharmacodynamic; PK, pharmacokinetic. Serum collection for ADA assessment occurs < 24 hours predose.

[0537] An interim analysis is conducted for each subtype or treatment (i.e., BA3011 in combination with nivolumab in patients with tumors that are CD20 positive or BA3011 in combination with nivolumab in patients with tumors that are CD20 negative) after at least 10 patients in the subtype or treatment have the potential to be followed for at least 12 weeks after the initiation of IP.

[0538] Approximately 150 additional patients may be enrolled for sarcoma subtypes that meet the threshold. Treatment for all enrolled patients continues until disease progression, unacceptable toxicity, or other reason for treatment discontinuation.

[0539] Pegfilgrastim or filgrastim (or biosimilar) may be used at the discretion of the Investigator either prophylactically for patients with lower pre-dosing neutrophil count or to treat occurrences of neutropenia. Patients with ANC levels below 3000/μL or 3 x 10_9/L prior to BA3011 administration may be at an increased risk of neutropenia. For these patients, prophylactic pegfilgrastim or filgrastim (or biosimilar) can be administered 48 to 72 hours after BA3011 administration.

Randomization and Blinding

[0540] Both phases are open-label and nonrandomized.

Study Drug Materials And Management

Study Drug

BA3011

[0541] BA3011 is anti-human Axl extracellular domain recombinant, full-length bivalent humanized mAb (IgG1) produced in Chinese Hamster Ovary cells and conjugated to MMAE using a cleavable linker.

Nivolumab

[0542] OPDIVO® (nivolumab) is a commercially available programmed death receptor-1 (PD-1) blocking antibody. For more information regarding the use of OPDIVO® (nivolumab), refer to the prescribing information.

BA3011 Dose Calculation

[0543] Dose calculations are based on weight rounded to the nearest whole kg. For patients with a weight of >100 kg, the dose of BA3011 should be calculated for 100 kg. The dose volume required will be calculated using the following formula:

$$\text{Total Dose Volume (mL)} = \frac{\text{Patient weight (kg)} \times \text{dose level (mg/kg)}}{10 \text{ (vial concentration (mg/mL))}}$$

[0544] Volumes should be rounded to the nearest tenth mL, e.g., for 0.24 mL round it to 0.2 mL and for 0.25 mL round it to 0.3 mL. The number of vials needed to supply the dose is calculated by dividing the total dose volume by the nominal fill volume per vial (5 mL), rounded up to the unit vial.

$$\text{\# Vials} = \frac{\text{Calculated Total Dose Volume}}{5 \text{ mL}}$$

[0545] Dose adjustments for each cycle are only needed if there is a greater than 10% change in body weight from the Day 1 dose.

Administration

BA3011

Phase 1 Dose Escalation: The total administration time of BA3011 should be approximately 90 (±15) minutes on Cycle 1 Day 1 for 100 mL IV bags with an infusion rate of 67 mL/hr. For all subsequent administrations, the total administration time should be approximately 60 (±15) minutes for 100 mL with an infusion rate of 100 mL/hr, provided the patient does not experience infusion-related reactions.

Phase 1 Dose Expansion: The total administration time of BA3011 should be approximately 60 (±15) minutes on Cycle 1 Day 1 and approximately 30 (±15) minutes for all subsequent administrations, provided the patient does not experience infusion-related reactions.

[0546] Phase 2: The total administration time of BA3011 for all infusions, Cycle 1 Day 1 and thereafter, should be approximately 30 (±15) minutes.

[0547] Ba3011 must not be administered as an i.v. push or bolus. BA3011 should be administered through a dedicated IV line using only non-PVC saline bag and infusion tubing sets. BA3011 cannot be mixed with other medications in the same saline bag.

[0548] Following the observation of dose-limiting neutropenia at higher BA3011 dose levels, administration of pegfilgrastim or filgrastim (or biosimilar) is required for patients in Phase 1 between 48 to 72 hours after every Day 1 infusion of BA3011. In view of BA3011's potential for myelosuppression, it is particularly important that patients are not infused until any neutrophil toxicity has resolved to at least Grade 1, or Grade 2 for patients who have received prophylactic pegfilgrastim or filgrastim.

Nivolumab

[0549] Phase 2 only: Patients enrolled to receive combination therapy will receive BA3011 in combination with nivolumab (i.e., for patients 18 years old and above: 240 mg Q2W; for patients 12-17 years old: 3 mg/kg Q2W IV infusion) (Davis 2020). A patient's nivolumab dose should remain the same for the duration of the patient's time on study.

[0550] When BA3011 and nivolumab are to be administered on the same day, separate infusion bags and filters must be used for each infusion. Nivolumab is to be administered first. The second infusion will always be BA3011 and should be administered at least 30 minutes after completion of the nivolumab infusion. Nivolumab is to be administered as a

30-minute IV infusion. Detailed instructions for administration of nivolumab are provided in the OPDIVO® (nivolumab) prescribing information.

Number of Patients

[0551] During Phase 1 dose escalation, approximately 35 to 78 DLT-evaluable patients with advanced solid tumors are treated, depending on cohort expansion and tolerability of BA3011. Each cohort requires a minimum of 3 and up to 6 patients, except for the single-patient cohorts. A minimum of 6 patients are enrolled at the MTD. Phase 1 dose expansion is conducted in approximately 30 additional patients with Axl-expressing, advanced solid tumors. Phase 2 enrolls a minimum of approximately 70 Axl-expressing (TmPS $\geq$ 70) patients in the BA3011 monotherapy arm (10 patients with advanced, refractory sarcoma in each of up to 7 sarcoma subtype groups) and approximately 20 Axl-expressing (TmPS $\geq$ 70) patients in the combination (BA3011 with PD-1) arm of the study (10 CD20 positive and 10 CD20 negative patients). Approximately 150 additional patients may be enrolled depending on observed efficacy at interim analysis.

Treatment Assignment

[0552] During Phase 1 dose escalation, patients will be assigned to cohorts of escalating BA3011 dose levels as outlined in Table 16 and Figure 18. BA3011 is administered via IV infusion once (1Q3W) or twice (2Q3W) every 3 weeks, on Day 1 only or Days 1 and 8 of each 21-day cycle, respectively, according to the dose level assigned to each cohort. The starting BA3011 dose level will be 0.3 mg/kg 1Q3W. Escalation to the next specified dose level will continue until the MTD is identified. The Phase 1 dose expansion is designed to assist in determining the BA3011 dose and treatment schedule patients will receive in Phase 2 (e.g., the RP2D) and will be conducted in patients with Axl-expressing, advanced solid tumors at a BA3011 dose and regimen determined to be appropriate by the PSC. It is the Sponsor's responsibility, after discussions with the designated Medical Monitor and Investigators, to determine the RP2D based on the totality of the data and not to exceed the MTD. Based on data from the Phase 1 part of the study, the dose of BA3011 for Phase 2 is 1.8 mg/kg Q2W. Rationale for the Q2W dosing regimen is detailed in Section 1.6. For Phase 2, patients who meet enrollment criteria will be assigned to receive BA3011 alone or in combination with nivolumab. Patients with tumors showing B cell infiltration (per CD20 IHC assay) will be preferentially assigned to receive BA3011 in combination with nivolumab.

Pegfilgrastim Administration:

Phase 1:

[0553] • Following the observation of dose-limiting neutropenia at higher BA3011 dose levels, administration of pegfilgrastim (or biosimilar) is required at the approved dose per applicable package insert and must occur between 48 to 72 hours after every Day 1 infusion of BA3011.

[0554] • For patients dosed at the 2Q3W schedule (i.e., patients receiving BA3011 on Days 1 and 8), administration of filgrastim no earlier than 24 hours after the Day 1 dose of each cycle is allowed, followed by optional administration of pegfilgrastim (or biosimilar) on Day 9. Initiation of filgrastim between 48 and 72 hours after the Day 1 dose of each cycle is recommended due to the slower release rate of MMAE when conjugated with an antibody compared with standard chemotherapy.

[0555] • In case of allergy to pegfilgrastim, filgrastim (or biosimilar) may be substituted.

[0556] • Starting at Cycle 3, pegfilgrastim or filgrastim can be self-administered.

Phase 2:

[0557] • Pegfilgrastim or filgrastim (or biosimilar) is not required during Phase 2 but may be used at the discretion of the Investigator either prophylactically for patients with lower pre-dosing neutrophil count or to treat occurrences of neutropenia.

[0558] • Patients with ANC levels below 3000/μL or 3 x 10_9/L prior to BA3011 administration may be at an increased risk of neutropenia. For these patients, prophylactic pegfilgrastim or filgrastim (or biosimilar) 48 to 72 hours after BA3011 administration is recommended.

Example 14: Interim Safety and Efficacy Results of Phase 1 Study of Mecbotamab Vedotin (BA3011), a CAB-AXL-ADC, in Advanced Sarcoma Patients

[0559] In the following study, the safety profile, recommended Phase 2 dose (RP2D), and preliminary evidence of antitumor activity of BA3011 in patients with advanced sarcoma or other solid tumors was identified.

Methods

[0560] Study BA3011-001 was an ongoing, multi-center, open-label, Phase 1/2 first-in-human trial of BA3011.

[0561] In Phase 1 (NCT03425279), BA3011 was administered once (Q3W) or twice (2Q3W) every 3 weeks via intravenous (IV) infusion.

[0562] Phase 2 (NCT03425279) is an ongoing an open label assessment of the efficacy and safety of BA3011 alone and in combination with a PD-1 inhibitor in patients 12 years of age or more with AXL-expressing tumor membrane percent score (TmPS) ≥ 50 with advanced refractory sarcoma who have measurable disease and documented progression.

Results

Patient Disposition and Baseline Demographics

[0563] Median (range) age of patients was 58.0 (24–80) years, 57.7% were female, 84.6% were white, with 69.2% having an ECOG score of 0 and 30.8% having a score of 1.

[0564] Phase 1 sarcoma patients had on average received 4 or more prior lines of therapy.

[0565] In Phase 1, a total of 60 patients received BA3011 at dose levels from 0.3 to 3.0 mg/kg Q3W, and 1.2 to 1.8 mg/kg 2Q3W, including 26 patients with sarcoma.

[0566] 227 sarcoma patients were tested for AXL tumor membrane expression as part of the IHC assay validation work and in phase 1 & 2 studies with approximately 50% having a TmPS ≥ 70. AXL appeared to be expressed at a consistent rate across all sarcoma subtypes tested.

Safety

[0567] No clinically meaningful on-target toxicity to normal AXL-expressing tissue was observed, with a low rate of constipation. Dose-limiting toxicities were limited to monomethyl auristatin E (MMAE) conjugate-associated toxicity at the highest dose tested, including reversible neutropenia.

[0568] In Phase 1 sarcoma patients, there were no treatment-emergent adverse events (TEAEs) leading to death, and treatment-related TEAEs in 2 (7.7%) patients led to treatment discontinuation (Table 20).

[0569] Eleven patients had grade 3 related TEAEs and 1 patient had grade 4 neutropenia, which generally were MMAE related, including reversible myelosuppression, transient liver enzyme elevations and metabolic disturbances (Table 21).

[0570] Transient grade 1-2 liver enzyme elevations seen during cycle 1 treatment generally did not re-occur upon re-treatment. Creatinine levels were generally unchanged throughout treatment.

[0571] In Phase 1 sarcoma patients, an SAE related to treatment (grade 2 hepatic encephalopathy) occurred in 1 patient (Table 20).

Table 20 – Overview of Adverse Events in Sarcoma Patients in Phase 1

Dose	0.3 mg/kg Q3W	1.8 mg/kg Q3W	2.4 mg/kg Q3W	1.2 mg/kg 2Q3W	1.8 mg/kg 2Q3W	Total
No. of Patients	1	2	2	2	19	26
Any TEAE	1 (100.0%)	2 (100.0%)	2 (100.0%)	2 (100.0)%	19 (100.0%)	26 (100.0)%
TEAE with CTCAE grade 3 or 4	0	1 (50.0%)	1 (50.0%)	2 (100.0%)	13 (68.4%)	17 (65.4%)
Related TEAEs with CTCAE grade 3 or 4	0	0	1 (50.0%)	0	11 (57.9%)	12 (46.2%)
Any serious TEAE	0	1 (50.0%)	1 (50.0%)	0	7 (36.8%)	9 (34.6%)
Any related serious TEAE[a]	0	0	0	0	1 (5.3%)	1 (3.8%)
TEAE leading to treatment discontinuation	0	0	0	0	2 (10.5%)	2 (7.7%)
Related TEAE leading to treatment discontinuation[b]	0	0	0	0	2 (10.5%)	2 (7.7%)0
TEAEs leading to death	0	0	0	0	0	0

CTCAE=Common Terminology Criteria for Adverse Events

Q3W=Every 3 weeks

2Q3W=Twice every 3 weeks

TEAE=Treatment Emergent Adverse Event

[a]Related serious TEAE: Hepatic encephalopathy, CTCAE grade 2

[b]Neuropathy peripheral, CTCAE grade2; fatigue, CTCAE grade 3

Table 21 – Most Frequent Treatment-Emergent Adverse Events (≥ 20%, All TEAE All Grades) and Any Related Grade ¾ TEAE in Sarcoma Patients in Phase 1

	All TEAEs			Related TEAEs		
	All Grades n (%)	Grade 3 n (%)	Grade 4 n (%)	All Grades n (%)	Grade 3 n (%)	Grade 4 n (%)
Patients with at least one TEAE	26 (100.0)	15 (57.7)	2 (7.7)	22 (84.6)	11 (42.3)	1 (3.8)
Fatigue	16 (61.5)	0	0	11 (42.3)	0	0
Nausea	14(58.3)	1 (3.8)	0	10 (38.5)	1 (3.8)	0
Alanine aminotransferase increased	12 (46.2)	0	0	10 (38.5)	0	0
Aspartate aminotransferase increased	11 (42.3)	1 (3.8)	0	11 (42.3)	1 (3.8)	0
Bone pain	7 (26.9)	0	0	1 (3.8)	0	0
Constipation	7 (26.9)	1 (3.8)	0	4 (15.4)	0	0
Diarrhea	7 (26.9)	0	0	5 (19.2)	0	0
Neutrophil count decreased	7 (26.9)	4 (15.4)	1 (3.8)	7 (26.9)	4 (15.4)	1 (3.8)
Alopecia	6(23.9)	0	0	6 (23.1)	0	0
Arthralgia	6(23.9)	0	0	1 (3.8)	0	0
Blood alkaline phosphatase increased	6(23.9)	0	0	5 (19.2)	0	0
Decreased appetite	6(23.9)	0	0	4 (15.4)	0	0
Vomiting	6(23.9)	1 (3.8)	0	5 (19.2)	1 (3.8)	0
Anemia	4 (15.4)	2 (7.7)	0	1 (3.8)	1 (3.8)	0
Hypokalemia	4 (15.4)	3 (11.5)	0	4 (15.4)	3 (11.5)	0
Peripheral neuropathy	4 (15.4)	1 (3.8)	0	4 (15.4)	1 (3.8)	0
Hyponatremia	3 (11.5)	2 (7.7)	0	1. (3.8)	1 (3.8)	0
Lymphocyte count decreased	2 (7.7)	1 (3.8)	0	2 (7.7)	1 (3.8)	0
Blood bilirubin increased	1 (3.8)	1 (3.8)	0	1 (3.8)	1 (3.8)	0
Stomatitis	1 (3.8)	1 (3.8)	0	1 (3.8)	1 (3.8)	0

Recommended Phase 2 Dose (RP2D) / Pharmacokinetics

[0572] The RP2D was determined to be 1.8 mg/kg Q2W based on an integrated evaluation of Phase 1 data, including PK modeling.

[0573] The PK profile of BA3011 was approximately dose proportional; in Phase 1 the half-life was determined to be approximately 4 days.

Efficacy

[0574] BA3011 antitumor activity correlated with higher levels of AXL tumor membrane expression in sarcoma patients.

[0575] Of 7 treatment refractory sarcoma patients with baseline TmPS ≥ 70 and dosed at 1.8 mg/kg (Q3w or 2Q3w), 4 had confirmed partial responses (Figs. 20, 22 and 23).

Table 22 – Subjects as shown in Fig. 20

Subject	Cancer Type	Dosage		Subject	Cancer Type	Dosage
1	Leiomyosarcoma	1.8 mg/kg D1,8 Q3W		10	Undifferentiated Pleomorphic Sarcoma	1.8 mg/kg D1,8 Q3W
2	Undifferentiated Pleomorphic Sarcoma	1.8 mg/kg D1,8 Q3W		11	Osteosarcoma	1.8 mg/kg D1,8 Q3W
3	Liposarcoma	2.4 mg/kg D1 Q3W		12	Leiomyosarcoma	1.8 mg/kg D1,8 Q3W
4	Leiomyosarcoma	2.4 mg/kg D1 Q3W		13	Leiomyosarcoma	1.2 mg/kg D1,8 Q3W
5	Synovial Sarcoma	1.8 mg/kg D1,8 Q3W		14	Leiomyosarcoma	1.8 mg/kg D1 Q3W
6	Leiomyosarcoma	1.8 mg/kg D1,8 Q3W		15	Undifferentiated Pleomorphic Sarcoma	1.8 mg/kg D1,8 Q3W
7	Osteosarcoma	0.3 mg/kg D1 Q3W		16	Undifferentiated Pleomorphic Sarcoma	1.8 mg/kg D1,8 Q3W
8	Chondrosarcoma	1.2 mg/kg D 1,8 Q3W		17	Ewing Sarcoma	1.8 mg/kg D1 Q3W
9	Liposarcoma	1.8 mg/kg D1,8 Q3W				

[0576] Prolonged response to therapy in sarcoma patients in this study was demonstrated in Fig. 24.

Conclusion

[0577] Based on preliminary efficacy and safety results from this study, the benefit-risk profile of BA3011 monotherapy appeared to be favorable in patients with sarcoma.

[0578] No clinically meaningful on-target toxicity was observed. In Phase 1 sarcoma patients, evidence of antitumor activity was observed, with higher AXL tumor membrane expression correlating with response. AXL appeared to be expressed at a consistent rate throughout all sarcoma subtypes tested.

[0579] It is to be understood, however, that even though numerous characteristics and advantages of the present invention have been set forth in the foregoing description, together with details of the structure and function of the invention, the disclosure is illustrative only, and changes may be made in detail, especially in matters of shape, size and arrangement of parts within the principles of the invention to the full extent indicated by the broad general meanings of the terms in which the appended claims are expressed.

[0580] All documents mentioned herein are hereby incorporated by reference in their entirety or alternatively to provide the disclosure for which they were specifically relied upon. The applicant(s) do not intend to dedicate any disclosed embodiments to the public, and to the extent any disclosed modifications or alterations may not literally fall within the scope of the claims, they are considered to be part hereof under the doctrine of equivalents.

WHAT IS CLAIMED IS:

1. A method of treating an Axl expressing tumor comprising administering to a human subject in need of such treatment a pharmaceutical composition comprising mAbBA301-cleavable linker-$MMAE_n$ and a pharmaceutically acceptable carrier,
wherein the pharmaceutical composition is administered at a dose of 1.8 mg/kg of the human subject weight on days 1 and 8 every 21 days by intravenous infusion;
wherein mAbBA301 is an antibody or antibody fragment having a heavy chain variable region comprising a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region comprising a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18, and a lcCDR3 of SEQ ID NO. 19; and
wherein n is an integer between 1 and 4, inclusive.

2. The method of claim 1, wherein the heavy chain variable region comprises SEQ ID NO. 20 and the light chain variable region comprises SEQ ID NO. 21.

3. The method of claim 1, wherein the cleavable linker is mc-vc-PAB.

4. The method of any one of claims 1-3, wherein the Axl expressing tumor is a sarcoma, an adenocarcinoma, or a non-small lung cell cancer.

5. The method of claim 4, wherein the Axl expressing tumor is a sarcoma.

6. The method of any one of claims 1-3, further comprising administering a programmed death receptor-1 (PD-1) blocking antibody.

7. The method of any one of claims 1-3, wherein the Axl expressing tumor has a tumor membrane P score of at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, or at least 95.

8. The method of claim 7, wherein the Axl expressing tumor has a tumor membrane P score of at least 70.

9. The method of any one of claims 1-3, further comprising administering a granulocyte colony stimulating factor or an analog thereof.

10. The method of any one of claims 1-3, wherein the pharmaceutically acceptable carrier has a pH of 6.0 and comprises 20 mM histidine-HCl, 70 mg/mL sucrose and 0.5 mg/mL polysorbate 80.

11. The method of any one of claims 1-3, wherein n equals 4.

12. A method of treating an Axl expressing tumor comprising administering to a human subject in need of such treatment a pharmaceutical composition comprising mAbBA301-cleavable linker-$MMAE_n$ and a pharmaceutically acceptable carrier,
wherein the pharmaceutical composition is administered at a dose of 1.8 mg/kg of the human subject weight every 21 days by intravenous infusion;
wherein mAbBA301 is an antibody or antibody fragment having a heavy chain variable region comprising a hcCDR1 of SEQ ID NO. 14, a hcCDR2 of SEQ ID NO. 15 and a hcCDR3 of SEQ ID NO. 16; and a light chain variable region comprising a lcCDR1 of SEQ ID NO. 17, a lcCDR2 of SEQ ID NO. 18 and a lcCDR3 of SEQ ID NO. 19; and
wherein n is an integer between 1 and 4, inclusive.

13. The method of claim 12, wherein the heavy chain variable region comprises SEQ ID NO. 20 and the light chain variable region comprises SEQ ID NO. 21.

14. The method of claim 12, wherein the cleavable linker is mc-vc-PAB.

15. The method of any one of claims 12-14, wherein the Axl expressing tumor is a sarcoma, an adenocarcinoma, or a non-small lung cell cancer.

16. The method of claim 15, wherein the Axl expressing tumor is a sarcoma.

17. The method of any one of claims 12-14, further comprising administering a programmed death receptor-1 (PD-1) blocking antibody.

18. The method of any one of claims 12-14, wherein the Axl expressing tumor has a tumor membrane P score of at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, or at least 95.

19. The method of claim 18, wherein the Axl expressing tumor has a tumor membrane P score of at least 70.

20. The method of any one of claims 12-14, further comprising administering a granulocyte colony stimulating factor or an analog thereof.

21. The method of any one of claims 12-14, wherein the pharmaceutically acceptable carrier has a pH of 6.0 and comprises 20 mM histidine-HCl, 70 mg/mL sucrose and 0.5 mg/mL polysorbate 80.

22. The method of any one of claims 12-14, wherein n equals 4.

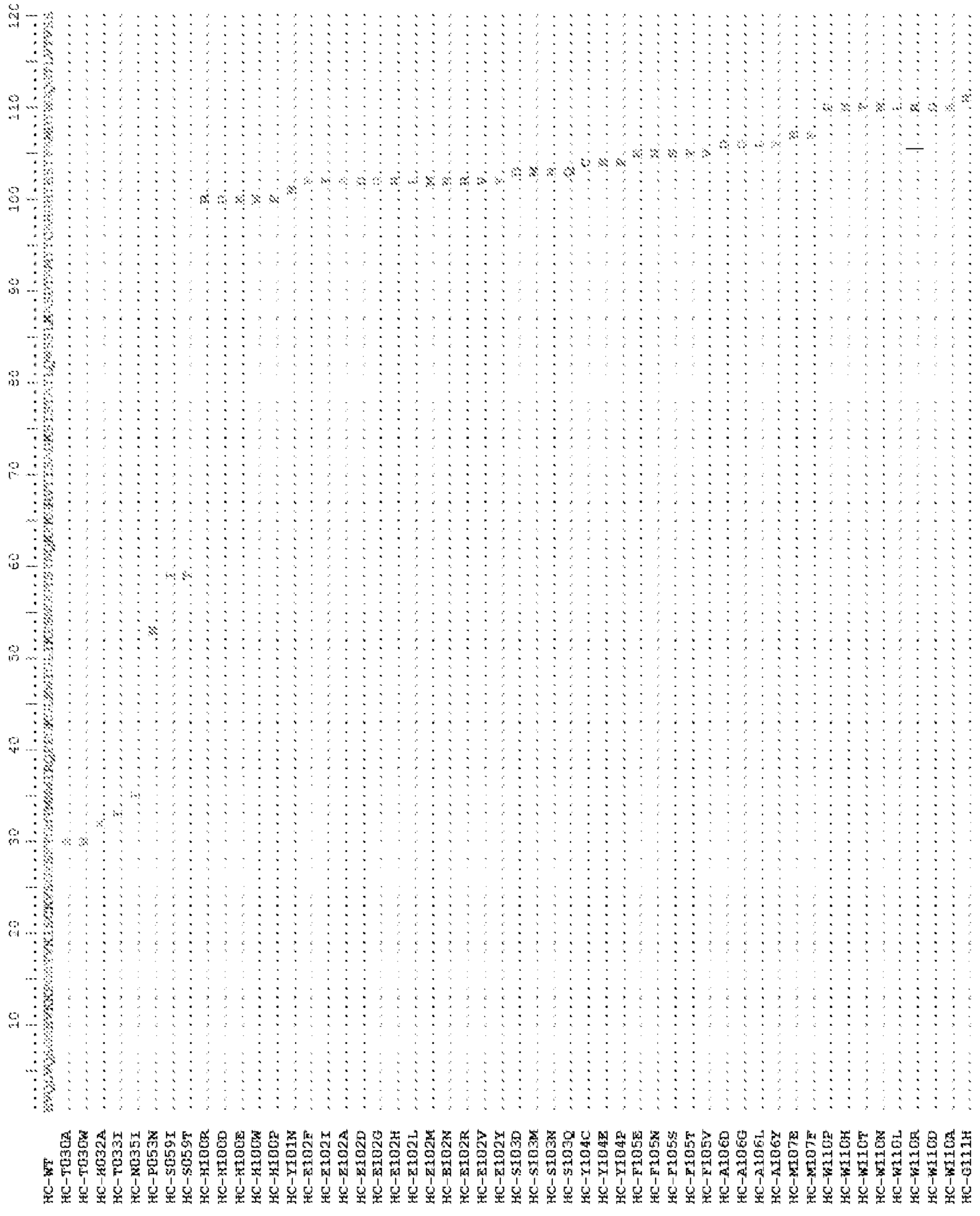

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FIG. 1A

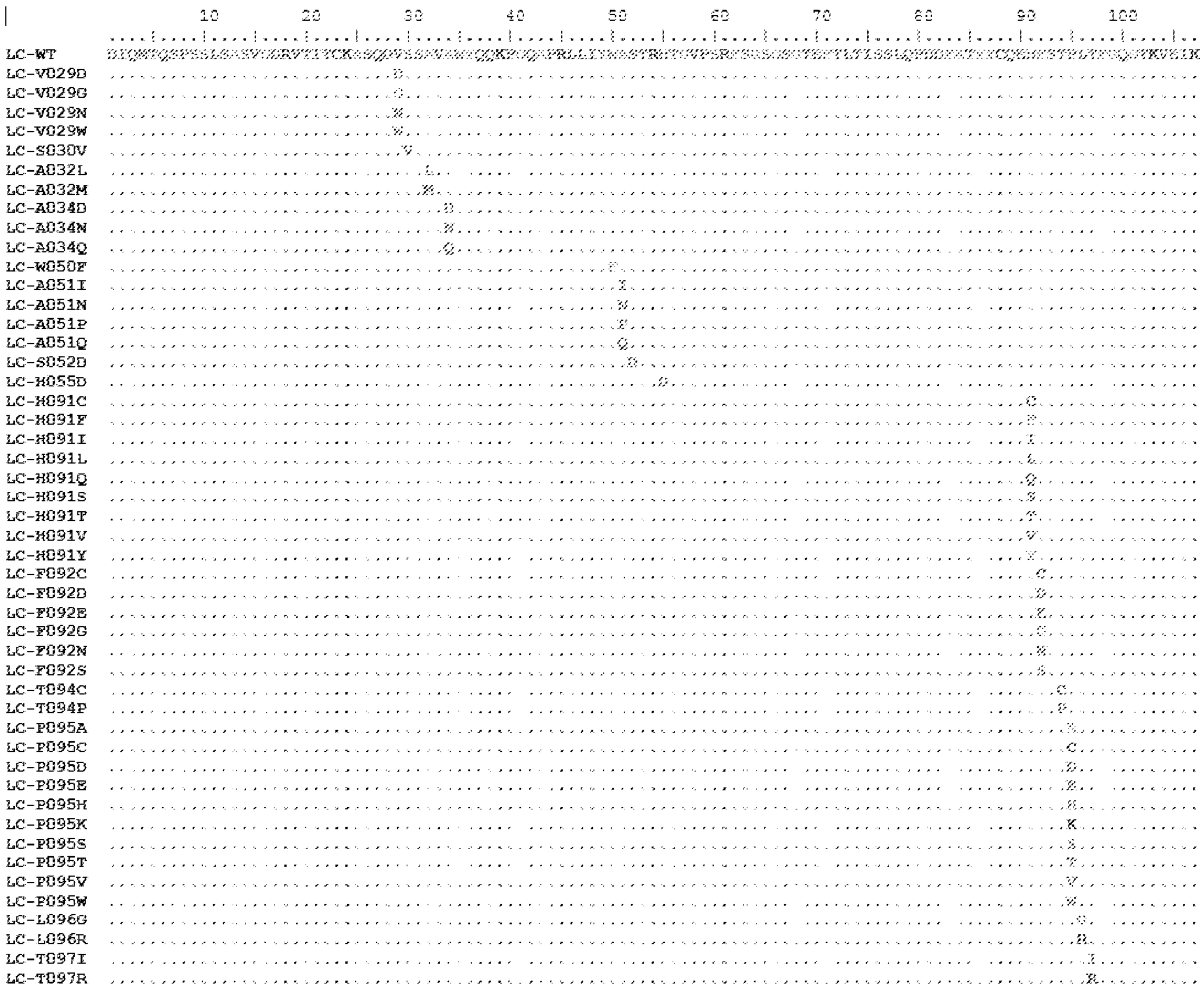

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FIG. 1B

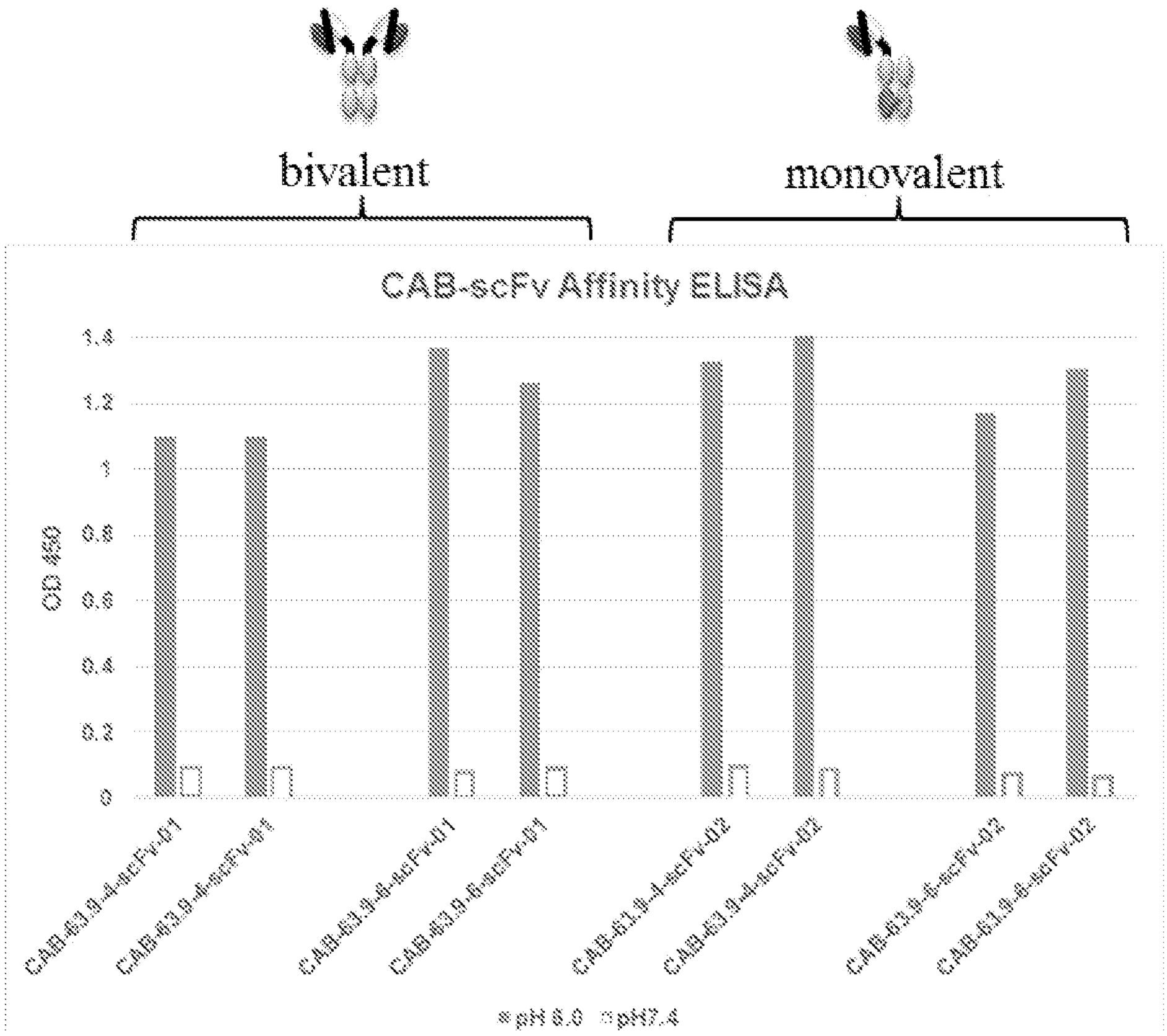

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pH7.4

FIG. 2

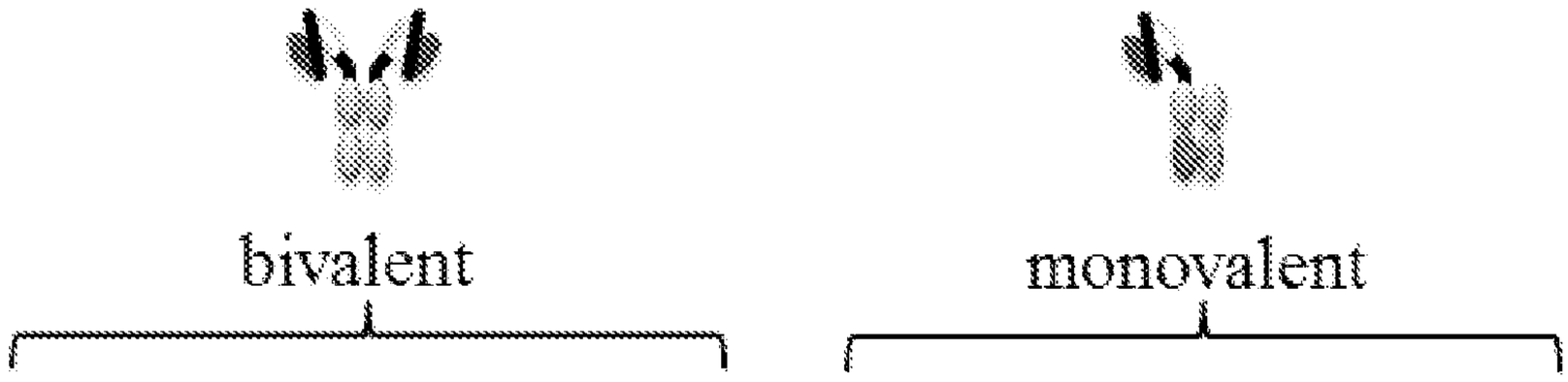

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FIG. 3

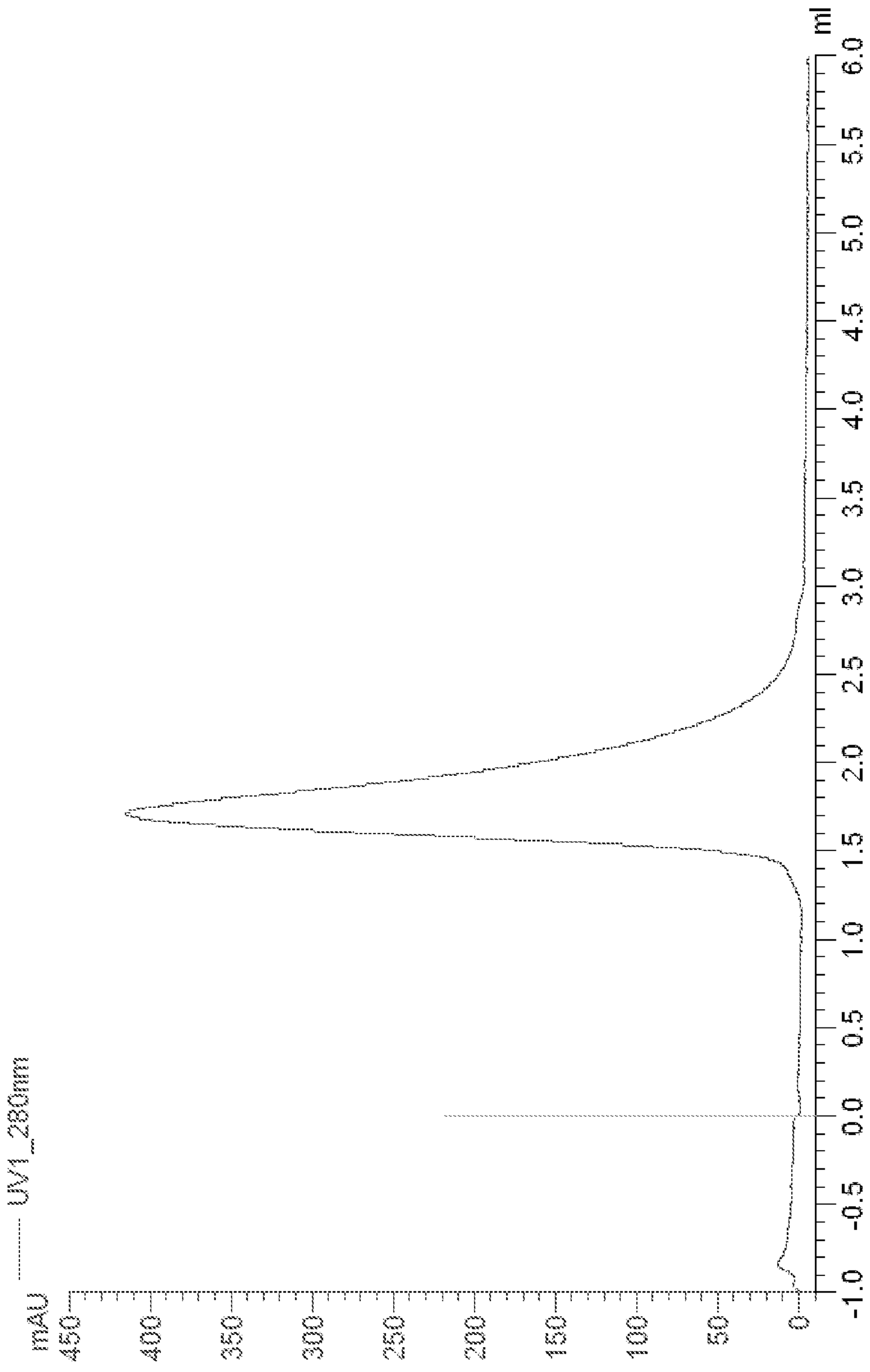

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3.5
4.0
4.5
5.0
5.5
6.0
ml

FIG. 4

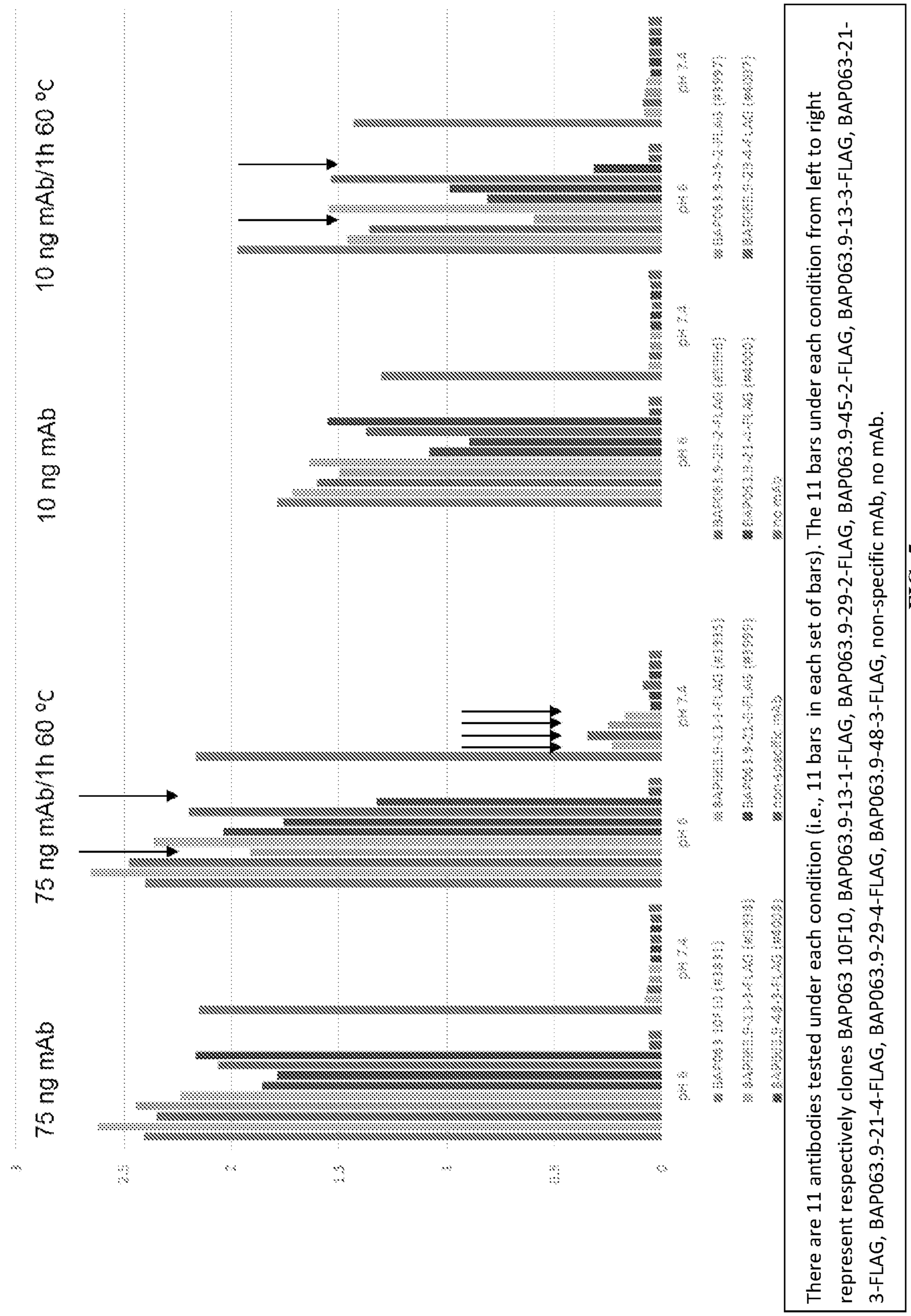

10 ng mAb/1h 60 °c
10 ng mAb
75 ng mAb/1h 60 °c
75 ng mAb
There are 11 antibodies tested under each condition (i.e., 11 bars in each set of bars). The 11 bars under each condition from left to right represent respectively clones BAP063 10F10, BAP063.9-13-1-FLAG, BAP063.9-29-2-FLAG, BAP063.9-45-2-FLAG, BAP063.9-21-4-FLAG, BAP063.9-29-4-FLAG, BAP063.9-48-3-FLAG, non-specific mAb, no mAb.

FIG. 5

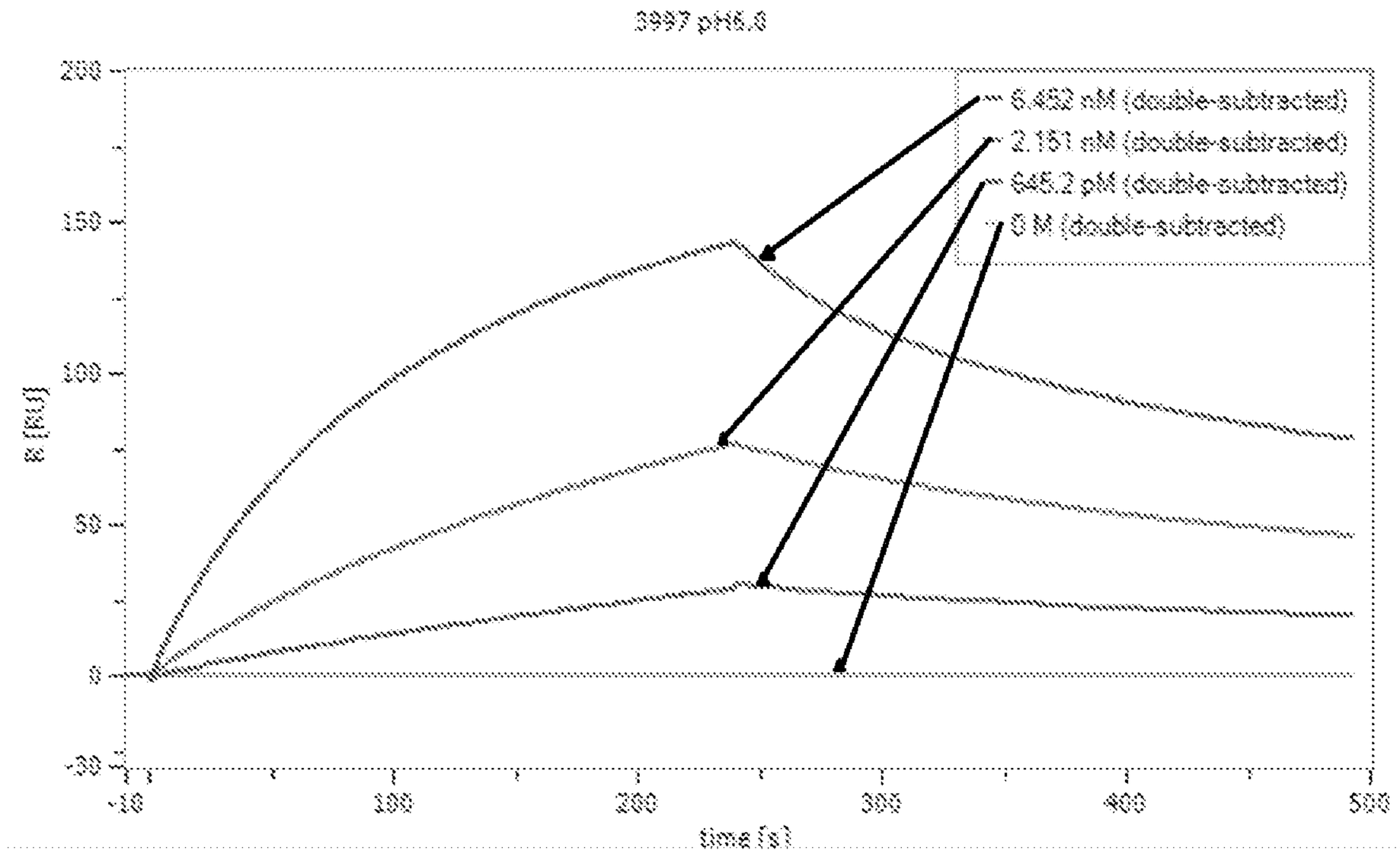

3997 pH6.0
200
150
100
50
0
-30
R [RU]
-10
100
200
300
400
500
time [s]
6.452 nM (double-subtracted)
2.151 nM (double-subtracted)
645.2 pM (double-subtracted)
0 M (double-subtracted)

FIG. 6A

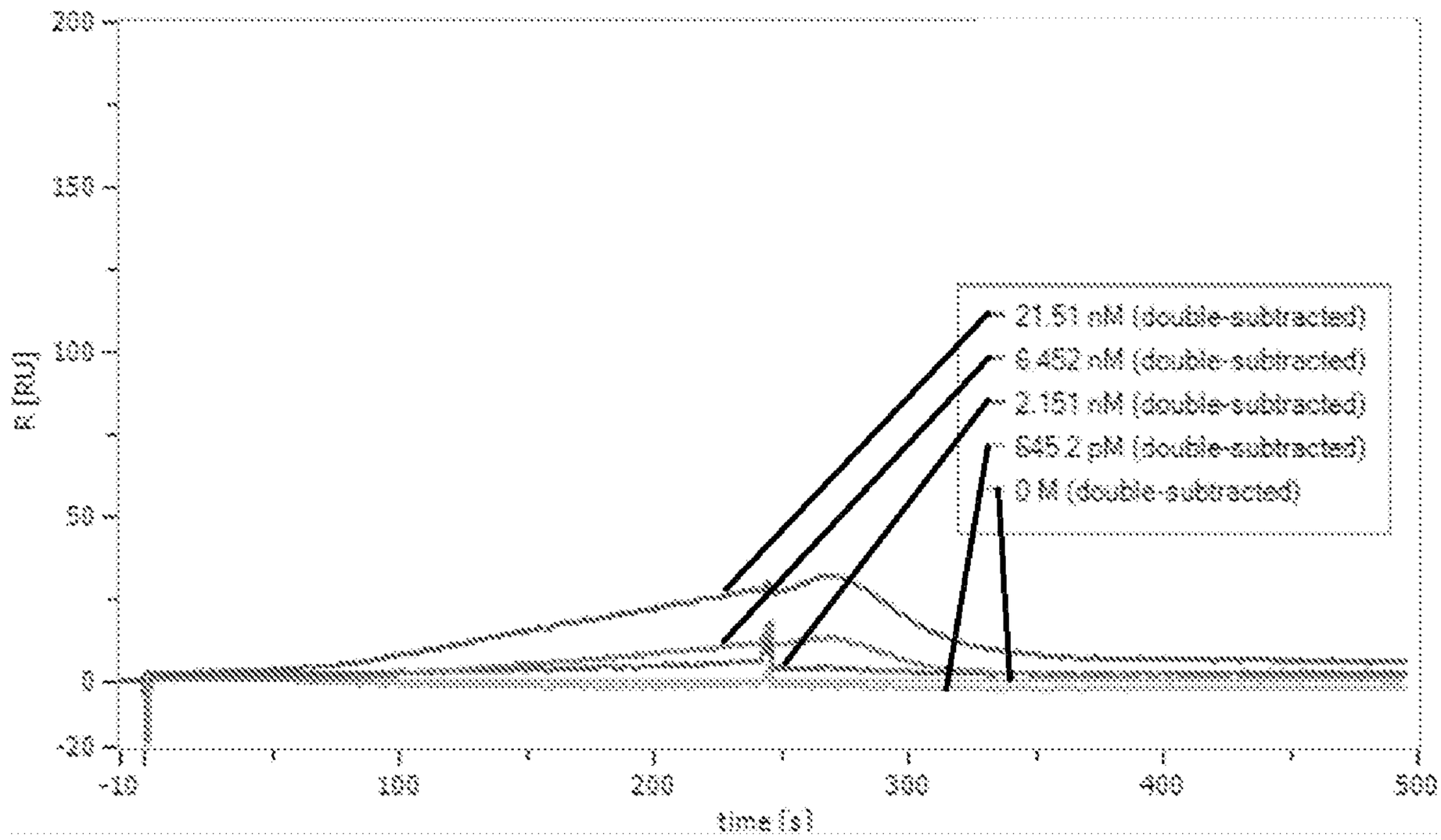

3997 pH7.4
200
150
100
50
0
-30
R [RU]
-10
100
200
300
400
500
time [s]
21.51 nM (double-subtracted)
6.452 nM (double-subtracted)
2.151 nM (double-subtracted)
645.2 pM (double-subtracted)
0 M (double-subtracted)

FIG. 6B

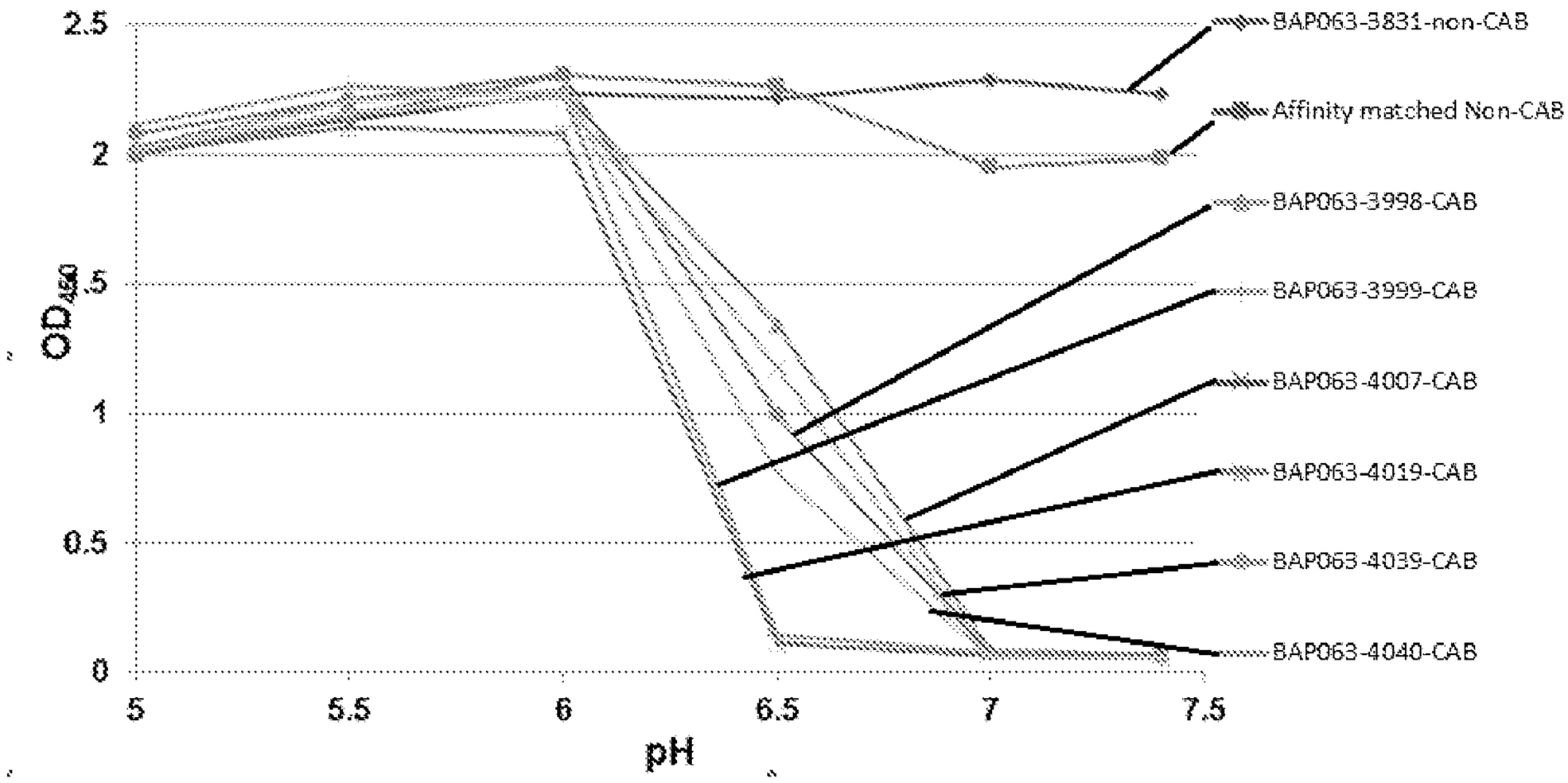

2.5
2
1.5
1
0.5
0
OD450
5
5.5
6
6.5
7
7.5
pH
BAP063-3831-non-CAB
Affinity matched Non-CAB
BAP063-3998-CAB
BAP063-3999-CAB
BAP063-4007-CAB
BAP063-4019-CAB
BAP063-4039-CAB
BAP063-4040-CAB

FIG. 7

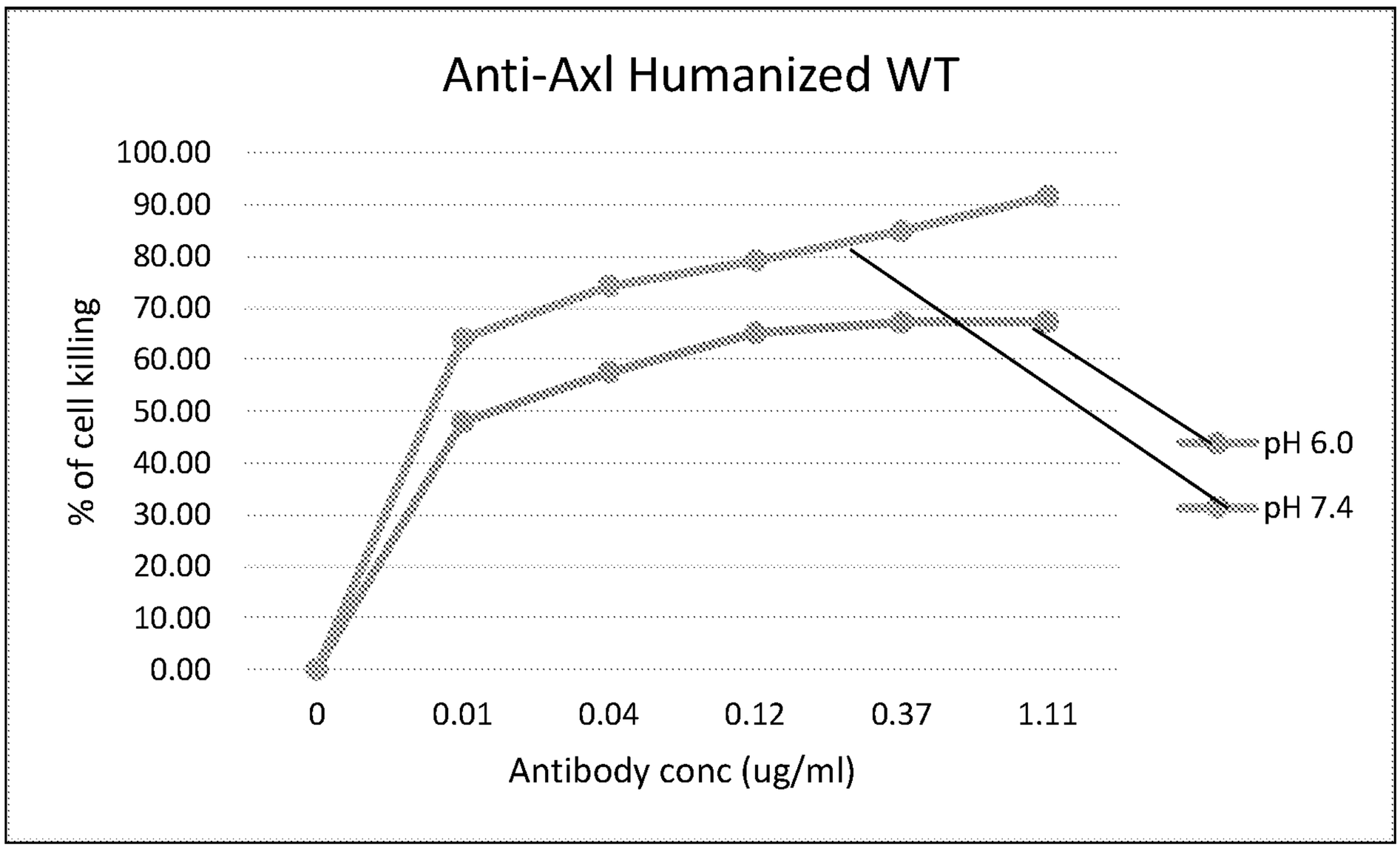

Anti-Axl Humanized WT
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
% of cell killing
0
0.01
0.04
0.12
0.37
1.11
Antibody conc (ug/ml)
pH 6.0
pH 7.4

FIG. 8A

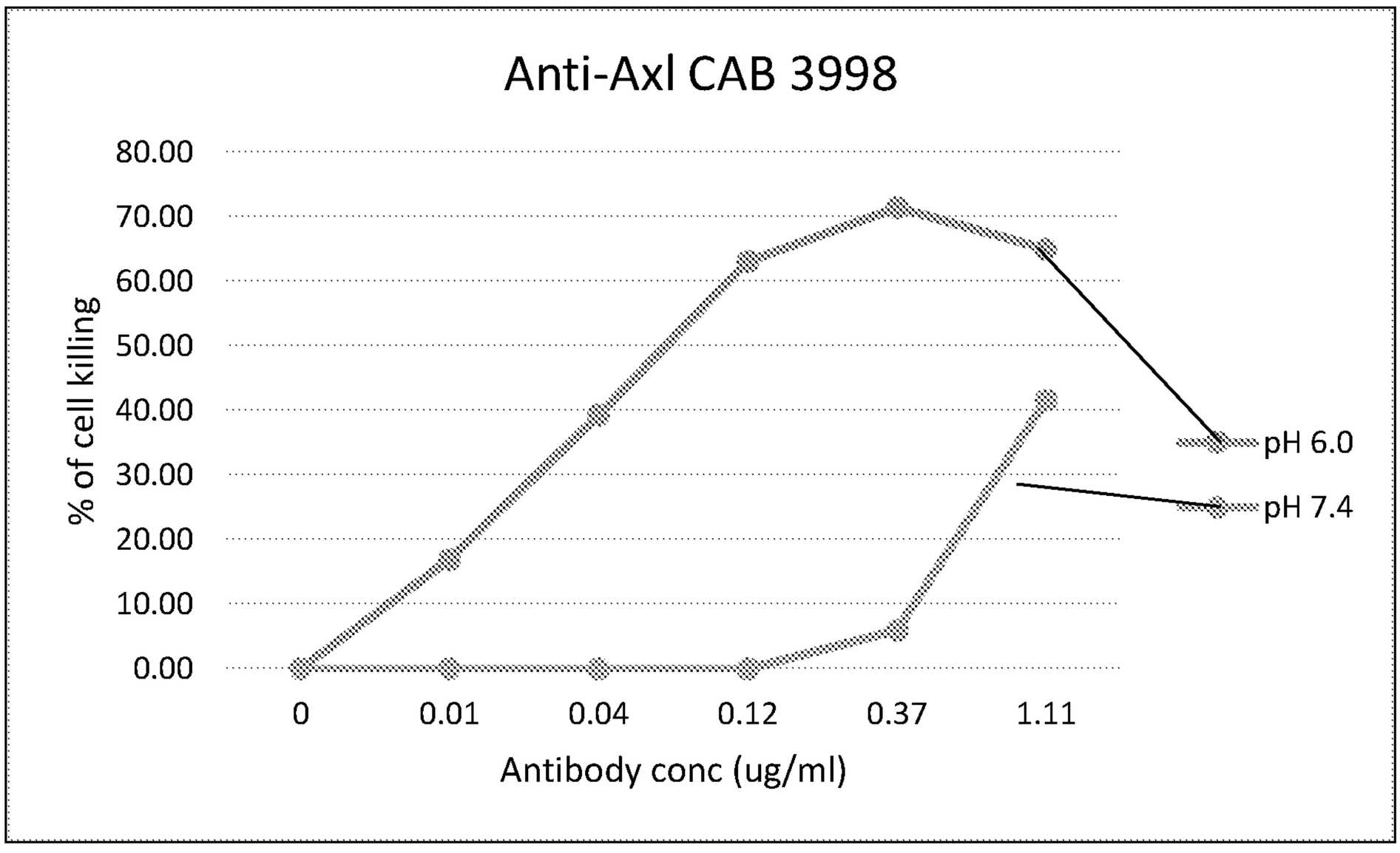

Anti-Axl CAB 3998
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
% of cell killing
0
0.01
0.04
0.12
0.37
1.11
Antibody conc (ug/ml)
pH 6.0
pH 7.4

FIG. 8B

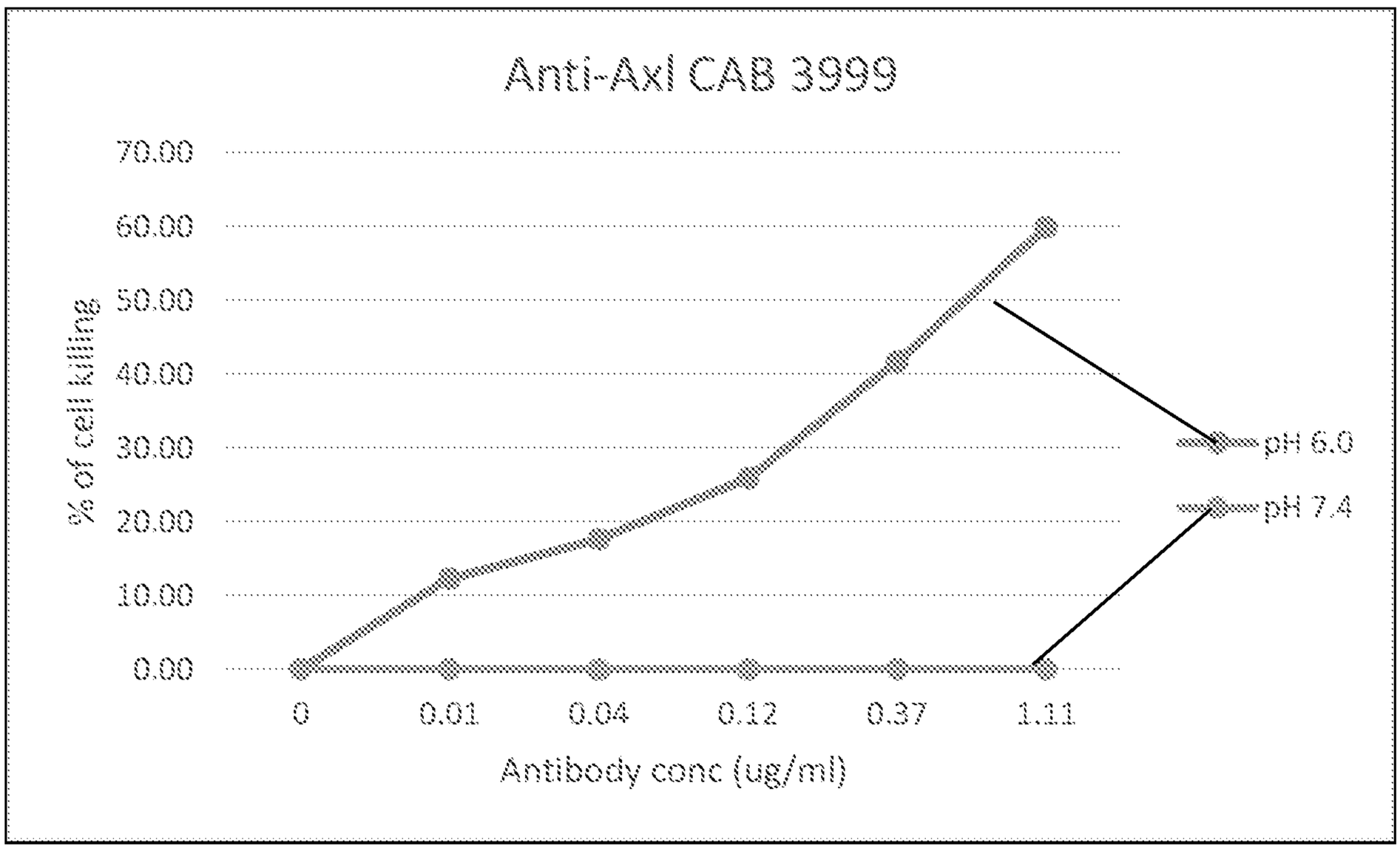

Anti-Axl CAB 3999
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
% of cell killing
0
0.01
0.04
0.12
0.37
1.11
Antibody conc (ug/ml)
pH 6.0
pH 7.4

FIG. 8C

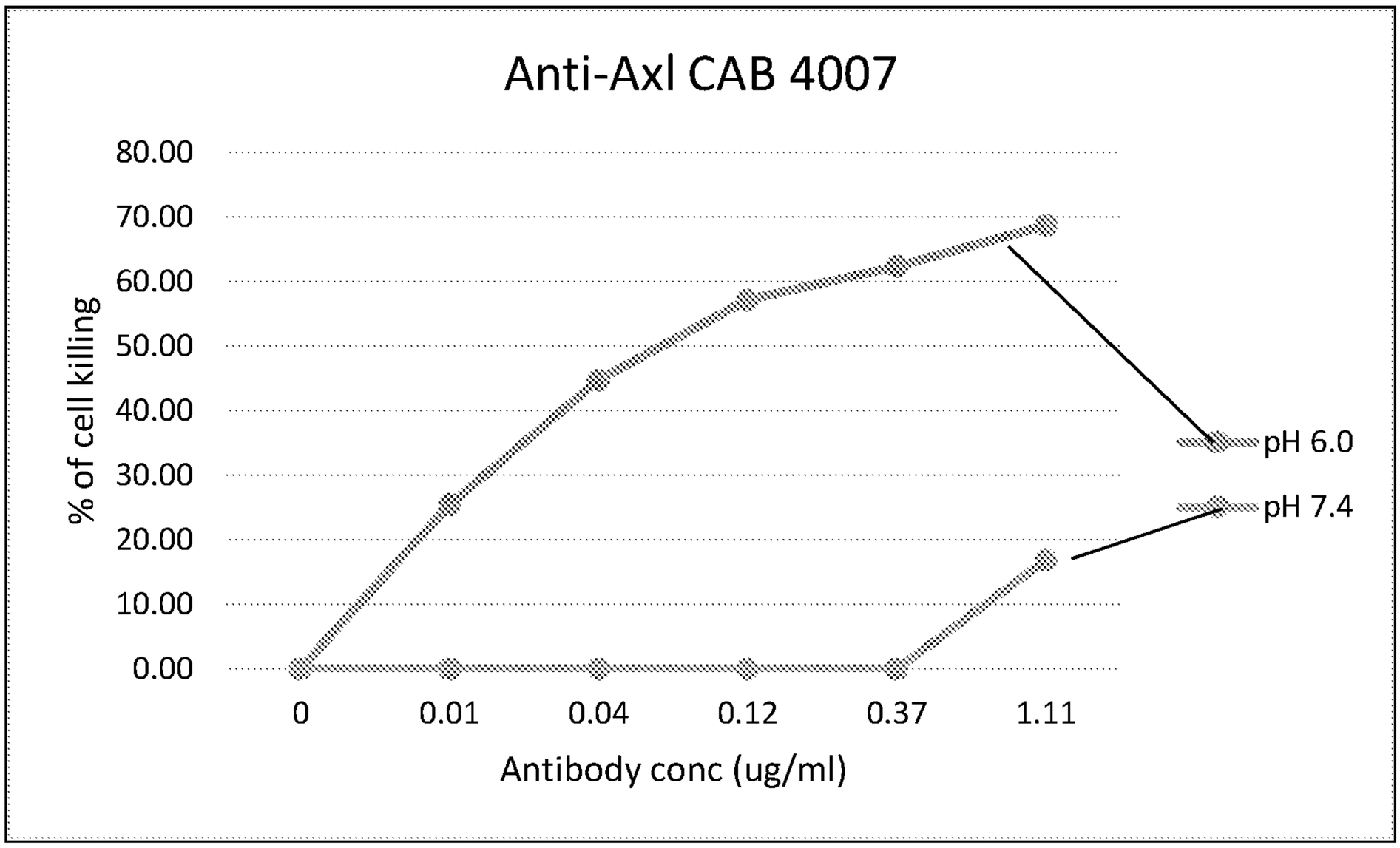

Anti-Axl CAB 4007
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
% of cell killing
0
0.01
0.04
0.12
0.37
1.11
Antibody conc (ug/ml)
pH 6.0
pH 7.4

FIG. 8D

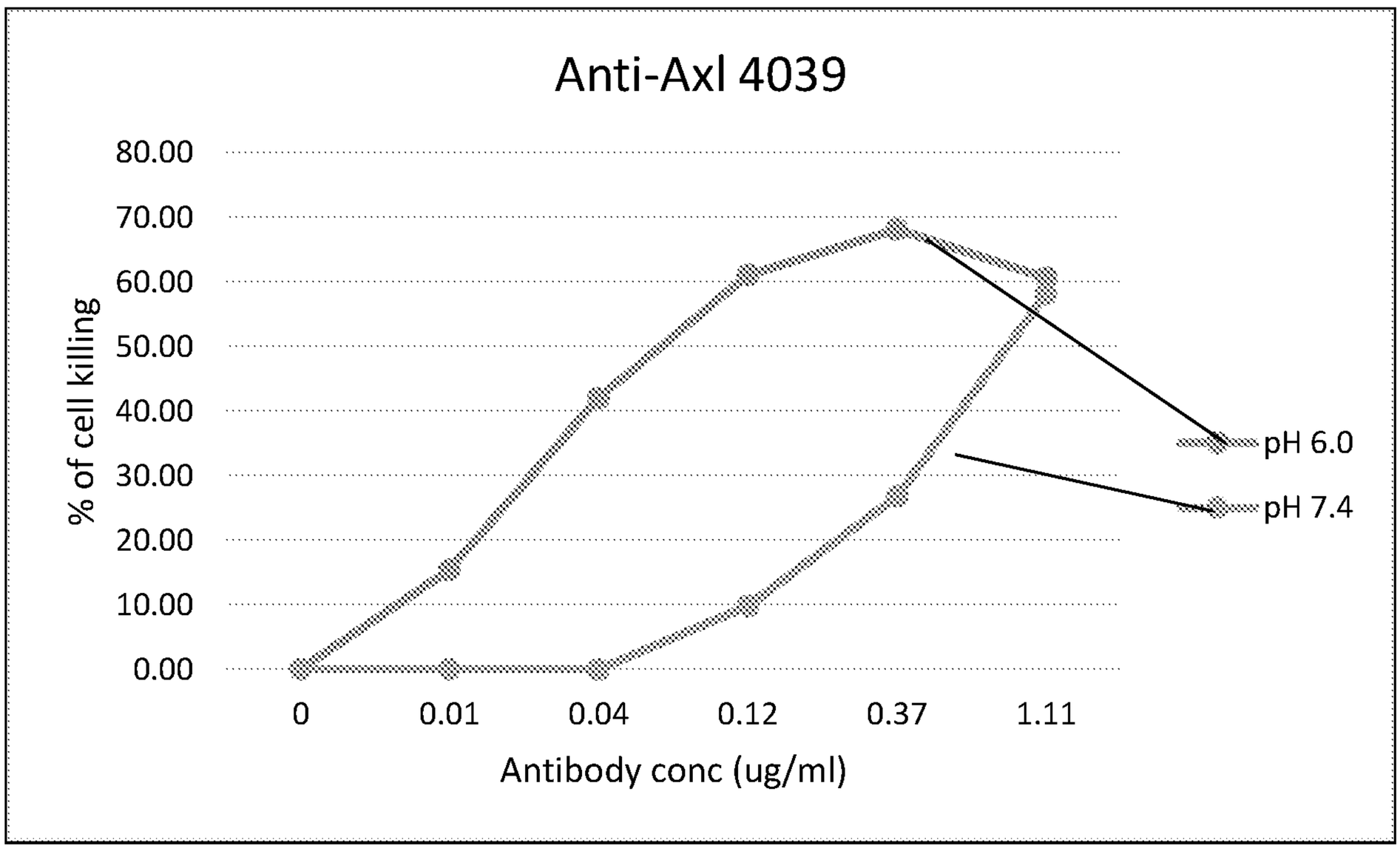

Anti-Axl 4039
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
% of cell killing
0
0.01
0.04
0.12
0.37
1.11
Antibody conc (ug/ml)
pH 6.0
pH 7.4

FIG. 8E

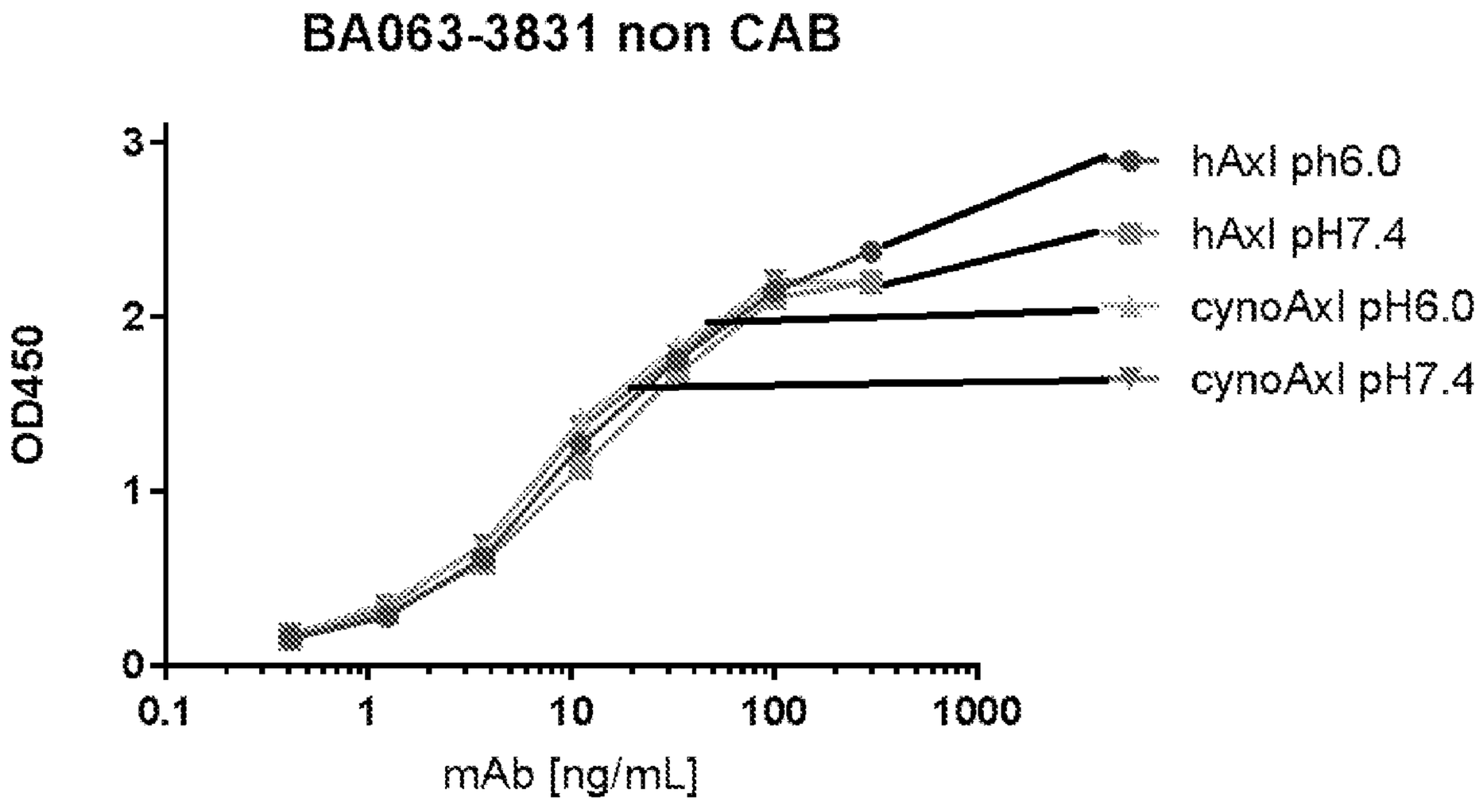

BA063-3831 non CAB
OD450
3
2
1
0
0.1
1
10
100
1000
mAb [ng/mL]
hAxl ph6.0
hAxl pH7.4
cynoAxl pH6.0
cynoAxl pH7.4

FIG. 9A

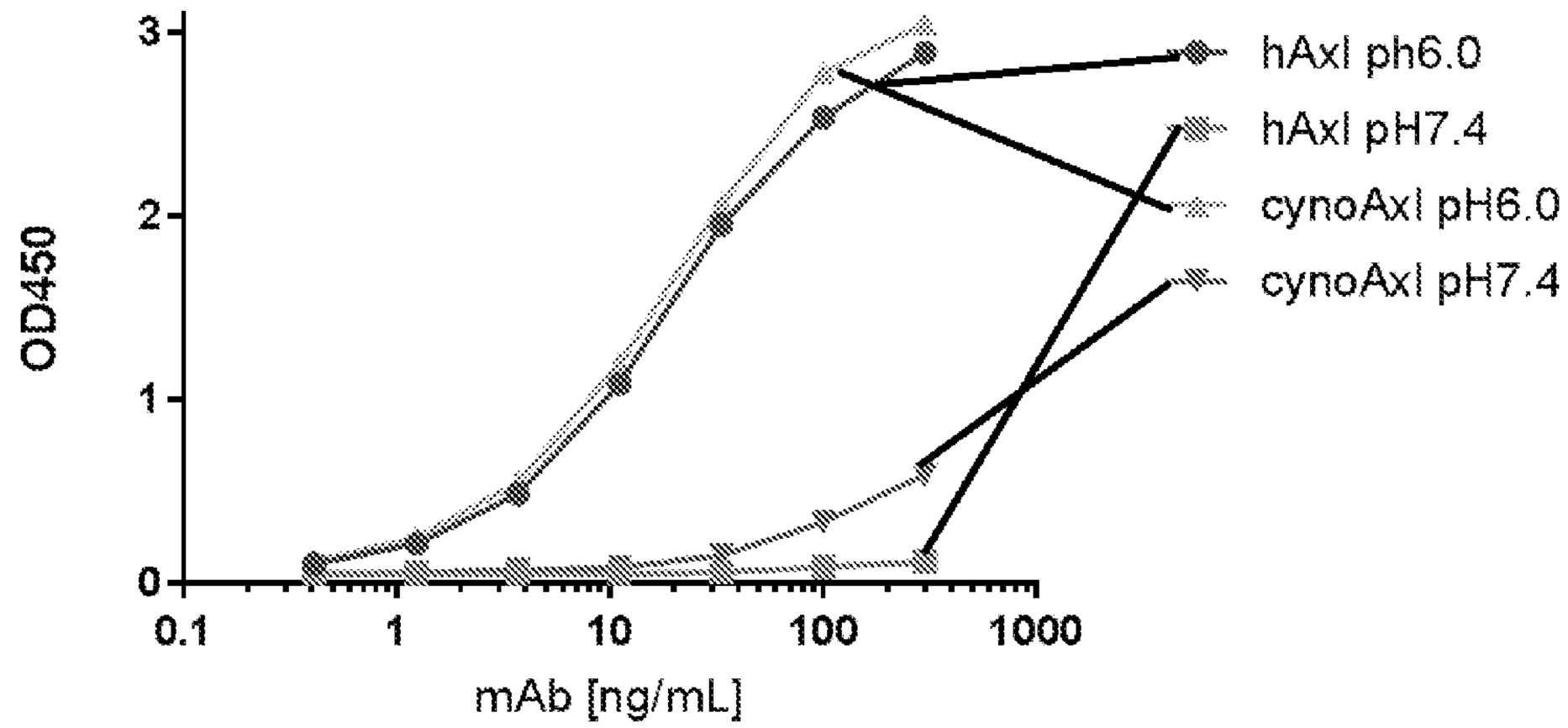

BA063-3998 CAB
OD450
3
2
1
0
0.1
1
10
100
1000
mAb [ng/mL]
hAxl ph6.0
hAxl pH7.4
cynoAxl pH6.0
cynoAxl pH7.4

FIG. 9B

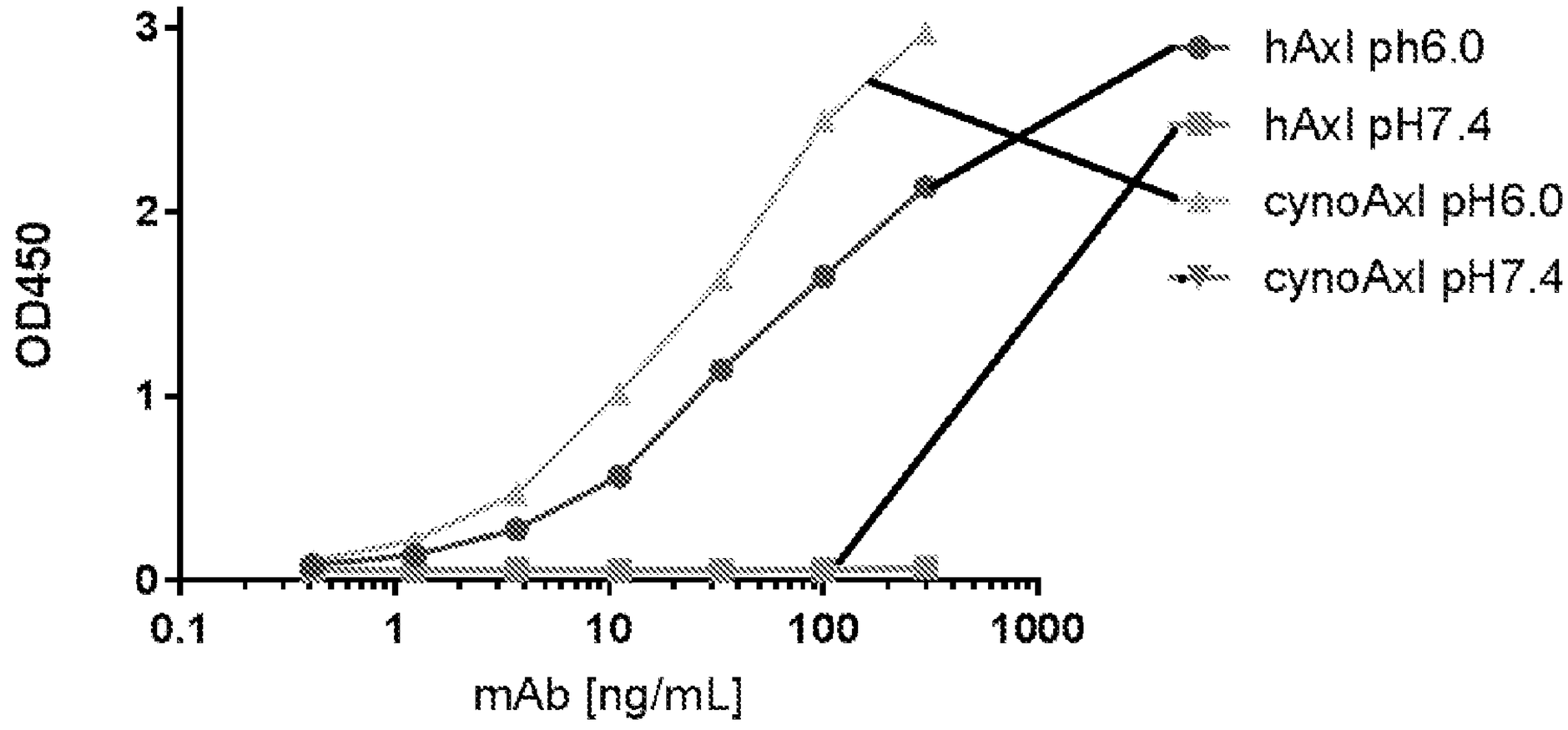

BA063-3999 CAB
OD450
3
2
1
0
0.1
1
10
100
1000
mAb [ng/mL]
hAxl ph6.0
hAxl pH7.4
cynoAxl pH6.0
cynoAxl pH7.4

FIG. 9C

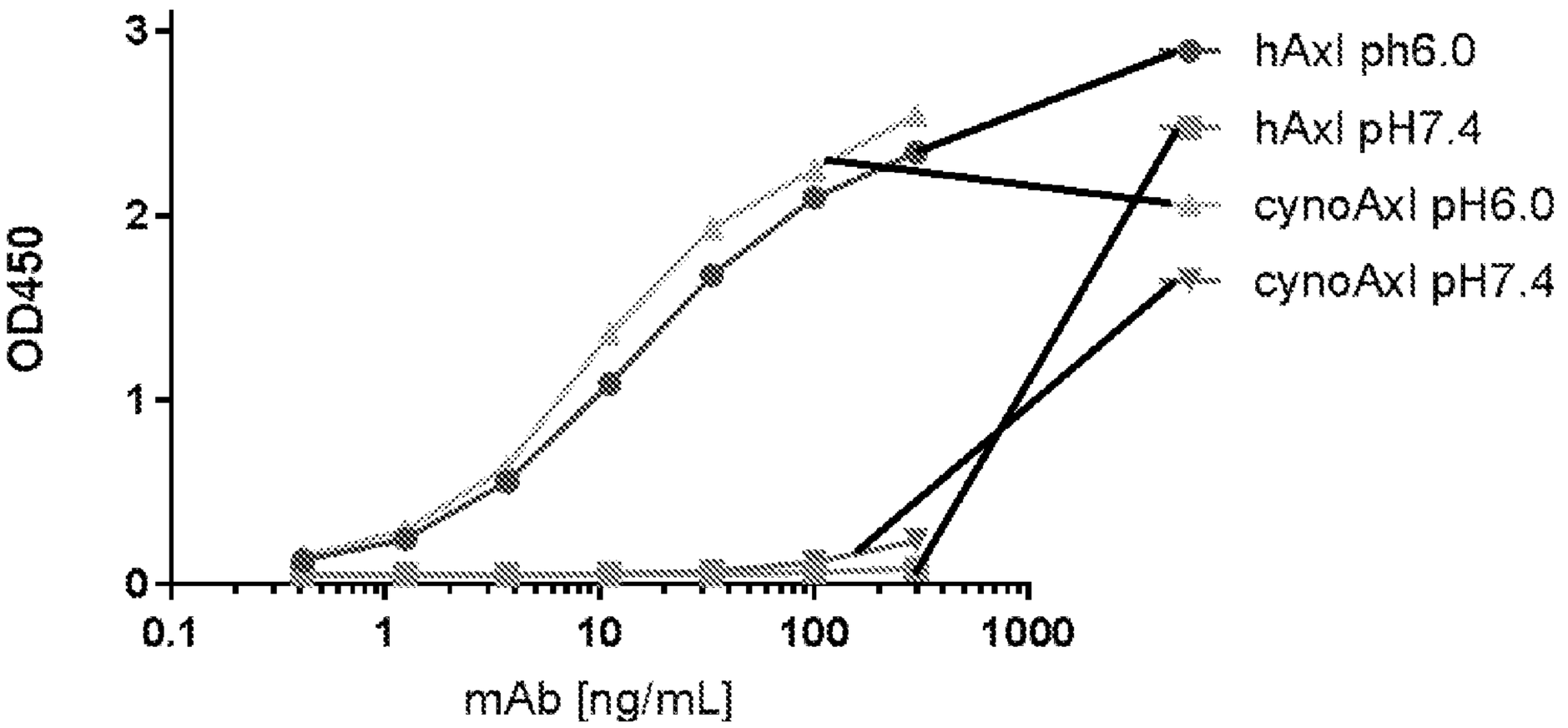

BA063-4007 CAB
OD450
3
2
1
0
0.1
1
10
100
1000
mAb [ng/mL]
hAxl ph6.0
hAxl pH7.4
cynoAxl pH6.0
cynoAxl pH7.4

FIG. 9D

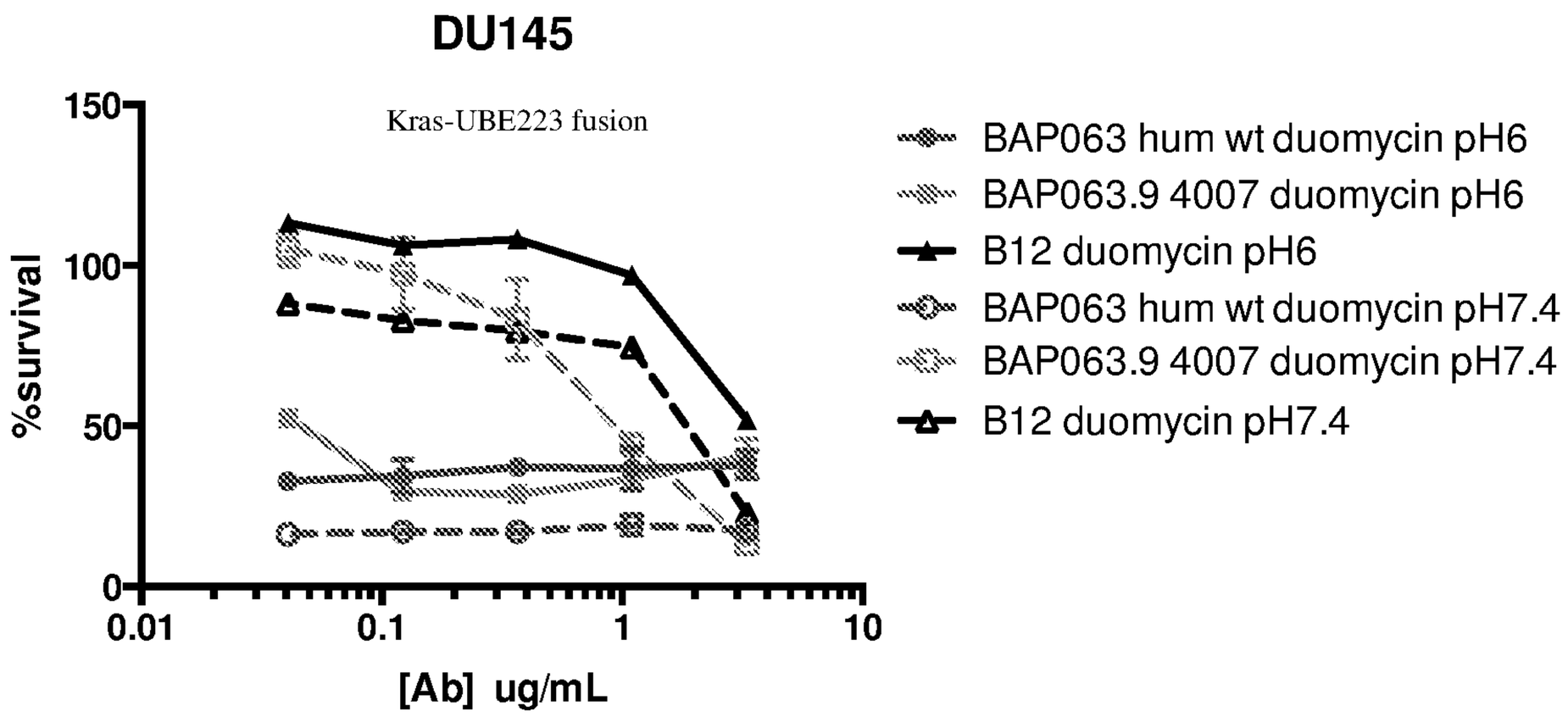


FIG. 10A

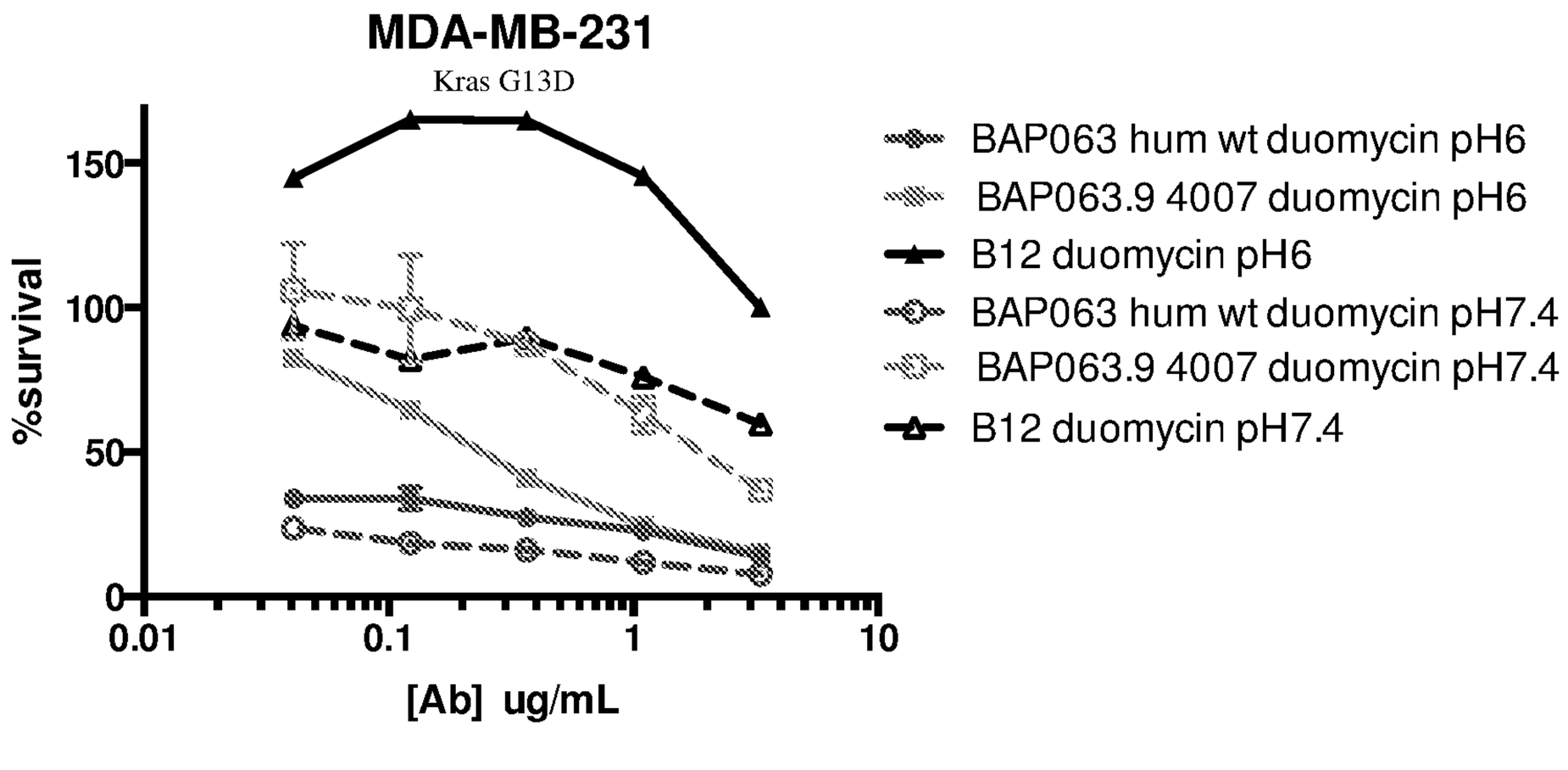


FIG. 10B

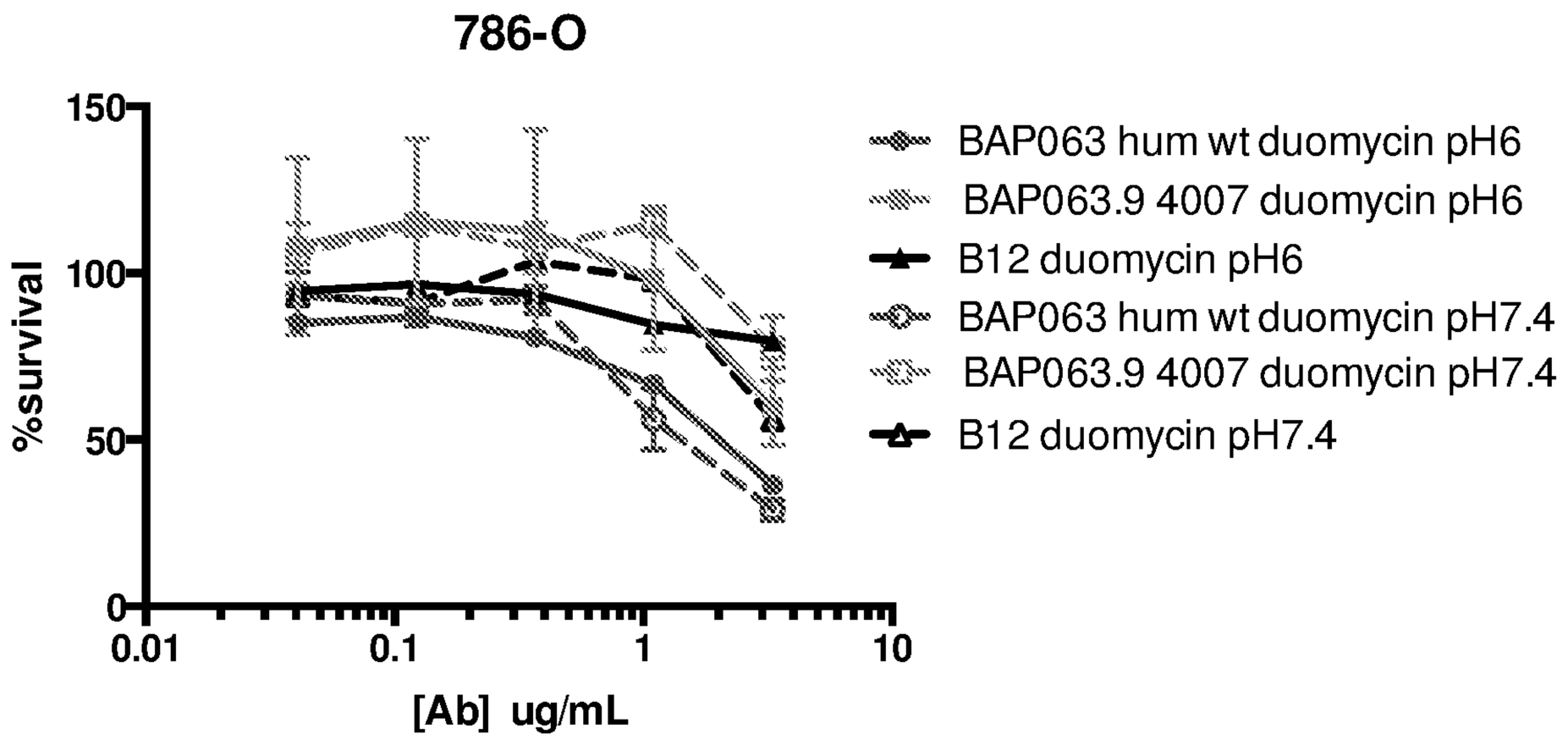


FIG. 10C

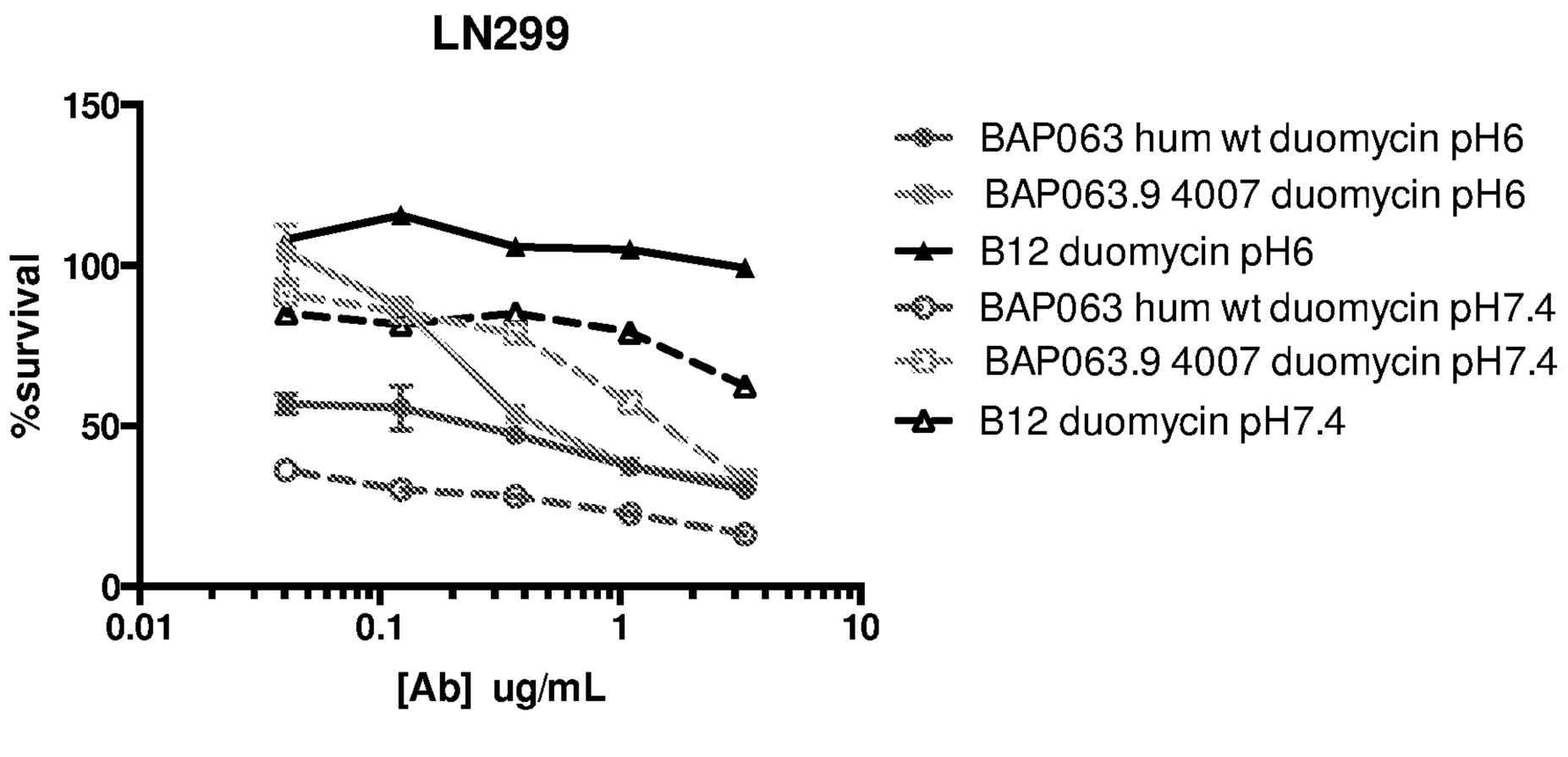


FIG. 10D

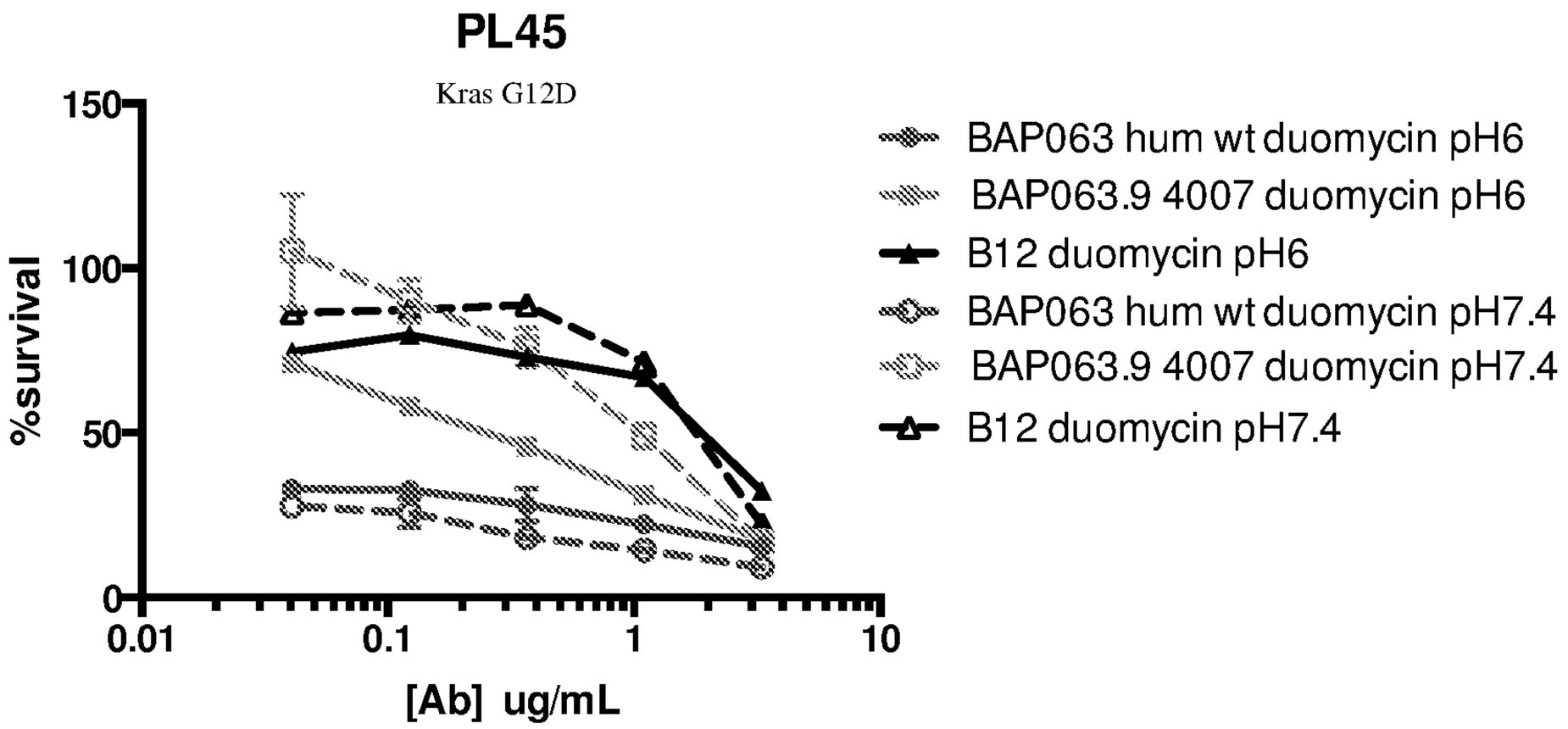


FIG. 10E

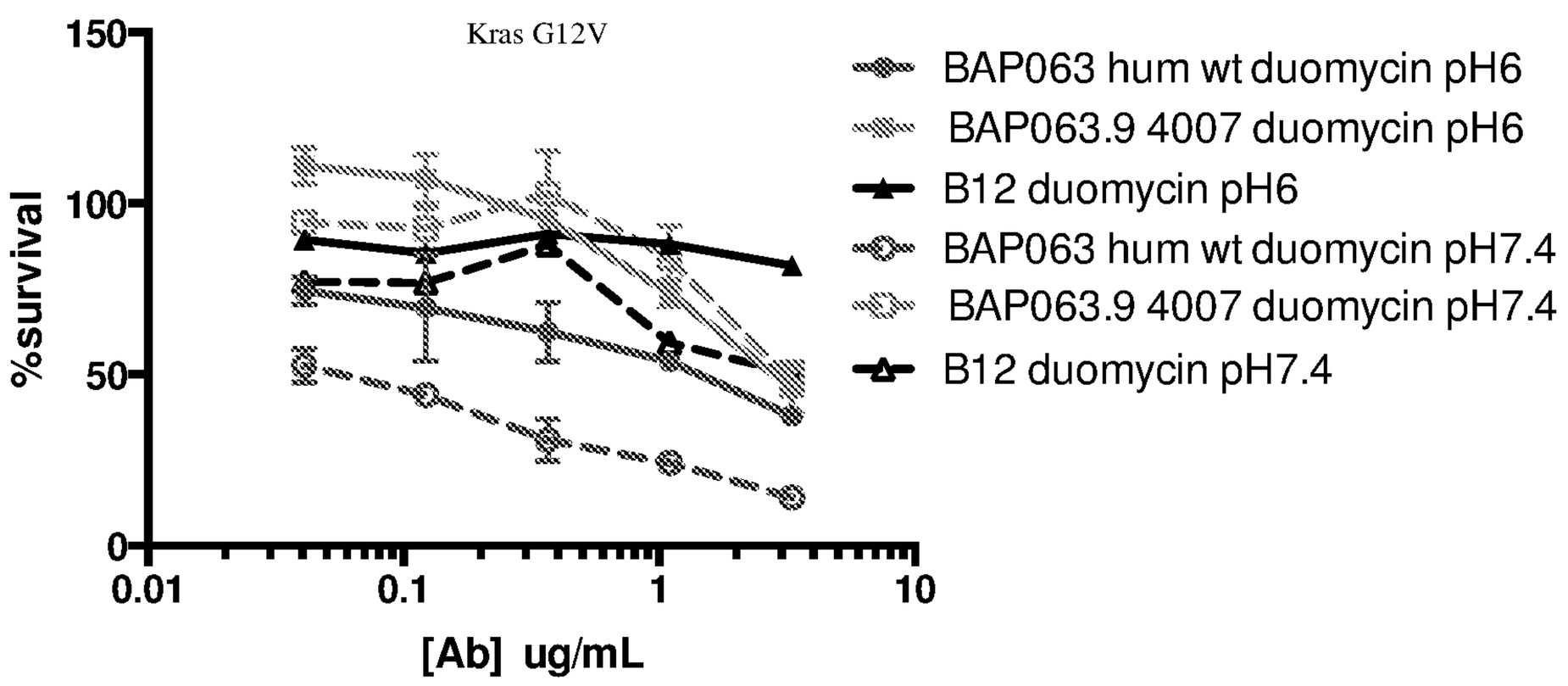


FIG. 10F

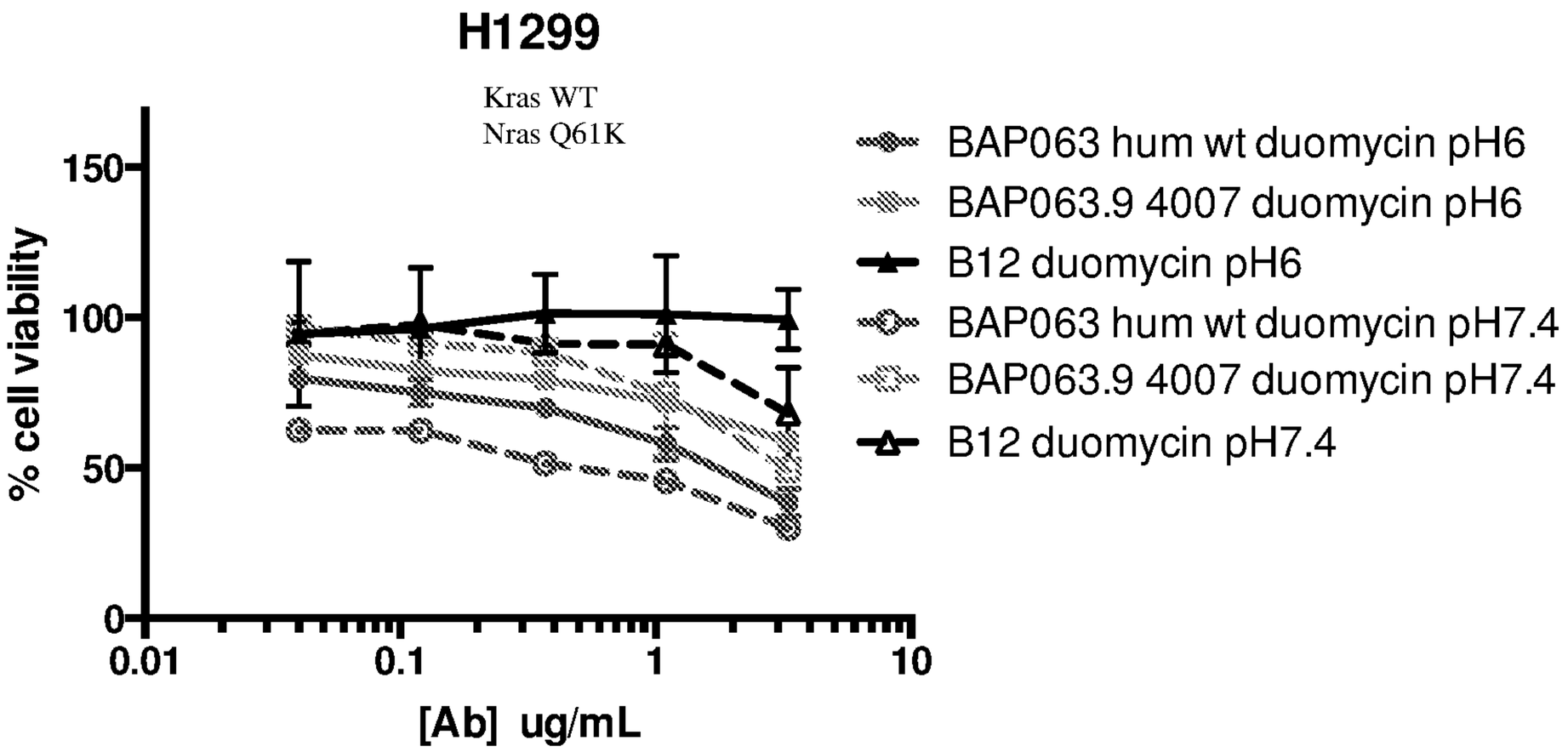


FIG. 10G

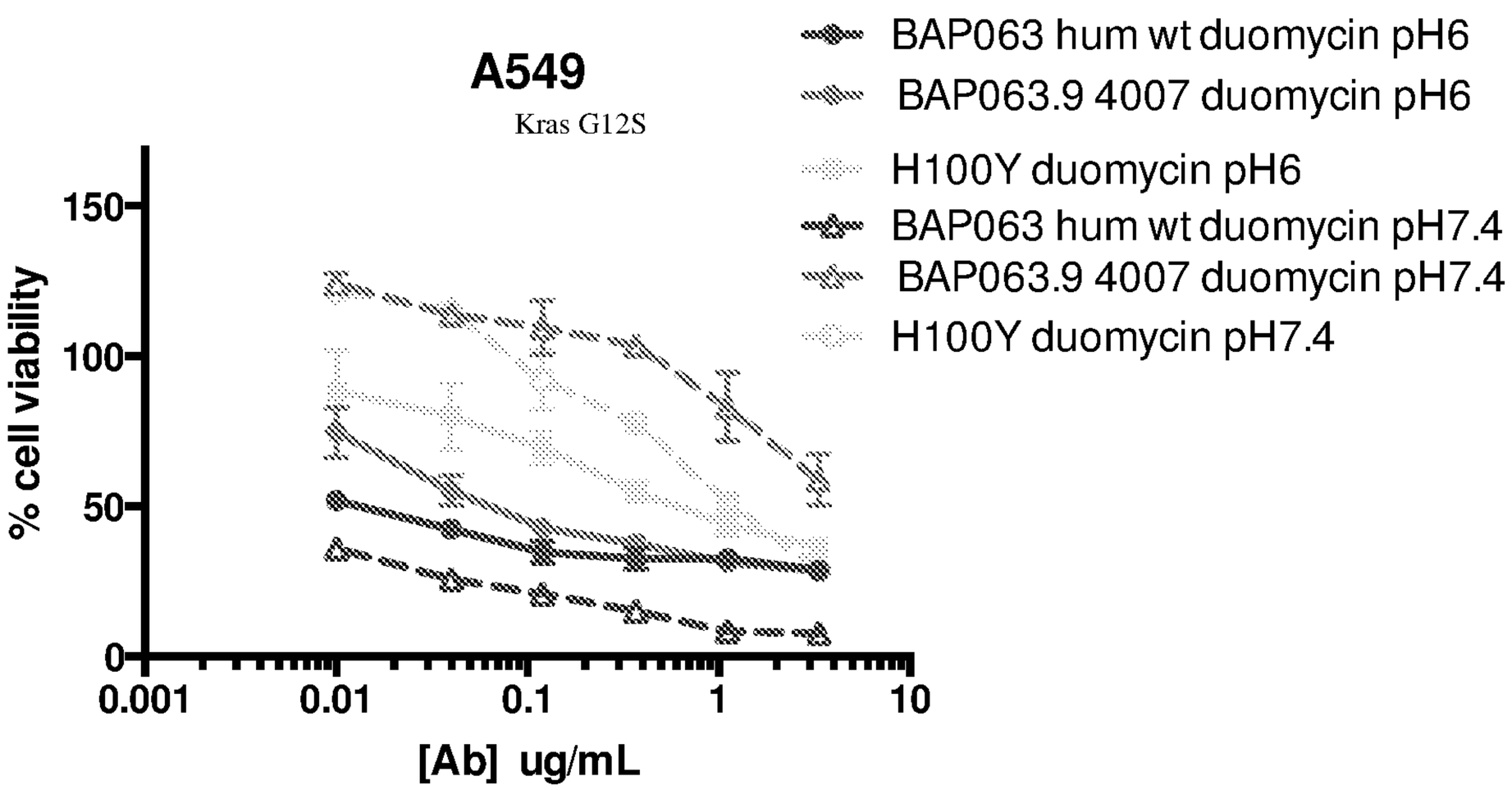


FIG. 10H

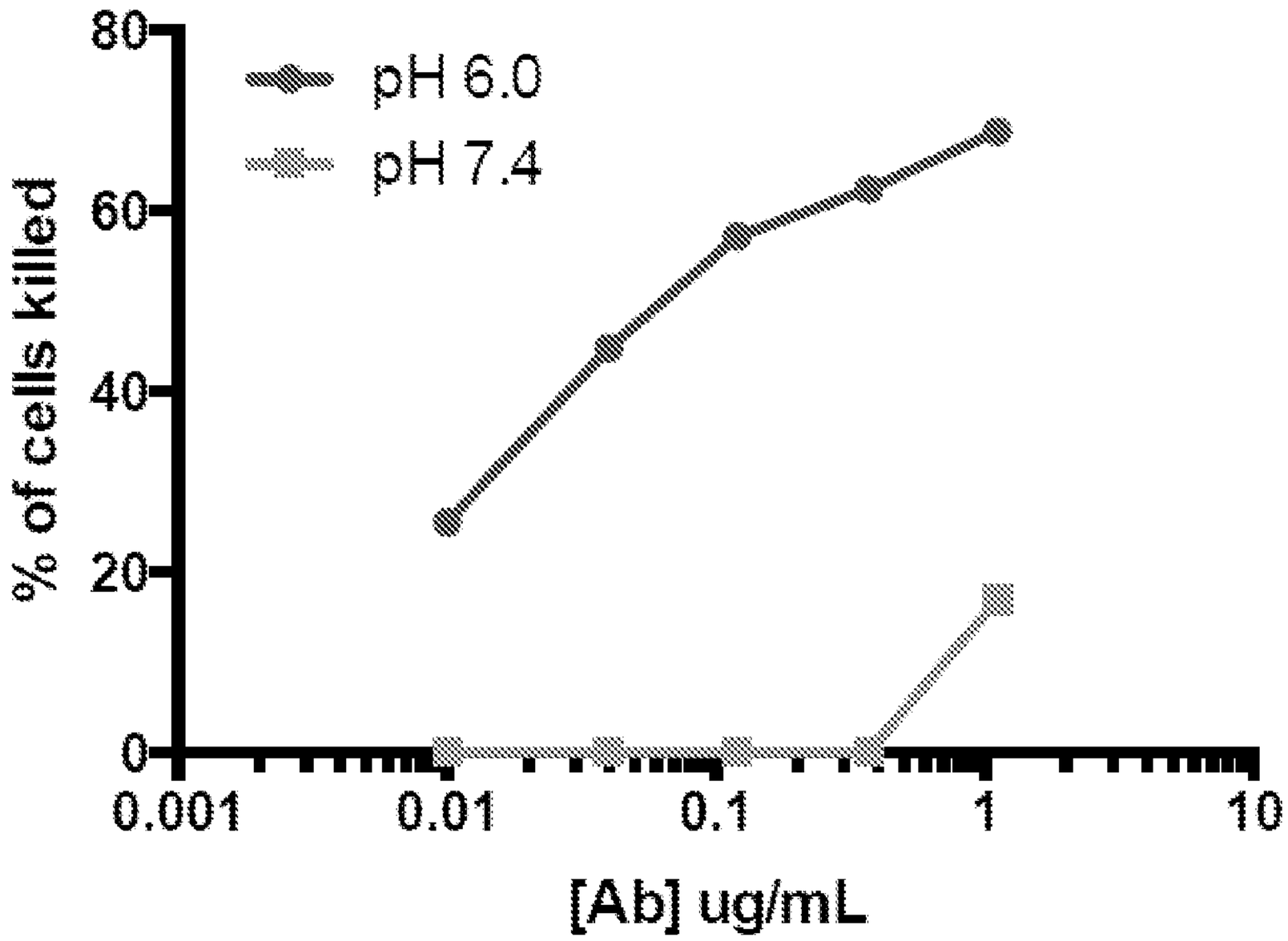

80
60
40
20
0
% of cells killed
pH 6.0
pH 7.4
0.001
0.01
0.1
1
10
[Ab] ug/mL

FIG. 11

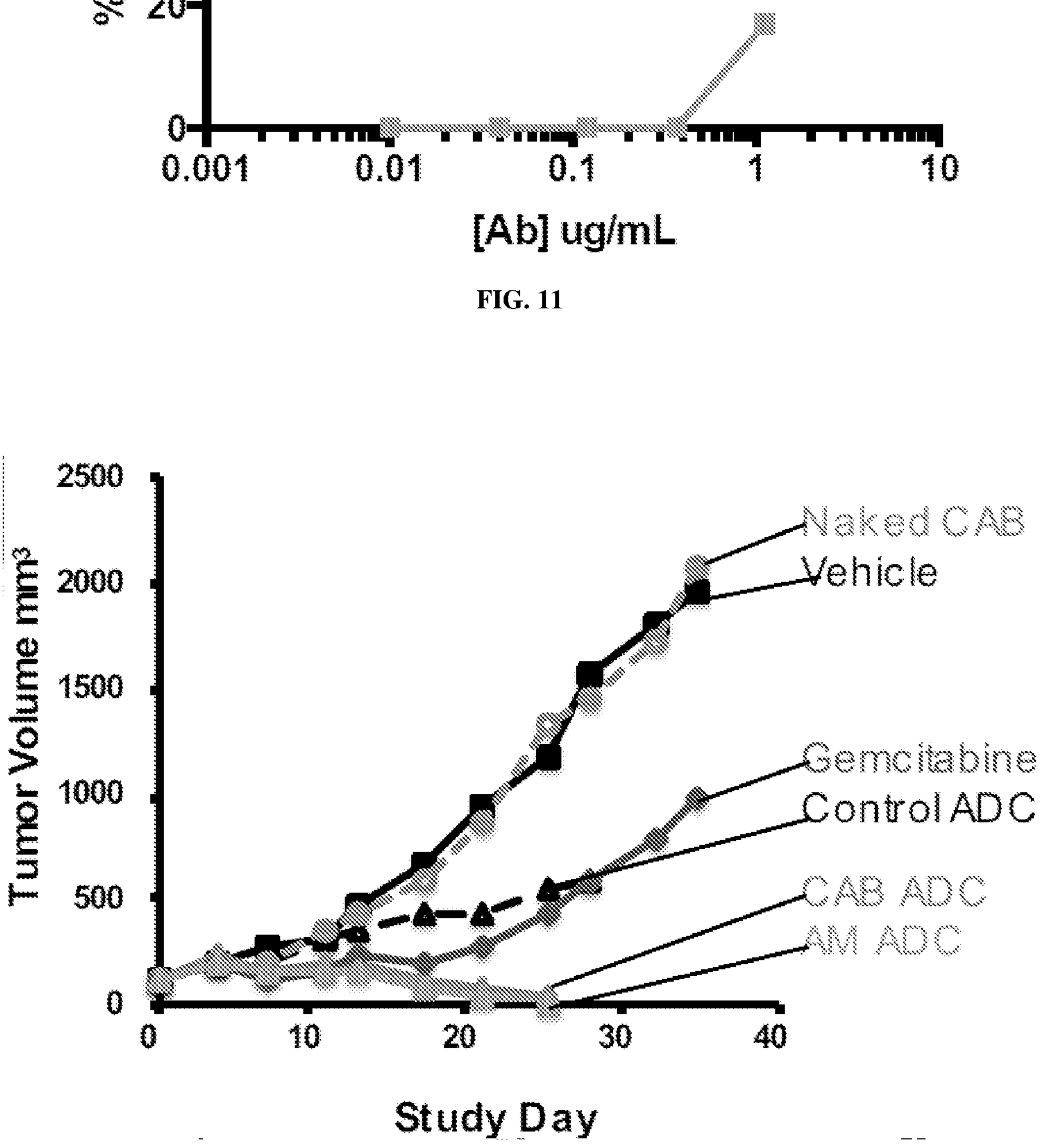

2500
2000
1500
1000
500
0
Tumor Volume mm3
Naked CAB
Vehicle
Gemcitabine
Control ADC
CAB ADC
AM ADC
0
10
20
30
40
Study Day

FIG. 12

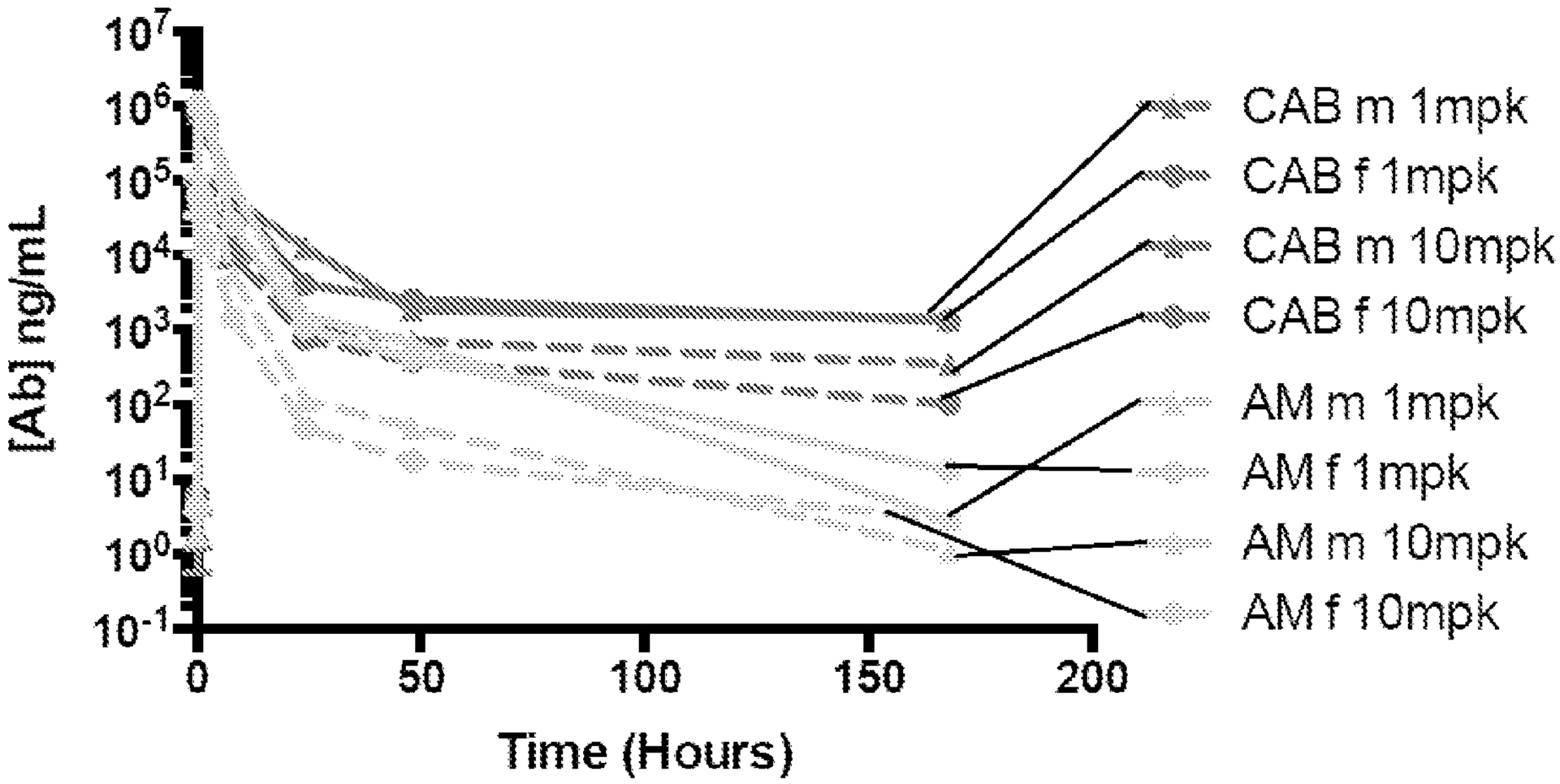


FIG. 13A

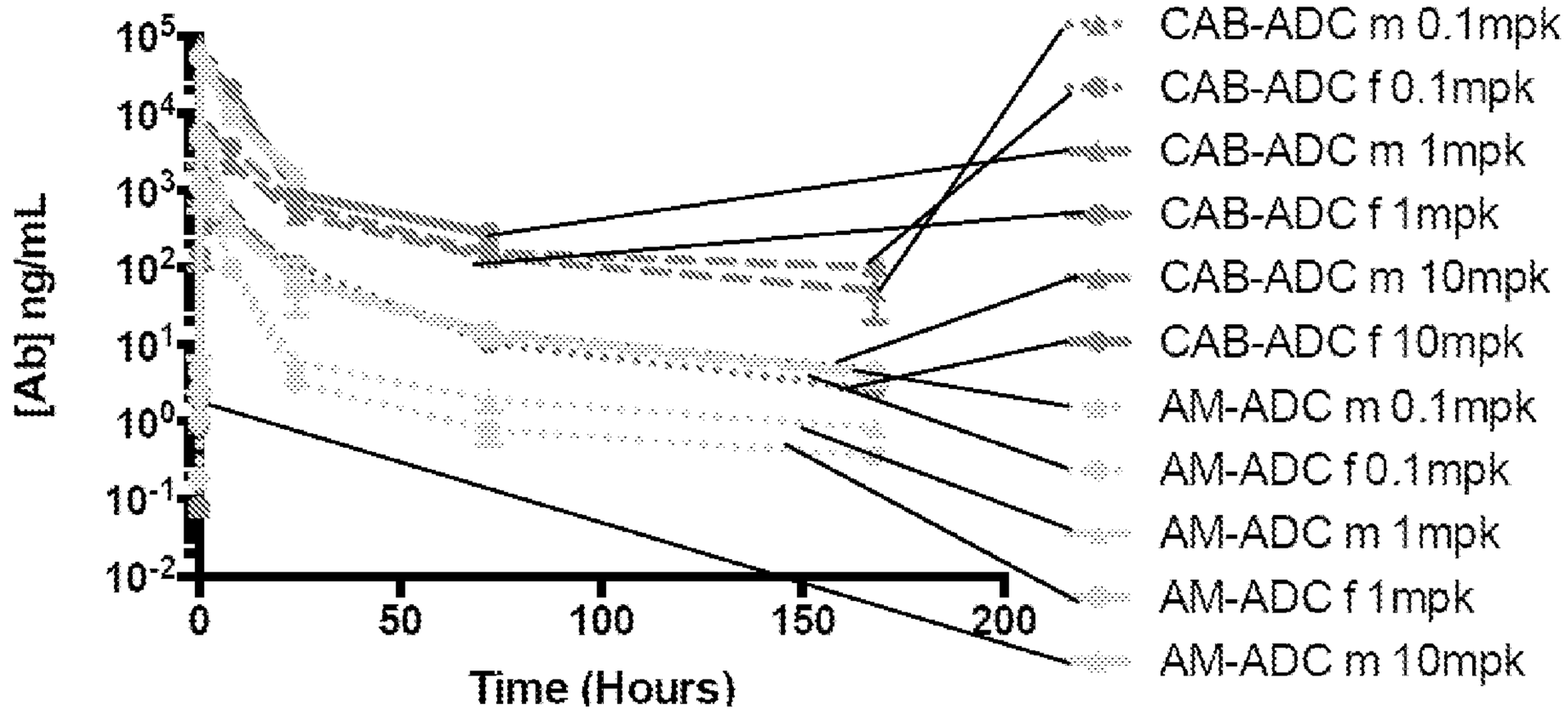


FIG. 13B

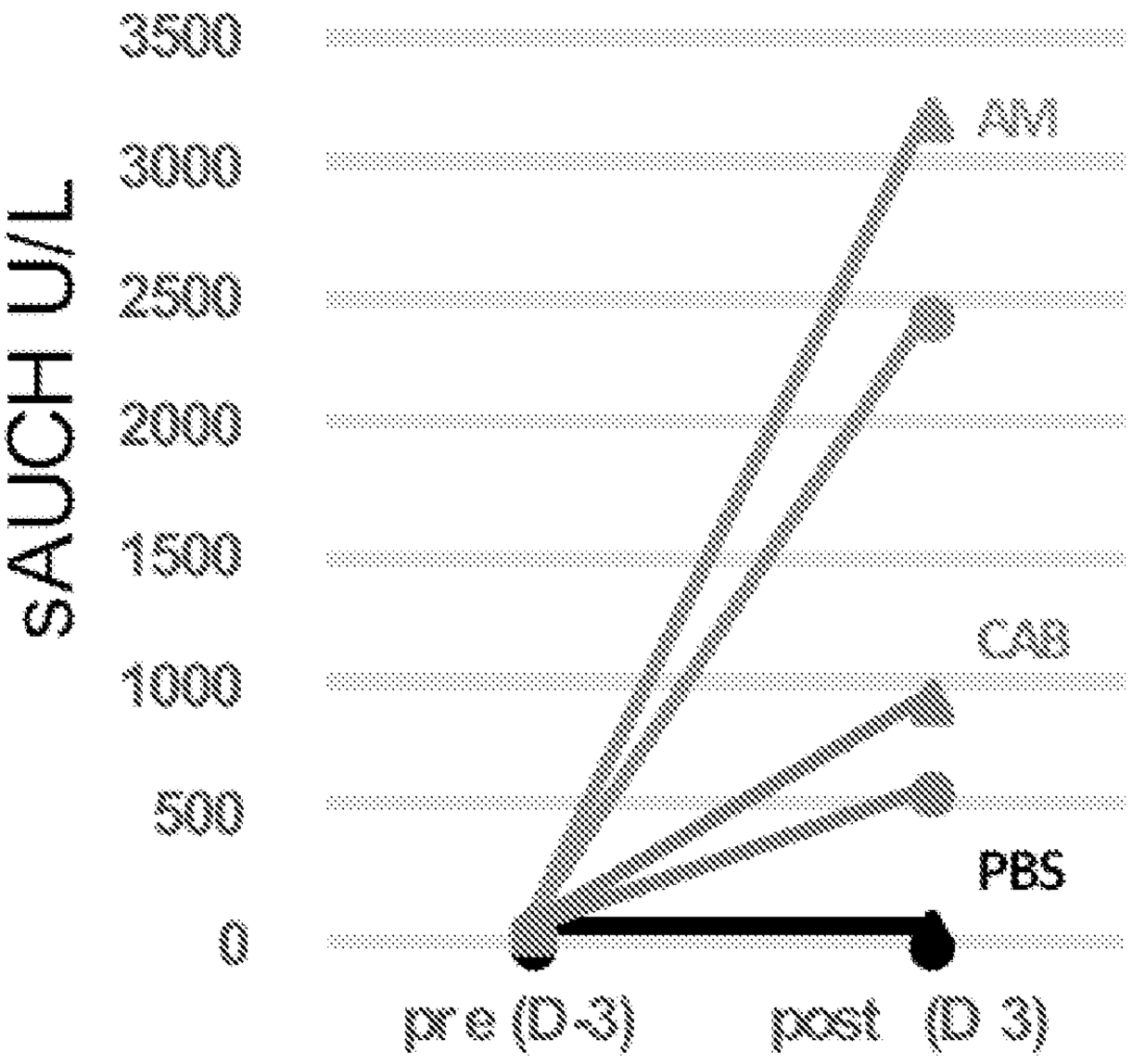


FIG. 14A

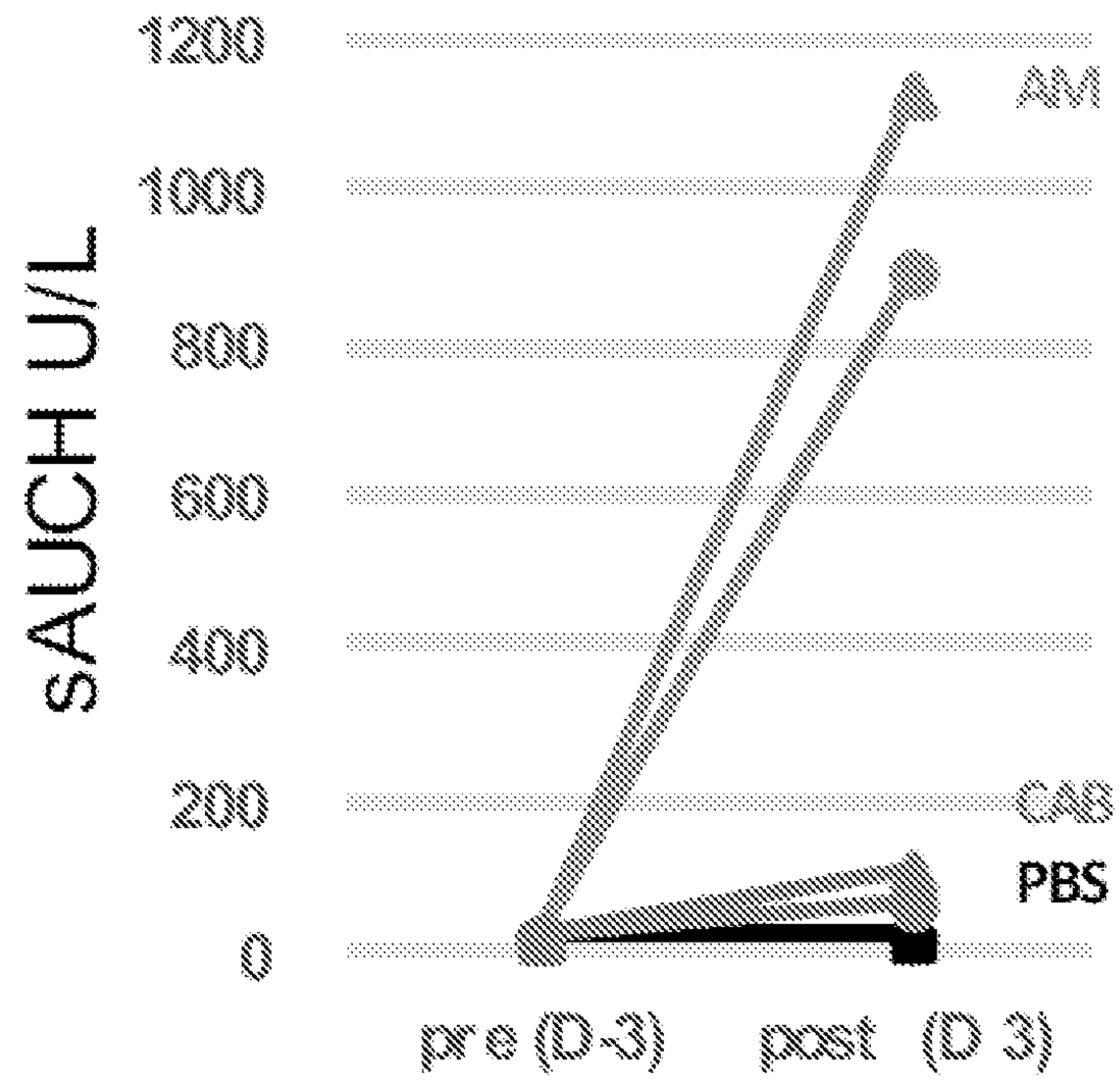


FIG. 14B

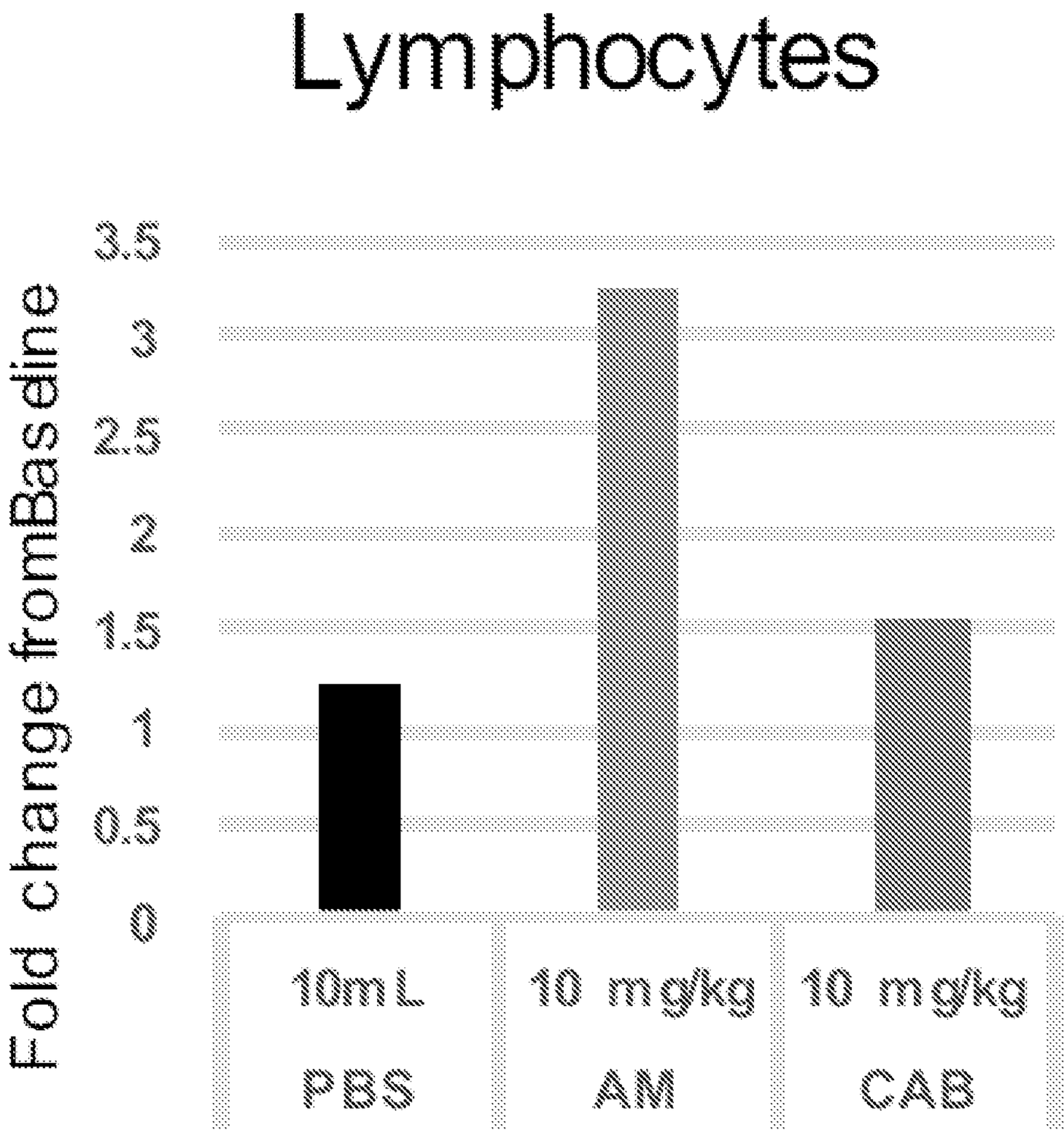


FIG. 15

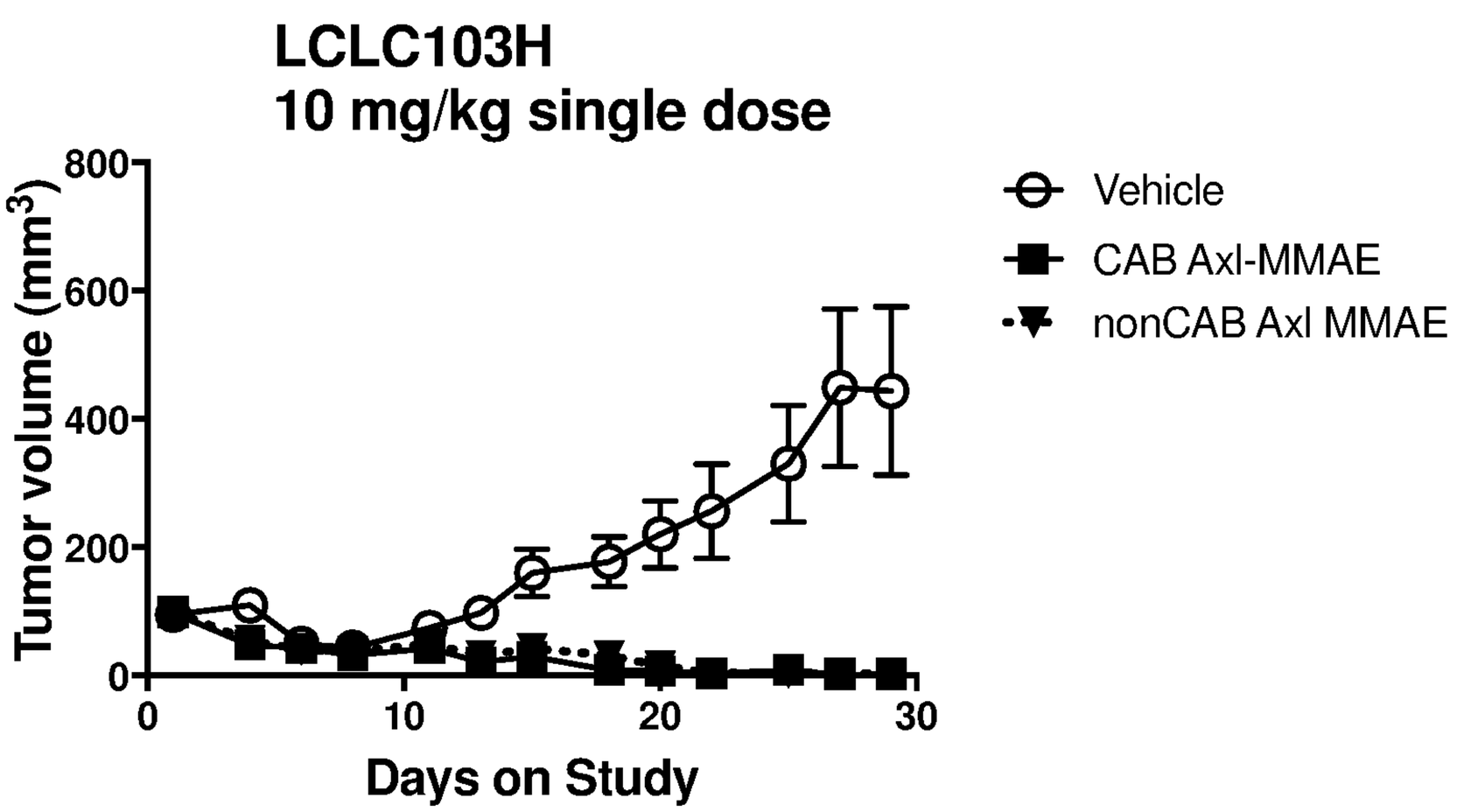


FIG. 16A

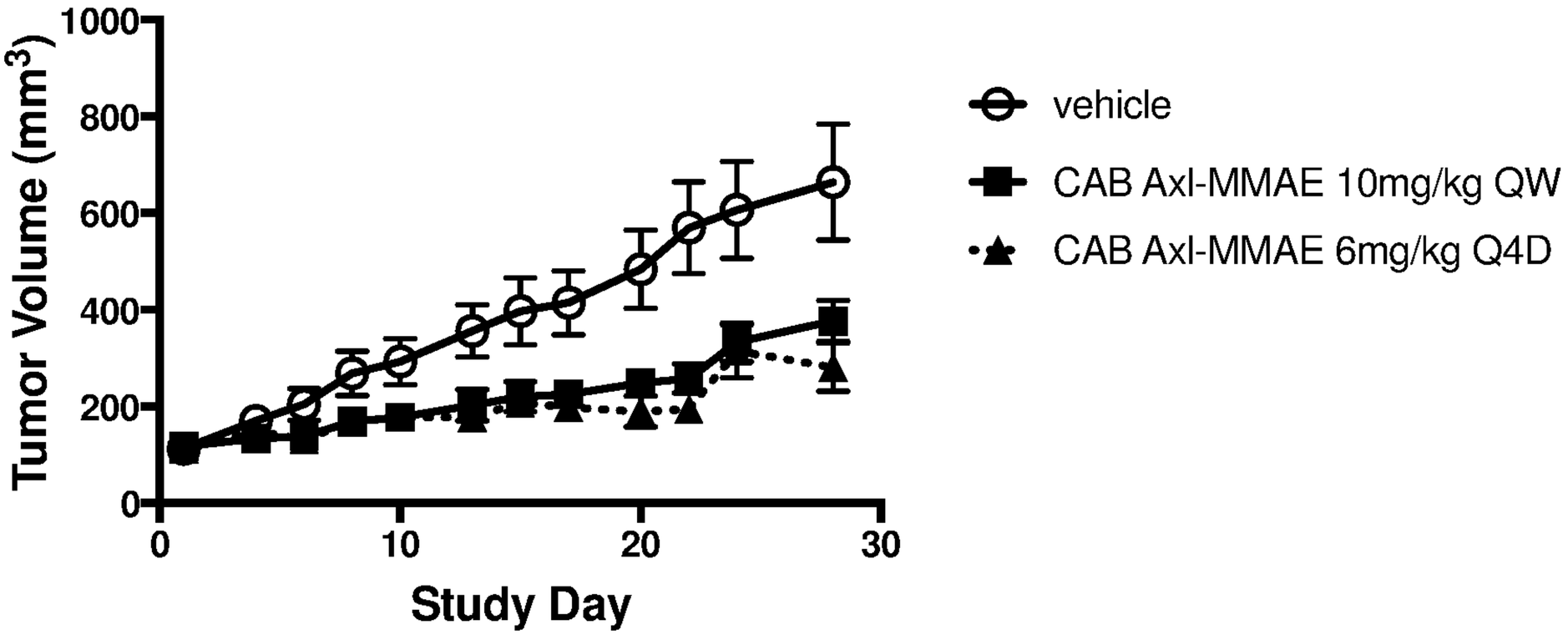


FIG. 16B

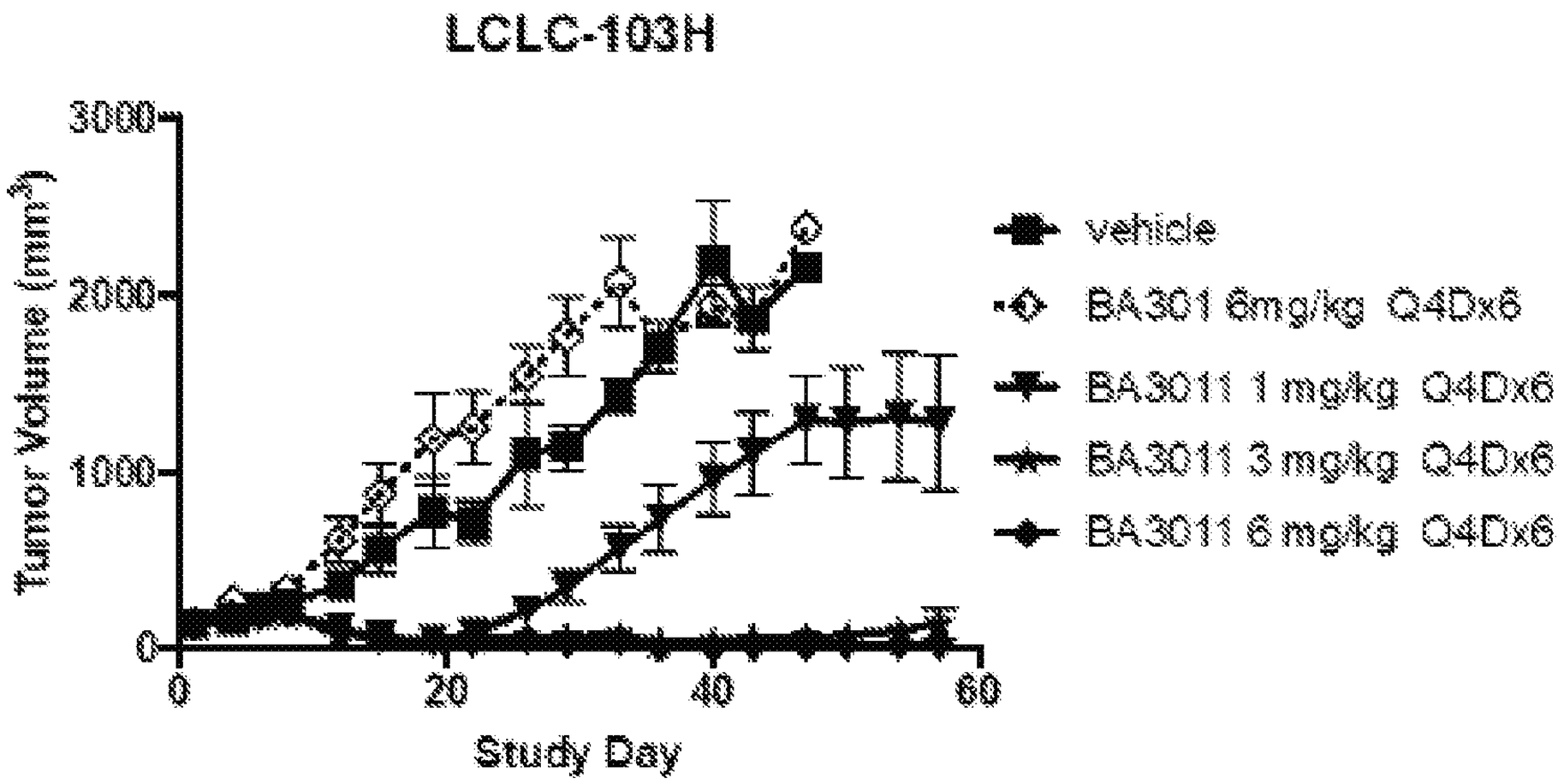


FIG. 17A

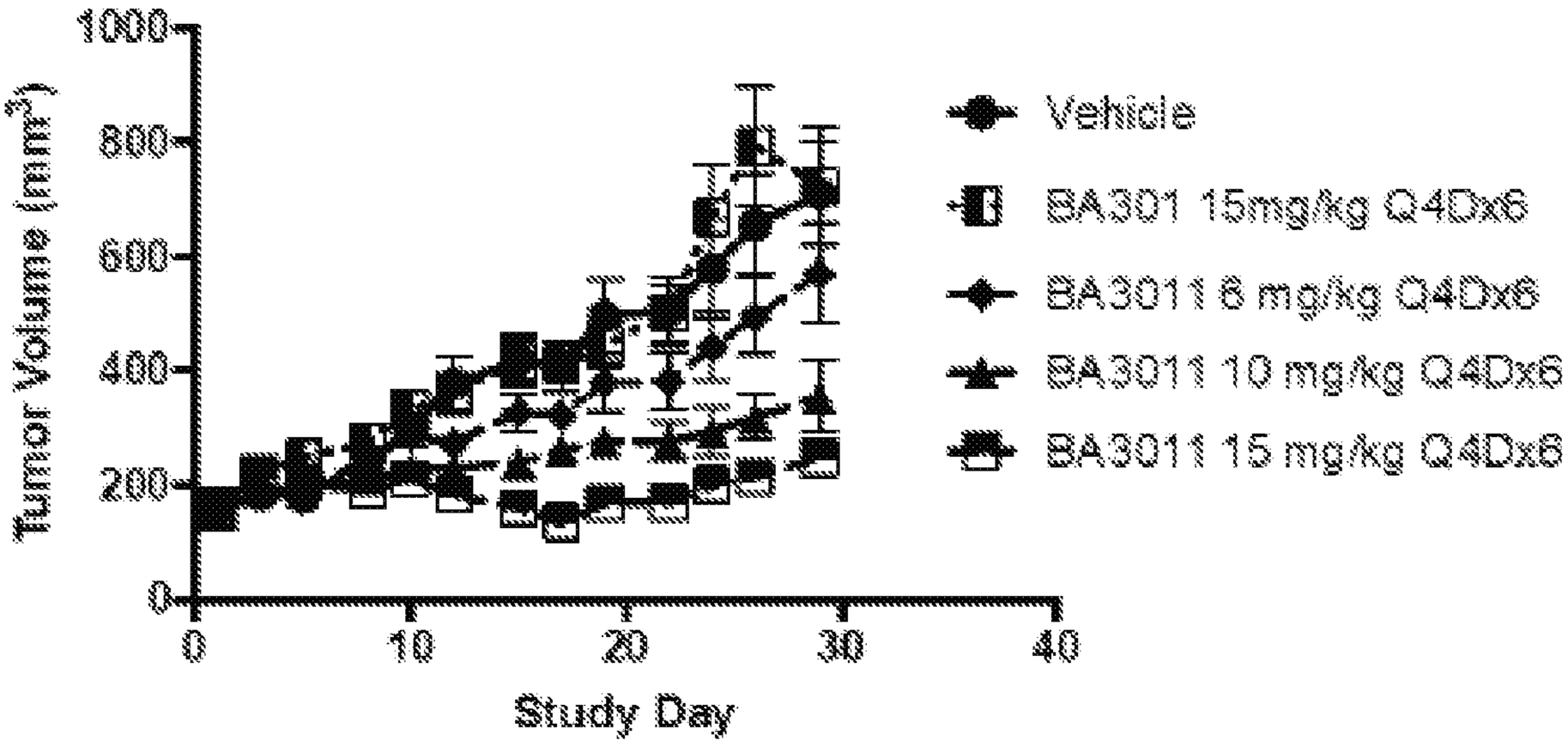


FIG. 17B

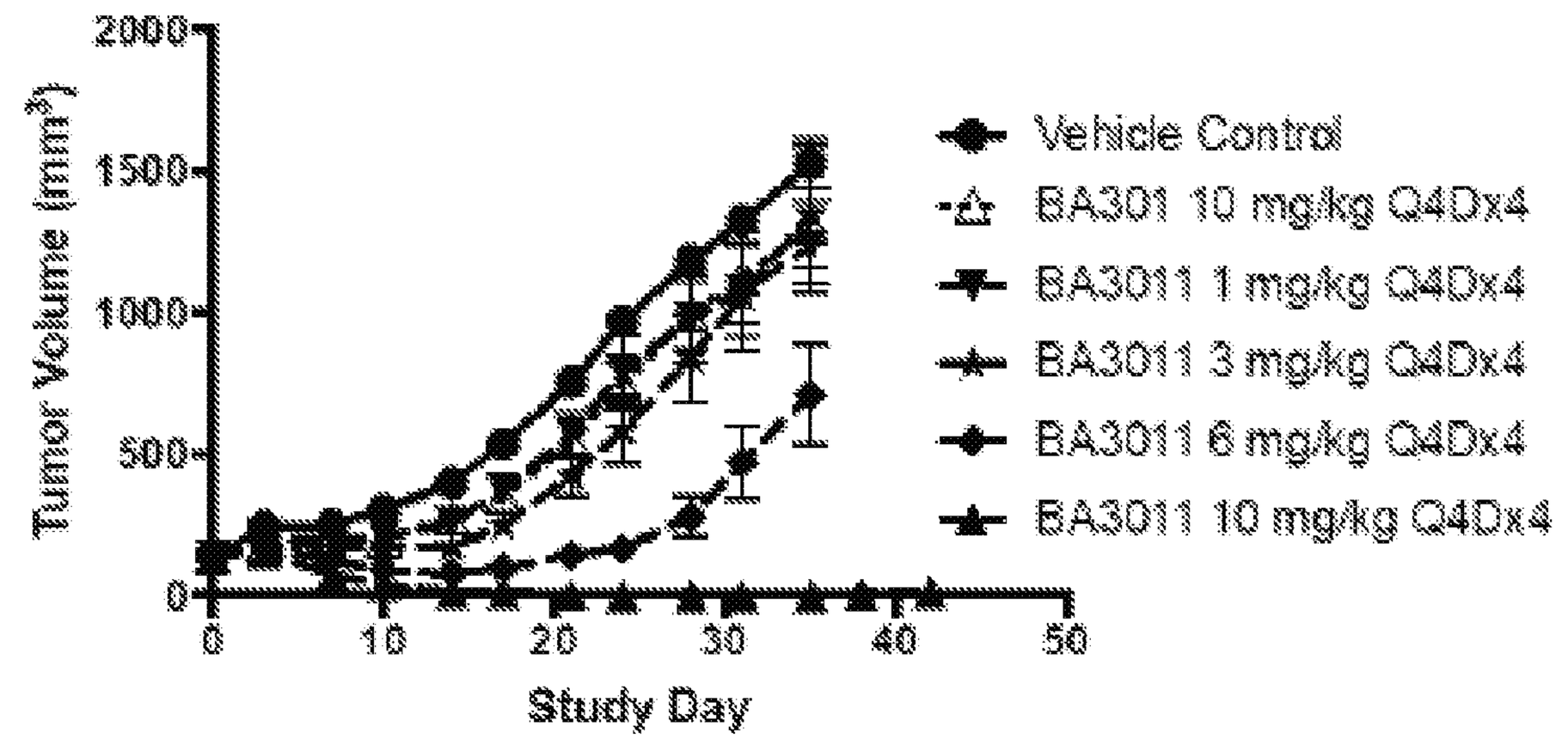


FIG. 17C

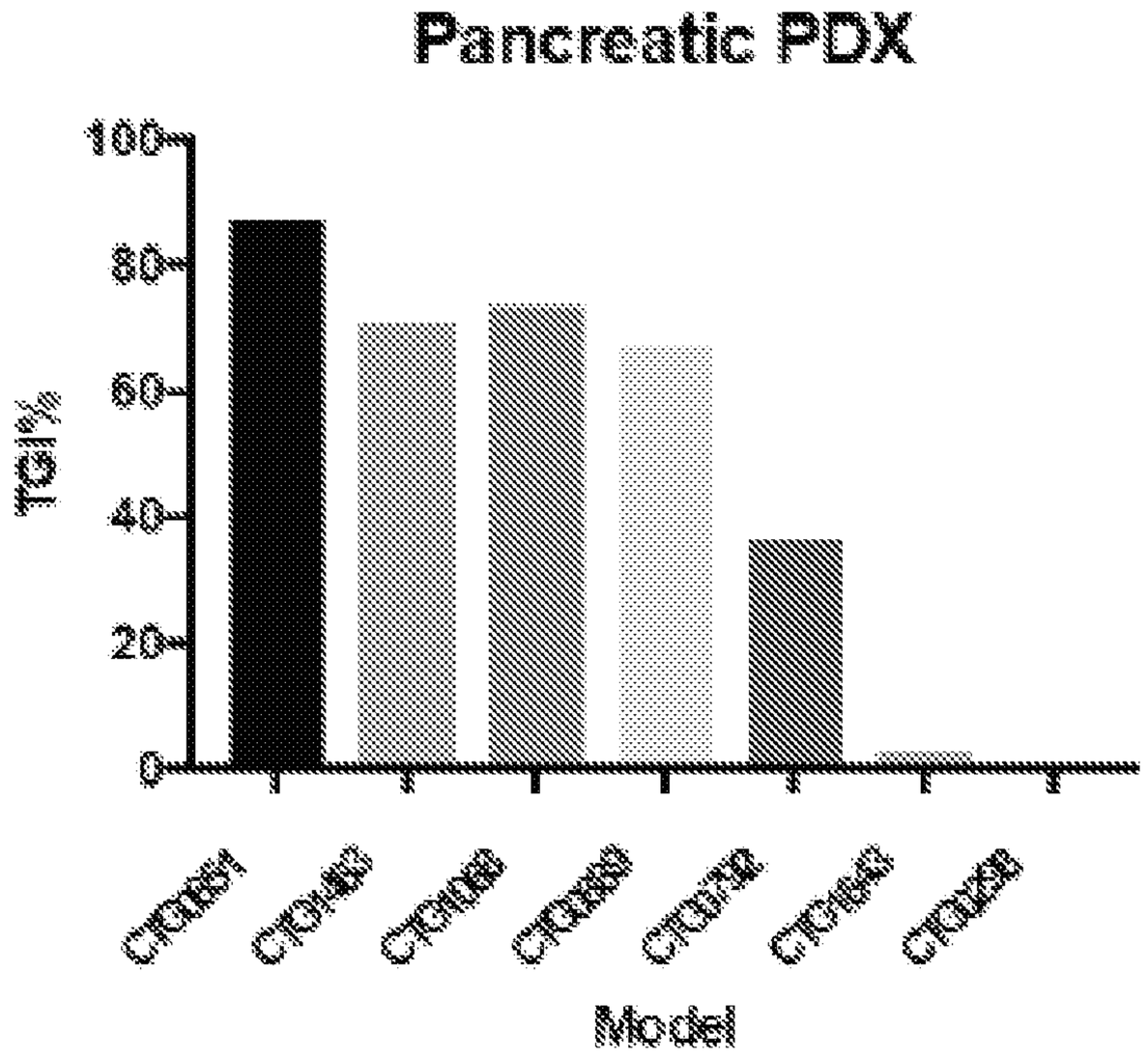


FIG. 17D

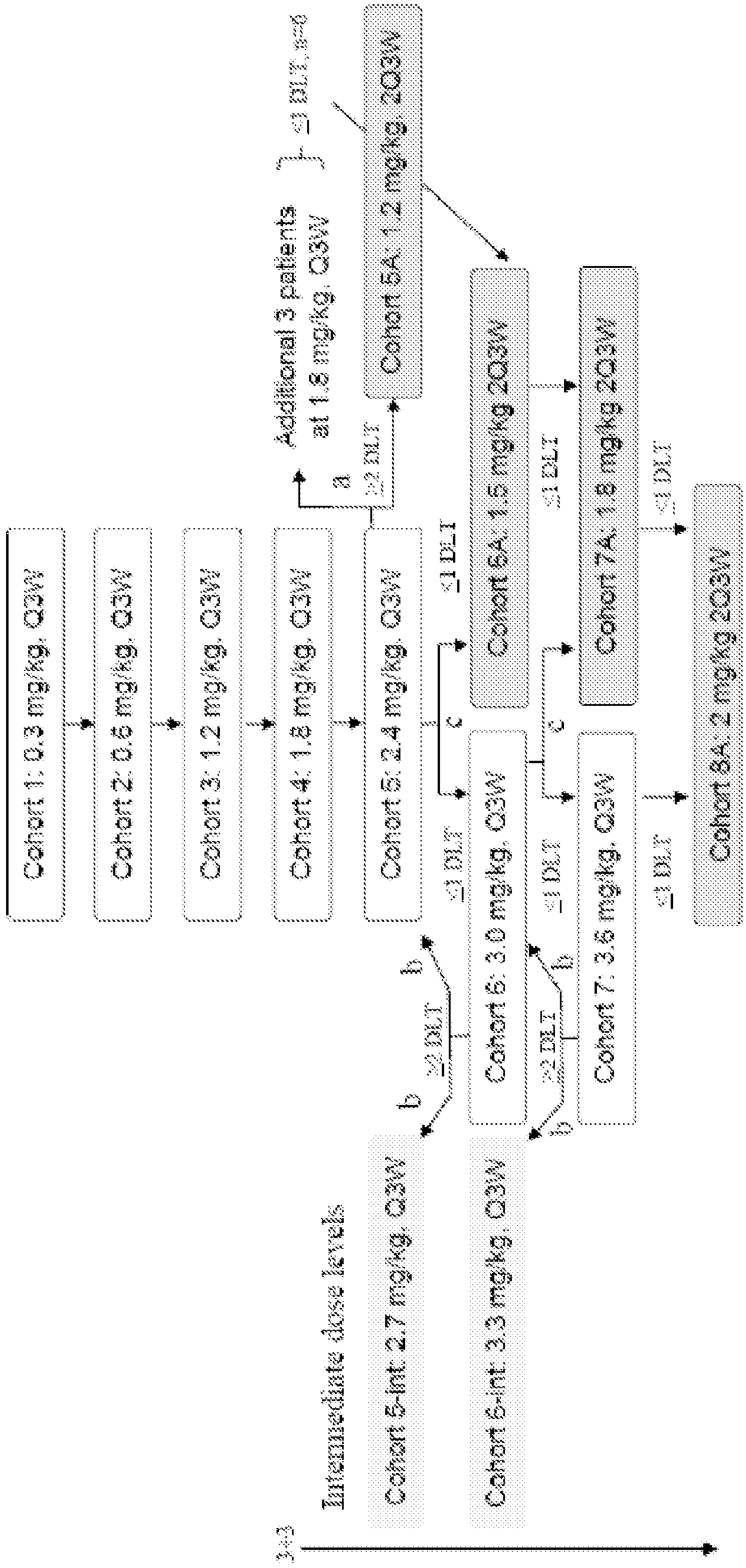

Cohort 1: 0.3 mg/kg, Q3W
Cohort 2: 0.6 mg/kg, Q3W
Cohort 3: 1.2 mg/kg, Q3W
Cohort 4: 1.8 mg/kg, Q3W
Cohort 5: 2.4 mg/kg, Q3W
≤1 DLT
≥2 DLT
a
Additional 3 patients at 1.8 mg/kg, Q3W
Cohort 5A: 1.2 mg/kg, Q3W
≤1 DLT
Cohort 6A: 1.5 mg/kg Q3W
≤1 DLT
Cohort 7A: 1.8 mg/kg Q3W
≤1 DLT
Cohort 8A: 2 mg/kg Q3W
b
≥2 DLT
Cohort 6: 3.0 mg/kg, Q3W
≥2 DLT
Cohort 7: 3.6 mg/kg, Q3W
Intermediate dose levels
Cohort 5-int: 2.7 mg/kg, Q3W
Cohort 6-int: 3.3 mg/kg, Q3W
3+3

FIG. 18

Caption for Fig. 18

a, If ≥2 DLT, enroll up to 6 patients in associated 2Q3W cohort and enroll 3 additional patients in previous cohort in parallel, if not previously enrolled

b, If ≥2 DLT, enroll up to 6 patients in the intermediate cohort and enroll 3 additional patients in previous cohort in parallel, if not previously enrolled

c, If ≤1 DLT, enroll up to 6 patients in dose cohorts in parallel

Cohort 7A opens if ≤1 DLT in both Cohort 6 & 6A; Similarly, Cohort 8A opens if ≤1 DLT in both Cohort 7 & 7A

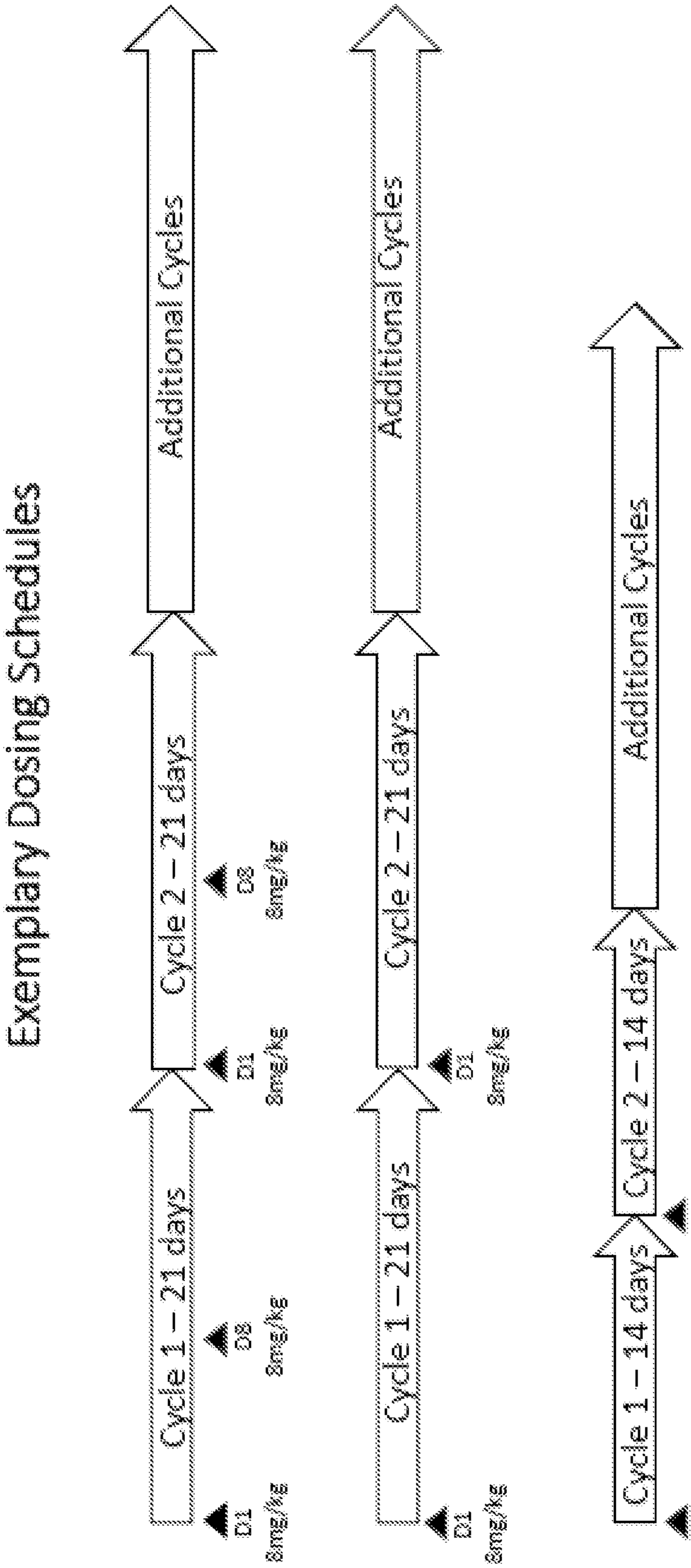
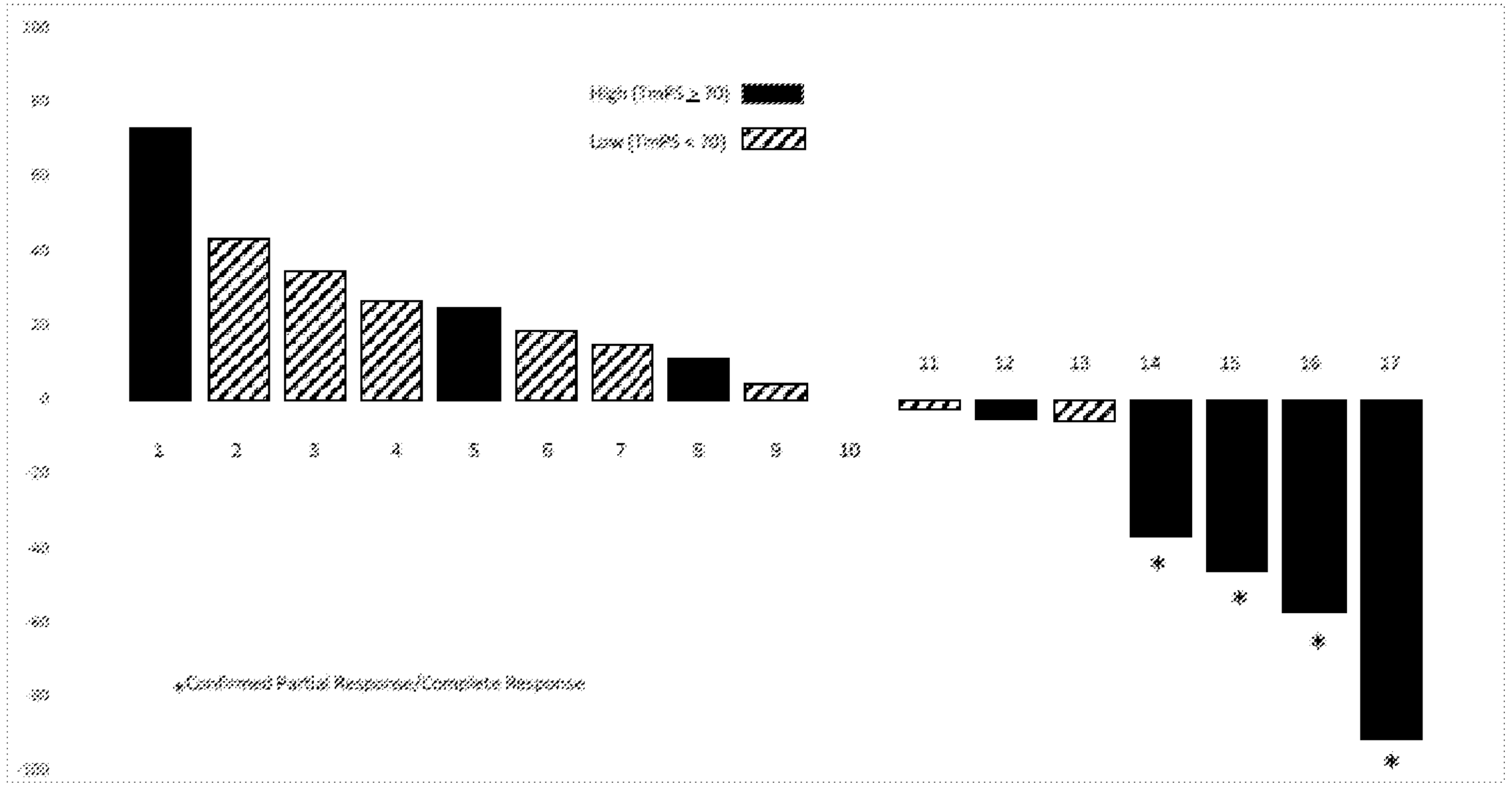

Exemplary Dosing Schedules
Cycle 1 – 21 days
D1
8mg/kg
D8
8mg/kg
Cycle 2 – 21 days
D1
8mg/kg
D8
8mg/kg
Additional Cycles
Cycle 1 – 21 days
D1
8mg/kg
Cycle 2 – 21 days
D1
8mg/kg
Additional Cycles
Cycle 1 – 14 days
D1
8mg/kg
Cycle 2 – 14 days
D1
8mg/kg
Additional Cycles

FIG. 19



Subject	Cancer Type	Dosage		Subject	Cancer Type	Dosage
1	Leiomyosarcoma	1.8 mg/kg D1,8 Q3W		10	Undifferentiated Pleomorphic Sarcoma	1.8 mg/kg D1,8 Q3W
2	Undifferentiated Pleomorphic Sarcoma	1.8 mg/kg D1,8 Q3W		11	Osteosarcoma	1.8 mg/kg D1,8 Q3W
3	Liposarcoma	3.4 mg/kg D1 Q3W		12	Leiomyosarcoma	1.8 mg/kg D1,8 Q3W
4	Leiomyosarcoma	3.4 mg/kg D1 Q3W		13	Leiomyosarcoma	1.8 mg/kg D1,8 Q3W
5	Synovial Sarcoma	1.8 mg/kg D1,8 Q3W		14	Leiomyosarcoma	1.8 mg/kg D1 Q3W
6	Leiomyosarcoma	1.8 mg/kg D1,8 Q3W		15	Undifferentiated Pleomorphic Sarcoma	1.8 mg/kg D1,8 Q3W
7	Osteosarcoma	0.3 mg/kg D1 Q3W		16	Undifferentiated Pleomorphic Sarcoma	1.8 mg/kg D1,8 Q3W
8	Chondrosarcoma	1.1 mg/kg D 1,8 Q3W		17	Ewing Sarcoma	1.8 mg/kg D1 Q3W
9	Liposarcoma	1.8 mg/kg D1,8 Q3W				

FIG. 20

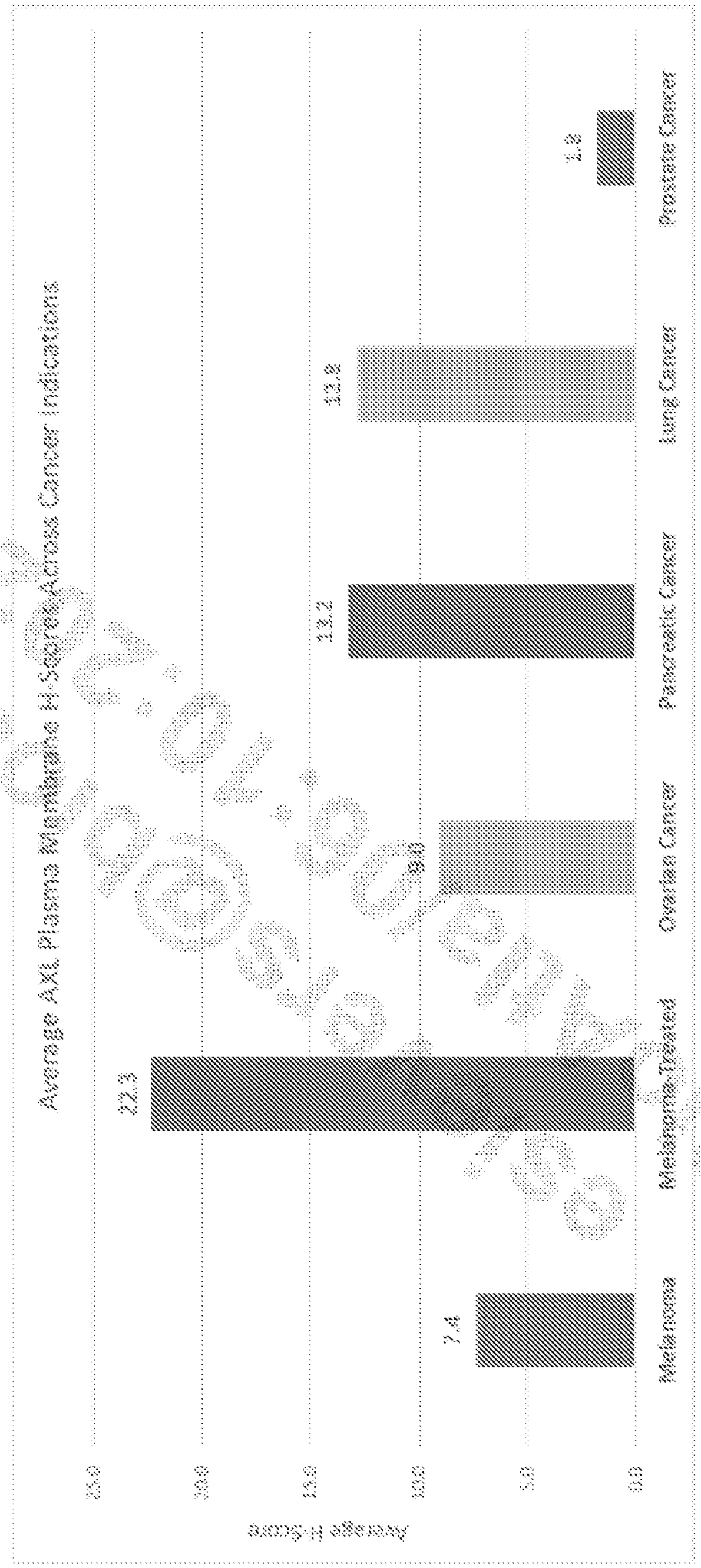

Average AXL H-Score Across Various Mammalian Tumors and Cancer Indications
Average H-score
0.0
20.0
40.0
60.0
80.0
100.0
120.0
Melanoma Treated
22.3
Melanoma
2.4
Pancreatic Cancer
13.2
Lung Cancer
12.4
Ovarian Cancer
9.0
Prostate Cancer
1.2

FIG. 21

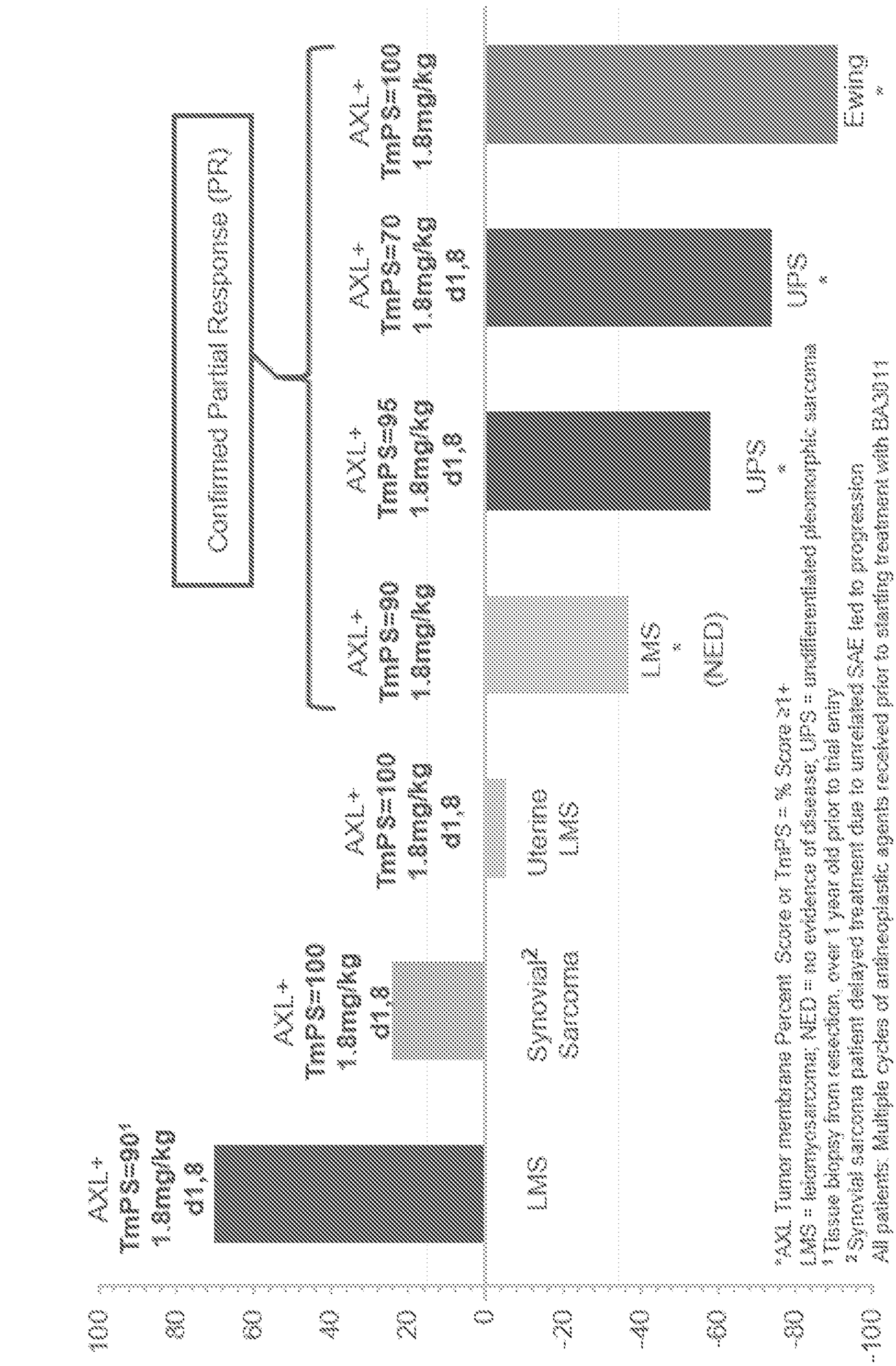
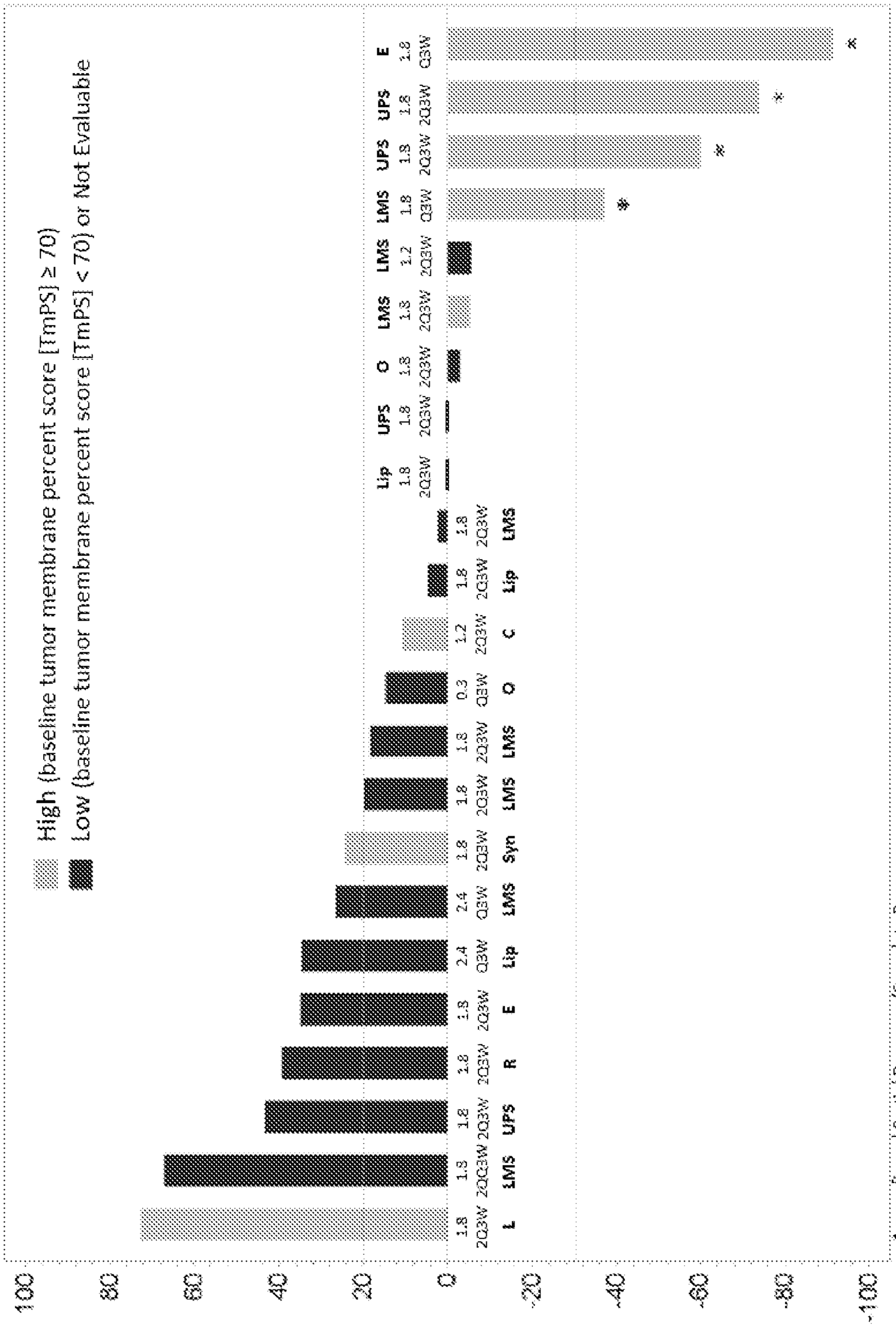

Maximum % change from Baseline in Sum of Target Lesions
100
80
60
40
20
0
-20
-40
-60
-80
-100
AXL+ TmPS=90 1.8mg/kg d1,8
LMS
AXL+ TmPS=100 1.8mg/kg d1,8
Synovial2 Sarcoma
AXL+ TmPS=100 1.8mg/kg d1,8
Uterine LMS
AXL+ TmPS=90 1.8mg/kg d1,8
(NED)
LMS *
AXL+ TmPS=95 1.8mg/kg d1,8
UPS *
AXL+ TmPS=70 1.8mg/kg d1,8
UPS *
Confirmed Partial Response (PR)
AXL+ TmPS=100 1.8mg/kg d1,8
Ewing *

FIG. 22


Maximum Change From Baseline (%)
100
80
60
40
20
0
-20
-40
-60
-80
-100
High (baseline tumor membrane percent score [TmPS] ≥ 70)
Low (baseline tumor membrane percent score [TmPS] < 70)
Not Evaluable or Not Evaluable
L 1.8 Q3W
LMS 1.8 2Q3W
UPS 1.8 2Q3W
R 1.8 2Q3W
E 1.8 Q3W
Lip 2.4 Q3W
LMS 2.4 Q3W
Syn 1.8 2Q3W
LMS 1.8 2Q3W
LMS 1.8 2Q3W
C 1.2 2Q3W
O 0.3 Q3W
Lip 1.8 2Q3W
UPS 1.8 2Q3W
O 1.8 2Q3W
LMS 1.8 2Q3W
E 1.8 Q3W
UPS 1.8 2Q3W
LMS 1.8 Q3W
*
*
*

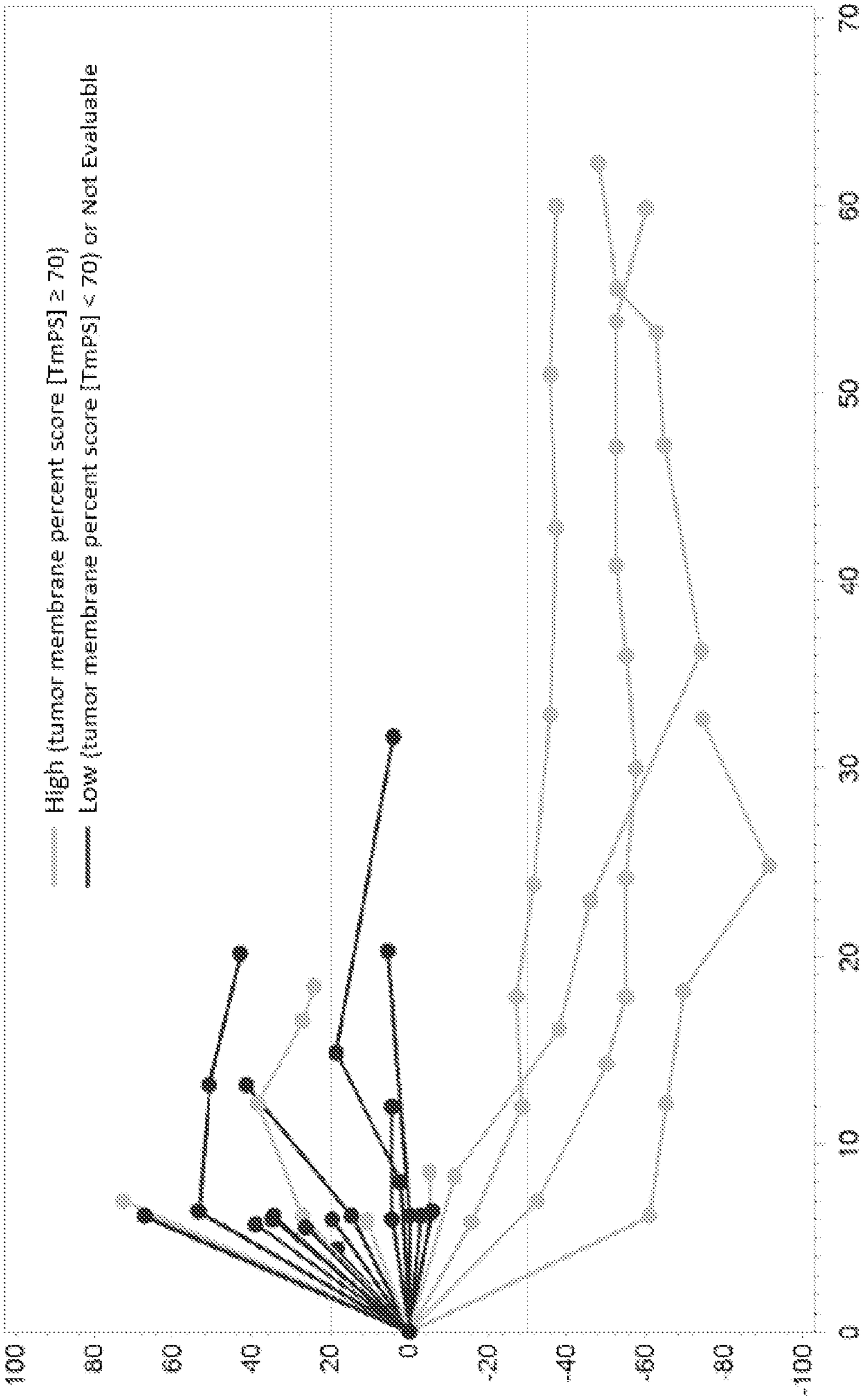

High (tumor membrane percent score [TmPS] ≥ 70)
Low (tumor membrane percent score [TmPS] < 70) or Not Evaluable
Time (weeks)
Change in Target Lesion From Baseline (%)
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
10
20
30
40
50
60
70

FIG. 24

SEQUENCE LISTING

<110> BioAtla, Inc.

<120> METHODS OF TREATING AXL-EXRPRESSING CANCERS WITH ANTI-AXL ANTIBODIES, ANTIBODY FRAGMENTS AND THEIR IMMUNOCONJUGATES

<130> BIAT-1034WO

<150> 63/113,040
<151> November 12, 2020

<160> 21

<170> PatentIn version 3.5

<210> 1
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<220>
<223> H1 sequence

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<220>
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<223> Xaa is H or A

<220>
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<223> Xaa is T or I

<220>
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<400> 1

Xaa Gly Xaa Xaa Met Xaa
1 5

<210> 2
<211> 17
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<220>
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<222> (10)..(10)
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Leu Ile Lys Xaa Ser Asn Gly Gly Thr Xaa Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

<210> 3
<211> 13
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<223> Xaa is E or A or D or F or G or H or I or L or M or N or R or V or Y

<220>
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<222> (5)..(5)

<223> Xaa is S or D or M or N or Q

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<222> (8)..(8)
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<222> (9)..(9)
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<400> 3

Gly Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asp Tyr Xaa Xaa
1   5   10

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<222> (7)..(7)
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<220>
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<222> (11)..(11)
<223> Xaa is A or D or N or Q

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1   5   10

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<212> PRT
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<220>
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Trp Xaa Xaa Thr Arg Xaa Thr
1   5

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<223> Xaa is T or C or P

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<223> Xaa is P or A or C or D or E or H or K or S or T or V or W

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<223> Xaa is T or I or R

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Gln Glu Xaa Xaa Ser Xaa Xaa Xaa Xaa
1 5

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atcacttgca aggccagtca ggatgtggtt tctgctgtag cctggtacca gcagaaacct 120

ggccaggctc ccaggctcct catctattgg caggataccc ggcacactgg agtcccatca 180

aggttcagcg gcagtggatc tgggacagaa ttcactctca ccatcagcag cctgcagcct 240

gatgattttg caacttatta ctgtcaggaa cattttagca ctccgctcac gttcggccaa 300

gggaccaagg tggaaatcaa acc 323

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<213> Artificial Sequence

<220>
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atcacttgca aggccagtca ggatgtgagt tctgctgtag cctggtacca gcagaaacct 120

ggccaggctc ccaggctcct catctattgg caggataccc ggcacactgg agtcccatca 180

aggttcagcg gcagtggatc tgggacagaa ttcactctca ccatcagcag cctgcagcct 240

gatgattttg caacttatta ctgtcaggaa cattttagcc ctccgctcac gttcggccaa 300

gggaccaagg tggaaatcaa acc 323

<210> 9
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<212> DNA
<213> Artificial Sequence

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atcacttgca aggccagtca ggatgtggtt tctgctgtag cctggtacca gcagaaacct 120

ggccaggctc ccaggctcct catctattgg caggataccc ggcacactgg agtcccatca 180

aggttcagcg gcagtggatc tgggacagaa ttcactctca ccatcagcag cctgcagcct 240

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gggaccaagg tggaaatcaa acc 323

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atcacttgca aggccagtca ggatgtggtt tctgctgtag cctggtacca gcagaaacct 120

ggccaggctc ccaggctcct catctattgg caggataccc ggcacactgg agtcccatca 180

aggttcagcg gcagtggatc tgggacagaa ttcactctca ccatcagcag cctgcagcct 240

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gggaccaagg tggaaatcaa acc 323

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gaggtccagc tggtacagtc tggggctgag gtgaagaagc ctggggctac agtgaaaatc 60

tcctgcaagg tttctggtta ctcattcact ggcgctacca tgaactggat ccgccagccc 120

ccagggaagg ggctggagtg gattggtctt attaaacctt ccaatggtgg tactagttac 180

aaccagaagt tcaagggcag agtcaccatc tcagccgaca agtccatcag caccgcctac 240

ctgcagtgga gcagcctgaa ggcctcggac accgccatgt attactgtgc acatggtcac 300

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ccagggaagg ggctggagtg gattggtctt attaaacctt ccaatggtgg tactagttac 180

aaccagaagt tcaagggcag agtcaccatc tcagccgaca agtccatcag caccgcctac 240

ctgcagtgga gcagcctgaa ggcctcggac accgccatgt attactgtgc acatggtcac 300

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tcctgcaagg tttctggtta ctcattcact ggccacacca tgaactggat ccgccagccc 120

ccagggaagg ggctggagtg gattggtctt attaaacctt ccaatggtgg tactagttac 180

aaccagaagt tcaagggcag agtcaccatc tcagccgaca agtccatcag caccgcctac 240

ctgcagtgga gcagcctgaa ggcctcggac accgccatgt attactgtgc acatggtcac 300

tacgagagtt acgaggctat ggactactgg ggccagggaa cgctggtcac cgtcagctca 360

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1 5

<210> 15
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Leu Ile Lys Pro Ser Asn Gly Gly Thr Ser Tyr Asn Gln Lys Phe Lys
1 5 10 15

Gly

<210> 16
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Gly His Tyr Glu Ser Tyr Glu Ala Met Asp Tyr Trp Gly
1 5 10

<210> 17
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Lys Ala Ser Gln Asp Val Val Ser Ala Val Ala
1 5 10

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Trp Gln Asp Thr Arg His Thr

1 5

<210> 19
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Gln Glu His Phe Ser Pro Pro Leu Thr
1 5

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Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Val Ser Gly Tyr Ser Phe Trp Gly Ala
20 25 30

Thr Met Asn Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Leu Ile Lys Pro Ser Asn Gly Gly Thr Ser Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Arg Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala His Gly His Tyr Glu Ser Tyr Glu Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
        115   120

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Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1   5   10   15

Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Val Ser Ala
            20   25   30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
        35   40   45

Tyr Trp Gln Asp Thr Arg His Thr Gly Val Pro Ser Arg Phe Ser Gly
    50   55   60

Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65   70   75   80

Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Glu His Phe Ser Pro Pro Leu
                85   90   95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
            100   105