ADAM9 Antibody-Drug Conjugates

FIELD

[0001] The present disclosure is directed to an ADAM9 antibody drug conjugate ("**ADAM9-ADC**") that comprises the human ADAM9 binding domain of a humanized anti-human ADAM9 antibody conjugated to at least one drug moiety. The disclosure is also directed to pharmaceutical compositions that contain such **ADAM9-ADCs**, and to methods involving the use of any of such **ADAM9-ADCs** in the treatment of cancer and other diseases and conditions associated with or characterized by the expression of ADAM9.

BACKGROUND

I. ADAM Superfamily and ADAM9

[0002] ADAM is a family of proteins involved in various physiologic and pathologic processes (Amendola, R.S. *et al.* (2015) "*ADAM9 Disintegrin Domain Activates Human Neutrophils Through An Autocrine Circuit Involving Integrins And CXCR2*," J. Leukocyte Biol. 97(5):951-962; Edwars, D.R. *et al.* (2008) "*The ADAM Metalloproteases*," Molec. Aspects Med. 29:258-289). At least 40 gene members of the family have been identified, and at least 21 of such members are believed to be functional in humans (Li, J. *et al.* (2016) "*Overexpression of ADAM9 Promotes Colon Cancer Cells Invasion*," J. Invest. Surg. 26(3):127-133; Duffy, M.J. *et al.* (2011) "*The ADAMs Family Of Proteases: New Biomarkers And Therapeutic Targets For Cancer*?," Clin. Proteomics 8:9:1-13; see also US Patent Publication No. 2013/0045244).

[0003] ADAM family members have a well-conserved structure with 8 domains, among which are a metalloprotease domain and an integrin-binding (disintegrin) domain (Duffy, M.J. *et al.* (2009) "*The Role Of ADAMs In Disease Pathophysiology*," Clin. Chim. Acta 403:31-36). The ADAM metalloprotease domain acts as a sheddase and has been reported to modulate a series of biologic processes by cleaving transmembrane proteins, which then can act as soluble ligands and regulate cellular signaling (Amendola, R.S. *et al.* (2015) "*ADAM9 Disintegrin Domain Activates Human Neutrophils Through An Autocrine Circuit Involving Integrins And CXCR2*," J. Leukocyte Biol. 97(5):951-962; Ito, N. *et al.* (2004) "*ADAMs, A Disintegrin And*

Metalloproteinases, Mediate Shedding Of Oxytocinase," Biochem. Biophys. Res. Commun. 314 (2004) 1008–1013).

[0004] ADAM9 is a member of the ADAM family of molecules. It is synthesized as an inactive form which is proteolytically cleaved to generate an active enzyme. Processing at the upstream site is particularly important for activation of the proenzyme. ADAM9 is expressed in fibroblasts (Zigrino, P. *et al.* (2011) "*The Disintegrin-Like And Cysteine-Rich Domains Of ADAM-9 Mediate Interactions Between Melanoma Cells And Fibroblasts*," J. Biol. Chem. 286:6801-6807), activated vascular smooth muscle cells (Sun, C. *et al.* (2010) "*ADAM15 Regulates Endothelial Permeability And Neutrophil Migration Via Src/ERK1/2 Signalling*," Cardiovasc. Res. 87:348-355), monocytes (Namba, K. *et al.* (2001) "*Involvement Of ADAM9 In Multinucleated Giant Cell Formation Of Blood Monocytes*," Cell. Immunol. 213:104-113), activated macrophages (Oksala, N. *et al.* (2009) "*ADAM-9, ADAM-15, And ADAM-17 Are Upregulated In Macrophages In Advanced Human Atherosclerotic Plaques In Aorta And Carotid And Femoral Arteries – Tampere Vascular Study*," Ann. Med. 41:279-290).

[0005] ADAM9's metalloprotease activity participates in the degradation of matrix components, to thereby allow migration of tumor cells (Amendola, R.S. *et al.* (2015) "*ADAM9 Disintegrin Domain Activates Human Neutrophils Through An Autocrine Circuit Involving Integrins And CXCR2*," J. Leukocyte Biol. 97(5):951-962). Its disintegrin domain, which is highly homologous to many snake-venom disintegrins, allows the interaction between ADAM9 and integrins, and enables ADAM9 to modulate, positively or negatively, cell adhesion events (Zigrino, P. *et al.* (2011) "*The Disintegrin-Like And Cysteine-Rich Domains Of ADAM-9 Mediate Interactions Between Melanoma Cells And Fibroblasts*," J. Biol. Chem. 286:6801-6807; Karadag, A. *et al.* (2006) "*ADAM-9 (MDC-9/Meltringamma), A Member Of The A Disintegrin And Metalloproteinase Family, Regulates Myeloma-Cell-Induced Interleukin-6 Production In Osteoblasts By Direct Interaction With The Alpha(v)Beta5 Integrin*," Blood 107:3271-3278; Cominetti, M.R. *et al.* (2009) "*Inhibition Of Platelets And Tumor Cell Adhesion By The Disintegrin Domain Of Human ADAM9 To Collagen I Under Dynamic Flow Conditions*," Biochimie 91:1045-1052). The ADAM9 disintegrin domain has been shown to interact with the α6β1, α6β4, αvβ5 and α9β1 integrins.

II. ADAM9 Expressing Tumors

[0006] The expression of ADAM9 has been found to be relevant to disease, especially cancer. ADAM9 has been found to cleave and release a number of molecules with important roles in tumorigenesis and angiogenesis, such as TEK, KDR, EPHB4, CD40, VCAM1 and CDH5. ADAM9 is expressed by many types of tumor cells, including tumor cells of breast cancers, colon cancers, gastric cancers, gliomas, liver cancers, non-small cell lung cancers, melanomas, myelomas, pancreatic cancers and prostate cancers (Yoshimasu, T. *et al.* (2004) "*Overexpression Of ADAM9 In Non-Small Cell Lung Cancer Correlates With Brain Metastasis*," Cancer Res. 64:4190-4196; Peduto, L. *et al.* (2005) "*Critical Function For ADAM9 In Mouse Prostate Cancer*," Cancer Res. 65:9312-9319; Zigrino, P. *et al.* (2005) "*ADAM-9 Expression And Regulation In Human Skin Melanoma And Melanoma Cell Lines*," Int. J. Cancer 116:853-859; Fritzsche, F.R. *et al.* (2008) "*ADAM9 Is Highly Expressed In Renal Cell Cancer And Is Associated With Tumour Progression*," BMC Cancer 8:179:1-9; Fry, J.L. *et al.* (2010) "*Secreted And Membrane-Bound Isoforms Of Protease ADAM9 Have Opposing Effects On Breast Cancer Cell Migration*," Cancer Res. 70, 8187-8198; Chang, L. *et al.* (2016) "*Combined Rnai Targeting Human Stat3 And ADAM9 As Gene Therapy For Non-Small Cell Lung Cancer*," Oncology Letters 11:1242-1250; Fan, X. *et al.* (2016) "*ADAM9 Expression Is Associate with Glioma Tumor Grade and Histological Type, and Acts as a Prognostic Factor in Lower-Grade Gliomas*," Int. J. Mol. Sci. 17:1276:1-11).

[0007] Significantly, increased ADAM9 expression has been found to correlate positively with tumor malignancy and metastatic potential (Amendola, R.S. *et al.* (2015) "*ADAM9 Disintegrin Domain Activates Human Neutrophils Through An Autocrine Circuit Involving Integrins And CXCR2*," J. Leukocyte Biol. 97(5):951-962; Fan, X. *et al.* (2016) "*ADAM9 Expression Is Associate with Glioma Tumor Grade and Histological Type, and Acts as a Prognostic Factor in Lower-Grade Gliomas*," Int. J. Mol. Sci. 17:1276:1-11; Li, J. *et al.* (2016) "*Overexpression of ADAM9 Promotes Colon Cancer Cells Invasion*," J. Invest. Surg. 26(3):127-133). Additionally, ADAM9 and its secreted soluble isoform seem to be crucial for cancer cells to disseminate (Amendola, R.S. *et al.* (2015) "*ADAM9 Disintegrin Domain Activates Human Neutrophils Through An Autocrine Circuit Involving Integrins And CXCR2*," J. Leukocyte Biol. 97(5):951-962; Fry, J.L. *et al.* (2010) "*Secreted And Membrane-Bound*

Isoforms Of Protease ADAM9 Have Opposing Effects On Breast Cancer Cell Migration," Cancer Res. 70, 8187–8198; Mazzocca, A. (2005) "*A Secreted Form Of ADAM9 Promotes Carcinoma Invasion Through Tumor-Stromal Interactions*," Cancer Res. 65:4728-4738; see also US Patent Nos. 9,150,656; 7,585,634; 7,829,277; 8,101,361; and 8,445,198 and US Patent Publication No. 2009/0023149).

[0008] A number of studies have thus identified ADAM9 as a potential target for anticancer therapy (Peduto, L. (2009) "*ADAM9 As A Potential Target Molecule In Cancer*," Curr. Pharm. Des. 15:2282-2287; Duffy, M.J. *et al.* (2009) "*Role Of ADAMs In Cancer Formation And Progression*," Clin. Cancer Res. 15:1140-1144; Duffy, M.J. *et al.* (2011) "*The ADAMs Family Of Proteases: New Biomarkers And Therapeutic Targets For Cancer*?" Clin. Proteomics 8:9:1-13; Josson, S. *et al.* (2011) "*Inhibition of ADAM9 Expression Induces Epithelial Phenotypic Alterations and Sensitizes Human Prostate Cancer Cells to Radiation and Chemotherapy*," Prostate 71(3):232-240; see also US Patent Publication Nos. 2016/0138113, 2016/0068909, 2016/0024582, 2015/0368352, 2015/0337356, 2015/0337048, 2015/0010575, 2014/0342946, 2012/0077694, 2011/0151536, 2011/0129450, 2010/0291063, 2010/0233079, 2010/0112713, 2009/0285840, 2009/0203051, 2004/0092466, 2003/0091568, and 2002/0068062, and PCT Publication Nos. WO 2016/077505, WO 2014/205293, WO 2014/186364, WO 2014/124326, WO 2014/108480, WO 2013/119960, WO 2013/098797, WO 2013/049704, and WO 2011/100362). Additionally, the expression of ADAM9 has also been found to be relevant to pulmonary disease and inflammation (see, *e.g.*, US Patent Publication Nos. 2016/0068909; 2012/0149595; 2009/0233300; 2006/0270618; and 2009/0142301). Antibodies that bind to ADAM9 are commercially available from Abcam, Thermofisher, Sigma-Aldrich, and other companies.

SUMMARY

[0009] The present disclosure is directed to an ADAM9 antibody drug conjugate ("**ADAM9-ADC**") that comprises the human ADAM9 binding domain of a humanized anti-human ADAM9 antibody conjugated to at least one drug moiety. The disclosure is directed to pharmaceutical compositions that contain such **ADAM9-ADCs,** and to methods involving the use of any of such **ADAM9-ADCs** in the treatment of cancer and other diseases and conditions.

[0010] In certain embodiments, the immunoconjugate of the present invention is represented by the following formula:

$$\mathbf{Ab\text{-}(LM)_m\text{-}(D)_n,}$$

wherein:

Ab is a humanized ADAM9 antibody or ADAM9 binding fragment thereof that binds to ADAM9 and comprises:

(i) the CDR_L1 sequence KASQSVDYSGDSYMN (**SEQ ID NO:24**), the CDR_L2 sequence AASDLES (**SEQ ID NO:25**) and the CDR_L3 sequence QQSHEDPFT (**SEQ ID NO:26**) in its Variable Light Chain (VL) domain, and

(ii) the CDR_H1 sequence SYWMH (**SEQ ID NO:29**), the CDR_H2 sequence EIIPIFGHTNYNEKFKS (**SEQ ID NO:30**) and the CDR_H3 sequence GGYYYYPRQGFLDY (**SEQ ID NO:31**) in its Variable Heavy Chain (VH) domain;

D is a camptothecin moiety;

LM is a Linker Molecule that covalently links **Ab** and **D**;

m is an integer between 1 and n and denotes the number of Linker Molecules of the **ADAM9-ADC**; and

n is an integer between 1 and 10 and denotes the number of cytotoxic camptothecin moieties covalently linked to the **ADAM9-ADC** molecule.

[0011] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the **Ab** comprises:

a) a humanized VL Domain comprising the amino acid sequence of **SEQ ID NO:22**, and

b) a humanized VH Domain comprising the amino acid sequence of **SEQ ID NO:27**.

[0012] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the **Ab** comprises:

a) a Light Chain comprising the amino acid sequence of **SEQ ID NO:23**, and

b) a Heavy Chain comprising the amino acid sequence of **SEQ ID NO:28**.

[0013] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the **Ab** is an antibody. In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the **Ab** is an antigen binding fragment of an antibody.

[0014] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the **Ab** comprises an Fc Domain of a human IgG. In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the human IgG is a human IgG1, IgG2, IgG3, or IgG4.

[0015] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the Fc Domain is a variant Fc Domain that comprises:

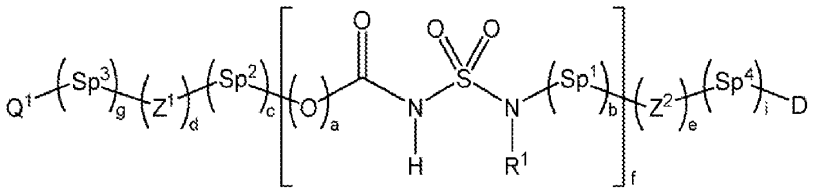
(a) one or more amino acid modifications that reduce the affinity of the variant Fc Domain for an FcγR; and/or

(b) one or more amino acid modifications that enhance the serum half-life of the variant Fc Domain.

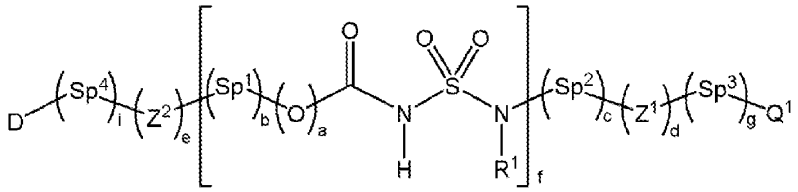
[0016] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the modifications that reduce the affinity of the variant Fc Domain for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat. In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the modifications that that enhance the serum half-life of the variant Fc Domain comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein the numbering is that of the EU index as in Kabat.

[0017] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the **LM** comprises a peptidic linker. In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the **LM** comprises a cleavable linker.

[0018] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the **LM** comprises the formula (4a) or (4b), or a salt thereof:



4a



4b

wherein:

a is independently 0 or 1;

b is independently 0 or 1;

c is 0 or 1;

d is 0 or 1;

e is 0 or 1;

f is an integer in the range of 1 to 150;

g is 0 or 1;

i is 0 or 1;

D is a cytotoxic camptothecin moiety;

Q^1 is an alkenyl group, (hetero)cycloalkenyl group, bicyclo triazole group or cycloalkenyl group;

wherein Q^1 is attached to a functional group of the antibody;

wherein Sp^1, Sp^2, Sp^3 and Sp^4 are independently selected from the group consisting of linear or branched C_1-C_{200} alkylene groups, C_2-C_{200} alkenylene groups, C_2-C_{200} alkynylene groups, C_3-C_{200} cycloalkylene groups, C_5-C_{200} cycloalkenylene groups, C_8-C_{200} cycloalkynylene groups, C_7-C_{200} alkylarylene groups, C_7-C_{200} arylalkylene groups, C_8-C_{200} arylalkenylene groups and C_9-C_{200} arylalkynylene groups, the alkylene groups, alkenylene groups, alkynylene groups, cycloalkylene groups, cycloalkenylene groups, cycloalkynylene groups,

alkylarylene groups, arylalkylene groups, arylalkenylene groups and arylalkynylene groups being optionally substituted and optionally interrupted by one or more heteroatoms selected from the group of O, S and NR^3, wherein R^3 is independently selected from the group consisting of hydrogen, C_1 - C_{24} alkyl groups, C_2 - C_{24} alkenyl groups, C_2 - C_{24} alkynyl groups and C_3 - C_{24} cycloalkyl groups, the alkyl groups, alkenyl groups, alkynyl groups and cycloalkyl groups being optionally substituted;

Z^1 is a connecting group that connects Q^1 or Sp^3 to Sp^2, O or C(O) or $N(R^1)$;

Z^2 is a connecting group that connects **D** or Sp^4 to Sp^1, $N(R^1)$, O or C(O);

wherein Z^1 and Z^2 are independently selected from the group consisting of -O-, -S-, $-NR^2-$, -N=N-, -C(O)-, $-C(O)NR^2-$, -O-C(O)- , -O-C(O)-O-, $-O-C(O)-NR^2$, $-NR^2-C(O)-$, $-NR^2-C(O)-O-$, $-NR^2-C(O)-NR^2-$, -S-C(O)-, -S-C(O)-O-, $-S-C(O)-NR^2-$, -S(O)-, $-S(O)_2-$, $-O-S(O)_2-$, $-O-S(O)_2-O-$, $-O-S(O)_2-NR^2-$, -O-S(O)-, -O-S(O)-O-, $-O-S(O)-NR^2-$, $-O-NR^2-C(O)-$, $-O-NR^2-C(O)-O-$, $-O-NR^2-C(O)-NR^2-$, $-NR^2-O-C(O)-$, $-NR^2-O-C(O)-O-$, $-NR^2-O-C(O)-NR^2-$, $-O-NR^2-C(S)-$, $-O-NR^2-C(S)-O-$, $-O-NR^2-C(S)-NR^2-$, $-NR^2-O-C(S)-$, $-NR^2-O-C(S)-O-$, $-NR^2-O-C(S)-NR^2-$, -O-C(S)-, -O-C(S)-O-, $-O-C(S)-NR^2-$, $-NR^2-C(S)-$, $-NR^2-C(S)-O-$, $-NR^2-C(S)-NR^2-$, $-S-S(O)_2-$, $-S-S(O)_2-O-$, $-S-S(O)_2-NR^2-$, $-NR^2-O-S(O)-$, $-NR^2-O-S(O)-O-$, $-NR^2-O-S(O)-NR^2-$, $-NR^2-O-S(O)_2-$, $-NR^2-O-S(O)_2-O-$, $-NR^2-O-S(O)_2-NR^2-$, $-O-NR^2-S(O)-$, $-O-NR^2-S(O)-O-$, $-O-NR^2-S(O)-NR^2-$, $-O-NR^2-S(O)_2-O-$, $-O-NR^2-S(O)_2-NR^2-$, $-O-NR^2-S(O)_2-$, $-O-P(O)(R^2)_2-$, $-S-P(O)(R^2)_2-$, $-NR^2-P(O)(R^2)_2-$ and combinations of two or more thereof, wherein R^2 is independently selected from the group consisting of hydrogen, C_1 - C_{24} alkyl groups, C_2 - C_{24} alkenyl groups, C_2 - C_{24} alkynyl groups and C_3 - C_{24} cycloalkyl groups, the alkyl groups, alkenyl groups, alkynyl groups and cycloalkyl groups being optionally substituted; and

R^1 is selected from the group consisting of hydrogen, C_1 - C_{24} alkyl groups, C_3 - C_{24} cycloalkyl groups, C_2 - C_{24} (hetero)aryl groups, C_3 - C_{24} alkyl(hetero)aryl groups and C_3 - C_{24} (hetero)arylalkyl groups, the C_1 - C_{24} alkyl groups, C_3 - C_{24} cycloalkyl groups, C_2 - C_{24} (hetero)aryl groups, C_3 - C_{24} alkyl(hetero)aryl groups and C_3 - C_{24} (hetero)arylalkyl groups optionally substituted and optionally

interrupted by one or more heteroatoms selected from O, S and NR^3 wherein R^3 is independently selected from the group consisting of hydrogen and C_1 - C_4 alkyl groups; or

R^1 is D, $-[(Sp^1)_b(Z^2)_e-(Sp^4)_i-D]$ or $-[(Sp^2)_c-(Z^1)_d-(Sp^3)_g-Q^1]$, wherein Sp^1, Sp^2, Sp^3, Sp^4, Z^1, Z^2, D, Q^1, b, c, d, e, g and i are as defined above.

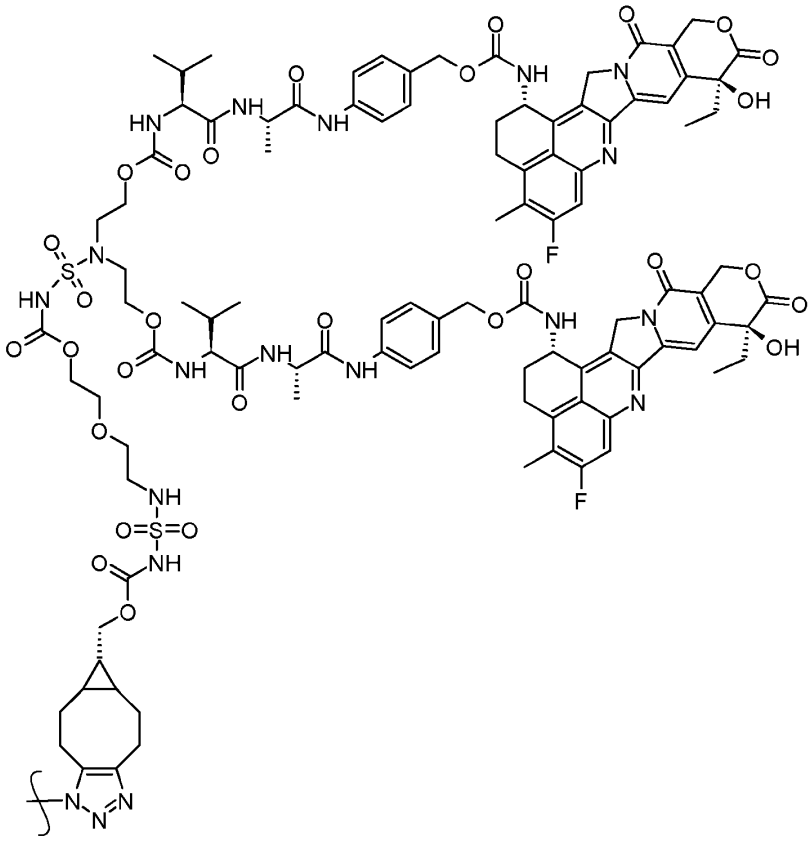
[0019] In aspects, the disclosure further provides such **ADAM9-ADC,** wherein Sp^1, Sp^2, Sp^3 and Sp^4, if present, are independently selected from the group consisting of linear or branched C_1-C_{20} alkylene groups, the alkylene groups being optionally substituted and optionally interrupted by one or more heteroatoms selected from the group consisting of O, S and NR^3, wherein R^3 is independently selected from the group consisting of hydrogen and C_1 - C_4 alkyl groups

[0020] In aspects, the disclosure further provides such **ADAM9-ADC**, where the **LM** comprises a Valine-Alanine (Val-Ala) amino acid linker. In aspects, the disclosure further provides such **ADAM9-ADC,** wherein the Val-Ala linker is a Val-Ala-PABC linker.

[0021] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the camptothecin moiety is selected from the group consisting of SN-38 (*S*-10-hydroxycamptothecin), topotecan (HYCAMPTIN; (S)-9-N,N-dimethylaminoethyl-10-hydroxycamptothecin), 9-aminocamptothecin (9-amino-20(S)-camptothecin), 9-nitrocamptothecin (also called rubitecan), lurtotecan (7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-camptothecin), exatecan, karenitecin, and a homocamptothecin.

[0022] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein the camptothecin moiety is exatecan.

[0023] In aspects, the disclosure further provides such **ADAM9-ADC**, wherein **LM** and **D** together comprise:



[0024] In aspects, the disclosure further provides an anti-ADAM9 antibody drug conjugate (**ADAM9-ADC**) that comprises the formula:

$$\mathbf{Ab\text{-}(LM)_m\text{-}(D)_n,}$$

wherein:

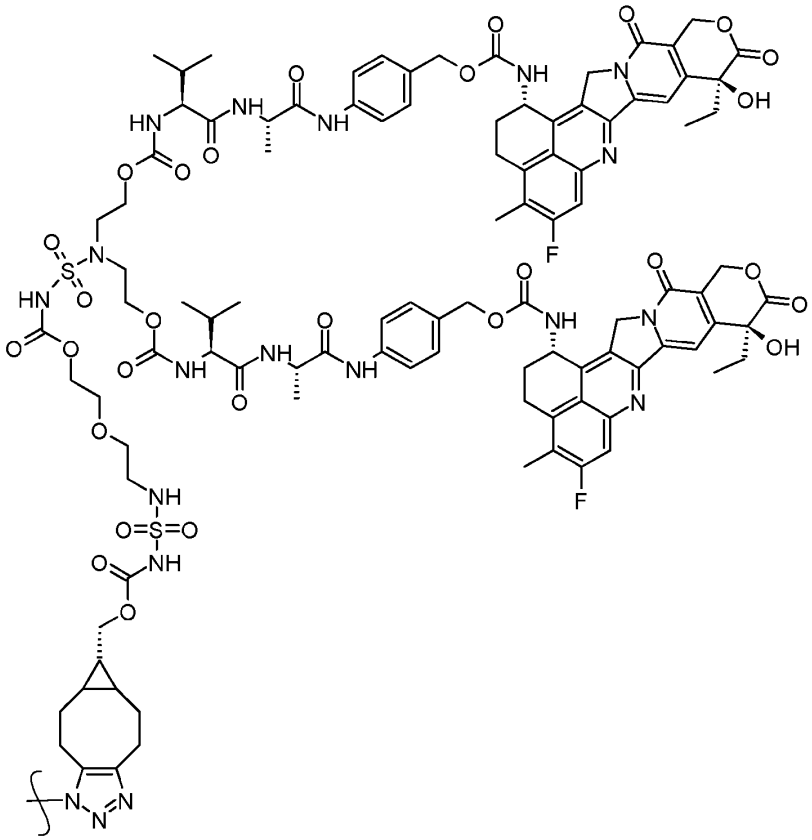
Ab is a humanized ADAM9 antibody or ADAM9 binding fragment thereof that binds to ADAM9 and comprises:

(i) the CDR_L1 sequence KASQSVDYSGDSYMN (**SEQ ID NO:24**), the CDR_L2 sequence AASDLES (**SEQ ID NO:25**) and the CDR_L3 sequence QQSHEDPFT (**SEQ ID NO:26**) in its Variable Light Chain (VL) domain, and

(ii) the CDR_H1 sequence SYWMH (**SEQ ID NO:29**), the CDR_H2 sequence EIIPIFGHTNYNEKFKS (**SEQ ID NO:30**) and the CDR_H3 sequence

GGYYYYPRQGFLDY (**SEQ ID NO:31**) in its Variable Heavy Chain (VH) domain;

D and **LM** together comprise:



m is an integer between 0 and n and denotes the number of Linker Molecules of the **ADAM9-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic camptothecin moieties covalently linked to the **ADAM9-ADC** molecule.

[0025] In aspects, the disclosure further provides such **ADAM9-ADC** molecule, wherein the **Ab** comprises a humanized VL Domain comprising the amino acid sequence of **SEQ ID NO:22**, and a humanized VH Domain comprising the amino acid sequence of **SEQ ID NO:27.**

[0026] In aspects, the disclosure further provides such **ADAM9-ADC** molecule, wherein the **Ab** comprises a Light Chain comprising the amino acid sequence of **SEQ ID NO:23**, and a Heavy Chain comprising the amino acid sequence of **SEQ ID NO:28**.

[0027] In aspects, the disclosure further provides a pharmaceutical composition that comprises an effective amount of the **ADAM9-ADC** and a pharmaceutically acceptable carrier, excipient or diluent.

[0028] In aspects, the disclosure further provides a use of the **ADAM9-ADC** or the pharmaceutical composition in the treatment of a disease or condition associated with or characterized by the expression of ADAM9.

[0029] In aspects, the disclosure further provides a method of treating a disease or condition associated with or characterized by the expression of ADAM9 comprising administering the **ADAM9-ADC** or the pharmaceutical composition to a subject.

[0030] In aspects, the disclosure further provides such use or method, wherein the disease or condition associated with or characterized by the expression of ADAM9 is cancer.

[0031] In aspects, the disclosure further provides such use or method, wherein the cancer is selected from the group consisting of: bladder cancer, brain cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gallbladder cancer, gastric cancer, head and neck cancer, liver cancer, lung cancer, myeloid cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, thyroid cancer, testicular cancer, and uterine cancer.

[0032] In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is selected from the group consisting of: bladder cancer, brain cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gallbladder cancer, gastric cancer, head and neck cancer, liver cancer, lung cancer, myeloid cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, thyroid cancer, testicular cancer, and uterine cancer.

[0033] In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is glioma. In aspects, the disclosure further provides such use or

method for treatment of a cancer, wherein the cancer is breast cancer or triple negative breast cancer. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is cervical cancer. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is colorectal cancer. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is cholangiocarcinoma. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is gastric cancer or gastroesophageal junction adenocarcinoma. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is squamous cell cancer of the head and neck. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is non-small cell lung cancer. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is oral cancer or oral squamous cell carcinoma. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is ovarian cancer or ovarian clear cell carcinoma. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is pancreatic cancer. In aspects, the disclosure further provides such use or method for treatment of a cancer, wherein the cancer is renal cell carcinoma.

BRIEF DESCRIPTION OF THE DRAWINGS

[0034] **FIGs. 1A-1C** show plots of percent cell viability vs. ADC concentration in *in vitro* cytotoxicity studies toward ADAM9-expressing A375.S2 human melanoma cells (1A), Calu-3 human lung cancer cells (1B), and HPAF-II human pancreatic cancer cells (1C) as described in Example 1.

[0035] **FIGs. 2A-2D** show plots of percent cytoxicity vs. monoclonal antibody concentration in ADCC assays of unconjugated MGA027, conjugated MGC028, and conjugated negative control ADC (Nontargeting Control ADC) in NCI-H1703 cells (2A), NCI-H1975 cells (2B), HPAF-II cells (2C), and Hs746T cells (2D) as described in Example 2.

[0036] **FIGs. 3A-3B** show plots of resonance units (RU) over time in surface plasmon resonance (SPR) assays of MGC028 and MGA027 with His-tagged human or cynomolgus

monkey ADAM9 extracellular domain proteins (3A) and His-tagged human CD16A extracellular domain proteins (3B) as described in Example 3.

[0037] **FIG. 4** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 and Nontargeting Control ADC against HPAF-II pancreatic adenocarcinoma tumor cells as described in Example 4.

[0038] **FIG. 5** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 and Nontargeting Control ADC against Capan-1 pancreatic adenocarcinoma tumor cells as described in Example 4.

[0039] **FIG. 6** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 and Nontargeting Control ADC against Hs746T gastric carcinoma tumor cells as described in Example 4.

[0040] **FIG. 7** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 and Nontargeting Control ADC against NCI-H1975 lung adenocarcinoma tumor cells as described in Example 4.

[0041] **FIG. 8** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 and Nontargeting Control ADC against Calu-3 non-small cell lung cancer (NSCLC) adenocarcinoma tumor cells as described in Example 4.

[0042] **FIG. 9** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 and Nontargeting Control ADC against NCI-H1703 non-small cell lung cancer (NSCLC) squamous tumor cells as described in Example 4.

[0043] **FIG. 10** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 and Nontargeting Control ADC against SW48 colorectal adenocarcinoma tumor cells as described in Example 4.

[0044] **FIG. 11** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 and Nontargeting Control ADC against Detroit 562 squamous cell carcinoma of the head and neck (SCCHN) tumor cells as described in Example 4.

[0045] **FIGs. 12A-12B** show plots of mean tumor volume over time showing the antitumor activity of vehicle control, targeting ADC (MGC028), unconjugated precursor ADAM9-targeting antibody (MGA027), unconjugated exatecan, and the combination of MGA027 and unconjugated exatecan against SW48 colorectal adenocarcinoma tumor cells (12A) and NCI-H1975 lung adenocarcinoma tumor cells (12B) as described in Example 4.

[0046] **FIG. 13** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 against cholangiocarcinoma patient-derived tumor fragments as described in Example 5.

[0047] **FIG. 14** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 against pancreatic adenocarcinoma patient-derived tumor fragments as described in Example 5.

[0048] **FIG. 15** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 against pancreatic adenocarcinoma patient-derived tumor fragments as described in Example 5.

[0049] **FIG. 16** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 against non-small cell lung cancer (NSCLC) adenocarcinoma patient-derived tumor fragments as described in Example 5.

[0050] **FIG. 17** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 against non-small cell lung cancer (NSCLC) adenocarcinoma patient-derived tumor fragments as described in Example 5.

[0051] **FIG. 18** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 against colorectal cancer patient-derived tumor fragments as described in Example 5.

[0052] **FIG. 19** shows a plot of mean tumor volume over time showing the antitumor activity of MGC028 against squamous cell carcinoma of the head and neck (SCCHN) patient-derived tumor fragments as described in Example 5.

[0053] **FIG. 20** shows a plot of drug to antibody ratio (DAR) level of MGC028 in human, mouse, or cynomolgus monkey K_2EDTA plasma over time as described in Example 6.

[0054] **FIGs. 21A-21F** show plots of free exatecan in mouse (21A-21B), human (21C-21D) or cynomolgus monkey (21E-21F) plasma as described in Example 6.

DETAILED DESCRIPTION

[0055] The present disclosure is directed to an anti-ADAM9 antibody drug conjugate (**ADAM9-ADC**) that comprises the human ADAM9 binding domain of a humanized anti-human ADAM9 antibody conjugated to at least one drug moiety. The disclosure is also directed to pharmaceutical compositions that contain such **ADAM9-ADCs**, and to methods involving the use of any of such **ADAM9-ADCs** in the treatment of cancer and other diseases and conditions. Certain **ADAM9-ADCs** and their uses in the treatment of cancer are described, for example, in PCT Publication No. WO 2018/119196, which is hereby expressly incorporated by reference herein.

I. Antibodies and Their Binding Domains

[0056] The antibodies of the present invention are immunoglobulin molecules capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, *etc.*, through at least one antigen recognition site, located in the Variable Domain of the immunoglobulin molecule. An **ADAM9-ADC** of the present disclosure thus comprises an antibody that binds to ADAM9. As used herein, the terms "antibody" and "antibodies" refer to monoclonal antibodies, multispecific antibodies, human antibodies, humanized antibodies, synthetic antibodies, chimeric antibodies, polyclonal antibodies, camelized antibodies, single-chain Fvs (scFv), single-chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked bispecific Fvs (sdFv), intrabodies, and epitope-binding fragments of any of the above. In particular, the term "antibody" includes immunoglobulin molecules and immunologically active fragments of immunoglobulin molecules, *i.e.*, molecules that contain an epitope-binding site. Immunoglobulin molecules can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), class (*e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1 and IgA_2) or subclass. Antibodies are capable of "immunospecifically binding" to a polypeptide or protein or a non-protein molecule (or of binding to such molecule in an "immunospecific manner") due to the presence on such molecule

of a particular domain or moiety or conformation (an "epitope"). An epitope-containing molecule may have immunogenic activity, such that it elicits an antibody production response in an animal; such molecules are termed "antigens".

[0057] As used herein, an antibody, diabody or other epitope-binding molecule is said to "immunospecifically" bind a region of another molecule (*i.e.*, an epitope) if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with that epitope relative to alternative epitopes. For example, an antibody that immunospecifically binds to a viral epitope is an antibody that binds this viral epitope with greater affinity, avidity, more readily, and/or with greater duration than it immunospecifically binds to other viral epitopes or non-viral epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that immunospecifically binds to a first target may or may not specifically or preferentially bind to a second target. As such, "immunospecific binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means "immunospecific" binding. Two molecules are said to be capable of binding to one another in a "physiospecific" manner, if such binding exhibits the specificity with which receptors bind to their respective ligands.

[0058] The term "monoclonal antibody" refers to a homogeneous antibody population wherein the monoclonal antibody is comprised of amino acids (naturally occurring or non-naturally occurring) that are involved in the selective binding of an antigen. Monoclonal antibodies are highly specific, being directed against a single epitope (or antigenic site). The term "monoclonal antibody" encompasses not only intact monoclonal antibodies and full-length monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv, *etc.*), single-chain (scFv) binding molecules, mutants thereof, fusion proteins comprising an antibody portion, humanized monoclonal antibodies, chimeric monoclonal antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity and the ability to bind to an antigen. It is not intended to be limited as regards to the source of the antibody or the manner in which it is made (*e.g.*, by hybridoma, phage selection, recombinant expression, transgenic animals, *etc.*). The term includes whole immunoglobulins as well as the fragments *etc.* described above under the definition of "antibody." Methods of making monoclonal antibodies are known in the art. One

method which may be employed is the method of Kohler, G. *et al.* (1975) "*Continuous Cultures Of Fused Cells Secreting Antibody Of Predefined Specificity*," Nature 256:495-497 or a modification thereof. Typically, monoclonal antibodies are developed in mice, rats or rabbits. The antibodies are produced by immunizing an animal with an immunogenic amount of cells, cell extracts, or protein preparations that contain the desired epitope. The immunogen can be, but is not limited to, primary cells, cultured cell lines, cancerous cells, proteins, peptides, nucleic acids, or tissue. Alternatively, existing monoclonal antibodies and any other equivalent antibodies that are immunospecific for a desired pathogenic epitope can be sequenced and produced recombinantly by any means known in the art. In one aspect, such an antibody is sequenced and the polynucleotide sequence is then cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. The polynucleotide sequence of such antibodies may be used for genetic manipulation to generate the monospecific or multispecific (*e.g.*, bispecific, trispecific and tetraspecific) molecules as well as an affinity optimized, a chimeric antibody, a humanized antibody, and/or a caninized antibody, to improve the affinity, or other characteristics of the antibody. The general principle in humanizing an antibody involves retaining the basic sequence of the antigen-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences.

[0059] Natural antibodies (such as IgG antibodies) are composed of two "Light Chains" complexed with two "Heavy Chains." Each Light Chain contains a Variable Domain ("VL") and a Constant Domain ("CL"). Each Heavy Chain contains a Variable Domain ("VH"), three Constant Domains ("CH1," "CH2" and "CH3"), and a "Hinge" Region ("H") located between the CH1 and CH2 Domains. The basic structural unit of naturally occurring immunoglobulins (*e.g.*, IgG) is thus a tetramer having two light chains and two heavy chains, usually expressed as a glycoprotein of about 150,000 Da. The amino-terminal ("N-terminal") portion of each chain includes a Variable Domain of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal ("C-terminal") portion of each chain defines a constant region, with light chains having a single Constant Domain and heavy chains usually having three Constant Domains and a Hinge Domain. Thus, the structure of the light chains of an IgG molecule is n-VL-CL-c and the structure of the IgG heavy chains is n-VH-CH1-H-CH2-

CH3-c (where n and c represent, respectively, the N-terminus and the C-terminus of the polypeptide).

A. Characteristics of Antibody Variable Domains

[0060] The Variable Domains of an IgG molecule consist of the complementarity determining regions ("CDR"), which contain the residues in contact with epitope, and non-CDR segments, referred to as framework segments ("FR"), which in general maintain the structure and determine the positioning of the CDR loops so as to permit such contacting (although certain framework residues may also contact antigen). Thus, the VL and VH Domains have the structure n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c. The amino acid sequences of the CDRs determine whether an antibody will be able to bind to a particular epitope. Interaction of an antibody light chain with an antibody heavy chain and, in particular, interaction of their VL and VH Domains, forms an epitope-binding site of the antibody.

[0061] Amino acids from the Variable Domains of the mature heavy and light chains of immunoglobulins are designated by the position of an amino acid in the chain. Kabat (SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, NH1, MD (1991)) described numerous amino acid sequences for antibodies, identified an amino acid consensus sequence for each subgroup, and assigned a residue number to each amino acid, and the CDRs and FRs are identified as defined by Kabat (it will be understood that CDR_{H1} as defined by Chothia, C. & Lesk, A. M. ((1987) "*Canonical structures for the hypervariable regions of immunoglobulins*," J. Mol. Biol. 196:901-917) begins five residues earlier). Kabat's numbering scheme is extendible to antibodies not included in his compendium by aligning the antibody in question with one of the consensus sequences in Kabat by reference to conserved amino acids. This method for assigning residue numbers has become standard in the field and readily identifies amino acids at equivalent positions in different antibodies, including chimeric or humanized variants. For example, an amino acid at position 50 of a human antibody light chain occupies the equivalent position to an amino acid at position 50 of a mouse antibody light chain. The positions within the VL and VH Domains at which the CDRs commence and end are thus well defined and can be ascertained by inspection of the sequences of the VL and VH Domains (see, e.g., Martin, C.R. (2010) "Protein Sequence and Structure Analysis of Antibody

Variable Domains," In: ANTIBODY ENGINEERING VOL. 2 (Kontermann, R. and Dübel, S. (eds.), Springer-Verlag Berlin Heidelberg, Chapter 3 (pages 33-51)).

[0062] Polypeptides that are (or may serve as) the first, second and third CDR of the Light Chain of an antibody are herein respectively designated as: CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain. Similarly, polypeptides that are (or may serve as) the first, second and third CDR of the Heavy Chain of an antibody are herein respectively designated as: CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain. Thus, the terms CDR_L1 Domain, CDR_L2 Domain, CDR_L3 Domain, CDR_H1 Domain, CDR_H2 Domain, and CDR_H3 Domain are directed to polypeptides that when incorporated into a protein cause that protein to be able to bind to a specific epitope regardless of whether such protein is an antibody having light and heavy chains or is a diabody or a single-chain binding molecule (*e.g.*, an scFv, a BiTe, *etc.*), or is another type of protein. Accordingly, as used herein, the term "epitope-binding fragment" denotes a fragment of a molecule capable of immunospecifically binding to an epitope. An epitope-binding fragment may contain any 1, 2, 3, 4, or 5 the CDR Domains of an antibody, or may contain all 6 of the CDR Domains of an antibody and, although capable of immunospecifically binding to such epitope, may exhibit an immunospecificity, affinity or selectivity toward such epitope that differs from that of such antibody. Preferably, however, an epitope-binding fragment will contain all 6 of the CDR Domains of such antibody. An epitope-binding fragment of an antibody may be a single polypeptide chain (*e.g.*, an scFv), or may comprise two or more polypeptide chains, each having an amino terminus and a carboxy terminus (*e.g.*, a diabody, a Fab fragment, an Fab_2 fragment, *etc.*). Unless specifically noted, the order of domains of the protein molecules described herein is in the "N-terminal to C-Terminal" direction.

[0063] The disclosure particularly encompasses single-chain Variable Domain fragments ("**scFv**") comprising a humanized anti-ADAM9-VL and/or VH Domain. Single-chain Variable Domain fragments comprise VL and VH Domains that are linked together using a short "Linker" peptide. Such Linkers can be modified to provide additional functions, such as to permit the attachment of a drug or to permit attachment to a solid support. The single-chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host

cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0064] The disclosure particularly encompasses binding molecules (including antibodies and diabodies) that comprise a VL and/or VH Domain of a humanized antibody. The term "humanized" antibody refers to a chimeric molecule, generally prepared using recombinant techniques, having an epitope-binding site of an immunoglobulin from a non-human species and a remaining immunoglobulin structure of the molecule that is based upon the structure and /or sequence of a human immunoglobulin. The polynucleotide sequence of the variable domains of such antibodies may be used for genetic manipulation to generate such derivatives and to improve the affinity, or other characteristics of such antibodies. It is known that the variable domains of both heavy and light chains contain three complementarity determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When non-human antibodies are prepared with respect to a particular antigen, the variable domains can be "reshaped" or "humanized." The general principle in humanizing an antibody involves retaining the basic sequence of the epitope-binding portion of the antibody, while swapping the non-human remainder of the antibody with human antibody sequences. There are four general steps to humanize a monoclonal antibody. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody or caninized antibody, *i.e.*, deciding which antibody framework region to use during the humanizing or canonizing process (3) the actual humanizing or caninizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patents Nos. 4,816,567; 5,807,715; 5,866,692; and 6,331,415. The term "optimized" antibody refers to an antibody having at least one amino acid which is different from the parent antibody in at least one complementarity determining region (CDR) in the light or heavy chain variable region, which confers a higher binding affinity, (e.g., a 2-fold or more fold) higher binding affinity, to human ADAM9 and/or cynomolgus monkey ADAM9 as compared to the parental antibody. It will be understood from the teaching provided herein that

the antibodies of the invention may be humanized, optimized, or both humanized and optimized.

[0065] A number of humanized antibody molecules comprising an epitope-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent Variable Domain and their associated complementarity determining regions (CDRs) fused to human constant domains (see, for example, Lobuglio *et al.* (1989) "*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224 (1989)). Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody Constant Domain (see, for example, Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; and Jones *et al.* (1986) "*Replacing The Complementarity-Determining Regions In A Human Antibody With Those From A Mouse*," Nature 321:522-525). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example, European Patent Publication No. 519,596. These "humanized" molecules are designed to minimize unwanted immunological response towards rodent anti-human antibody molecules, which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty *et al.* (1991) "*Polymerase Chain Reaction Facilitates The Cloning, CDR-Grafting, And Rapid Expression Of A Murine Monoclonal Antibody Directed Against The CD18 Component Of Leukocyte Integrins*," Nucl. Acids Res. 19:2471-2476 and in U.S. Patents Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692. In aspects, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other aspects, humanized antibodies have one or more CDRs (one, two, three, four, five, or six) which differ in sequence relative to the original antibody.

B. Characteristics of Antibody Constant Domains

1. Constant Domains of the Light Chain

[0066] As indicated above, each Light Chain of an antibody contains a Variable Domain ("VL") and a Constant Domain ("CL").

[0067] A representative CL Domain is a human IgG CL Kappa Domain. The amino acid sequence of a human CL Kappa Domain is (**SEQ ID NO:1**):

RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK
SFNRGEC

[0068] Another representative CL Domain is a human IgG CL Lambda Domain. The amino acid sequence of a human CL Lambda Domain is (**SEQ ID NO:2**):

QPKAAPSVTL FPPSSEELQA NKATLVCLIS DFYPGAVTVA WKADSSPVKA
GVETTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP
TECS

2. Constant Domains of the Heavy Chain

[0069] As indicated above, the heavy chains of an antibody may comprise CH1, Hinge Domain, CH2 and CH3 constant domains. The CH1 Domains of the two heavy chains of an antibody complex with the antibody's Light Chain's CL constant region and are attached to the heavy chains CH2 Domains via an intervening Hinge Domain.

[0070] A representative CH1 Domain is a human IgG1 CH1 Domain. The amino acid sequence of a human IgG1 CH1 Domain is (**SEQ ID NO:3**):

ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRV

[0071] Another representative CH1 Domain is a human IgG2 CH1 Domain. The amino acid sequence of a human IgG2 CH1 Domain is (**SEQ ID NO:4**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTV

[0072] Another representative CH1 Domain is a human IgG4 CH1 Domain. The amino acid sequence of a human IgG4 CH1 Domain is (**SEQ ID NO:5**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRV

[0073] A representative Hinge Domain is a human IgG1 Hinge Domain. The amino acid sequence of a human IgG1 Hinge Domain is (**SEQ ID NO:6**): EPKSCDKTHTCPPCP.

[0074] A representative Hinge Domain is a human IgG2 Hinge Domain. The amino acid sequence of a human IgG2 Hinge Domain is (**SEQ ID NO:7**): ERKCCVECPPCP.

[0075] Another representative Hinge Domain is a human IgG4 Hinge Domain. The amino acid sequence of a human IgG4 Hinge Domain is (**SEQ ID NO:8**): ESKYGPPCPSCP. An IgG4 Hinge Domain may comprise a stabilizing mutation such as the S228P substitution. The amino acid sequence of a S228P-stabilized human IgG4 Hinge Domain is (**SEQ ID NO:9**): ESKYGPPCPPCP.

[0076] The CH2 and CH3 Domains of the two heavy chains of an antibody interact to form an "Fc Domain," which is a domain that is recognized by cellular Fc Receptors, including but not limited to Fc gamma Receptors (FcγRs). As used herein, the term "Fc Domain" is used to define a C-terminal region of an IgG heavy chain. An Fc Domain is said to be of a particular IgG isotype, class or subclass if its amino acid sequence is most homologous to that isotype relative to other IgG isotypes. In addition to their known uses in diagnostics, antibodies have been shown to be useful as therapeutic agents.

[0077] The amino acid sequence of the CH2-CH3 Domain of a representative human IgG1 is (**SEQ ID NO:10**):

```
231       240         250         260         270         280
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD

          290         300         310         320         330
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA

          340         350         360         370         380
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

          390         400         410         420         430
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE

          440     447
ALHNHYTQKS LSLSPGX
```

as numbered by the EU index as set forth in Kabat, wherein **X** is a lysine (K) or is absent.

[0078] The amino acid sequence of the CH2-CH3 Domain of a representative human IgG2 is (**SEQ ID NO:11**):

```
231       240         250         260         270         280
APPVA-GPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFNWYVD

          290         300         310         320         330
GVEVHNAKTK PREEQFNSTF RVVSVLTVVH QDWLNGKEYK CKVSNKGLPA

          340         350         360         370         380
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDISVE

          390         400         410         420         430
WESNGQPENN YKTTPPMLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE

          440     447
ALHNHYTQKS LSLSPGX
```

as numbered by the EU index as set forth in Kabat, wherein **X** is a lysine (K) or is absent.

[0079] The amino acid sequence of the CH2-CH3 Domain of a representative human IgG3 is (**SEQ ID NO:12**):

```
231       240         250         260         270         280
APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVQFKWYVD

          290         300         310         320         330
GVEVHNAKTK PREEQYNSTF RVVSVLTVLH QDWLNGKEYK CKVSNKALPA

          340         350         360         370         380
PIEKTISKTK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE

          390         400         410         420         430
WESSGQPENN YNTTPPMLDS DGSFFLYSKL TVDKSRWQQG NIFSCSVMHE

          440     447
ALHNRFTQKS LSLSPGX
```

as numbered by the EU index as set forth in Kabat, wherein **X** is a lysine (K) or is absent.

[0080] The amino acid sequence of the CH2-CH3 Domain of a representative human IgG4 is (**SEQ ID NO:13**):

```
231       240         250         260         270         280
APEFLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSQED PEVQFNWYVD

          290         300         310         320         330
GVEVHNAKTK PREEQFNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKGLPS

          340         350         360         370         380
SIEKTISKAK GQPREPQVYT LPPSQEEMTK NQVSLTCLVK GFYPSDIAVE

          390         400         410         420         430
WESNGQPENN YKTTPPVLDS DGSFFLYSRL TVDKSRWQEG NVFSCSVMHE

          440     447
ALHNHYTQKS LSLSLGX
```

as numbered by the EU index as set forth in Kabat, wherein **X** is a lysine (K) or is absent.

[0081] Throughout the present specification, the numbering of the residues in the constant region of an IgG heavy chain is that of the EU index as in Kabat *et al.*, SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, NH1, MD (1991), expressly incorporated herein by reference. The term "EU index as in Kabat" refers to the numbering of the constant domains of human IgG1 EU antibody.

[0082] Polymorphisms have been observed at a number of different positions within antibody constant regions (*e.g.*, Fc positions, including but not limited to positions 270, 272, 312, 315, 356, and 358 as numbered by the EU index as set forth in Kabat), and thus slight differences between the presented sequence and sequences in the prior art can exist. Polymorphic forms of human immunoglobulins have been well-characterized. At present, 18 Gm allotypes are known: G1m (1, 2, 3, 17) or G1m (a, x, f, z), G2m (23) or G2m (n), G3m (5, 6, 10, 11, 13, 14, 15, 16, 21, 24, 26, 27, 28) or G3m (b1, c3, b3, b0, b3, b4, s, t, g1, c5, u, v, g5) (Lefranc, *et al.*, "*The Human IgG Subclasses: Molecular Analysis Of Structure, Function And Regulation.*" Pergamon, Oxford, pp. 43-78 (1990); Lefranc, G. *et al.*, 1979, Hum. Genet.: 50, 199-211). It is specifically contemplated that the antibodies of the present disclosure may incorporate any allotype, isoallotype, or haplotype of any immunoglobulin gene, and are not

limited to the allotype, isoallotype or haplotype of the sequences provided herein. Furthermore, in some expression systems the C-terminal amino acid residue (bolded above) of the CH3 Domain may be post-translationally removed. Accordingly, the C-terminal residue of the CH3 Domain is an optional amino acid residue. Specifically encompassed by the instant disclosure is an **ADAM9-ADC** lacking the C-terminal residue of the CH3 Domain. Also specifically encompassed by the instant disclosure are such constructs comprising the C-terminal lysine residue of the CH3 Domain.

[0083] The present disclosure particularly encompasses **ADAM9-ADCs** comprising anti-ADAM9 Variable Domains (*i.e.*, VL and/or VH Domains) that immunospecifically bind to an epitope of a human ADAM9 polypeptide. Such **ADAM9-ADCs** are capable of immunospecifically binding to human ADAM9. As used herein such ADAM9 Variable Domains are referred to as "anti- ADAM9-VL" and "anti- ADAM9-VH," respectively.

II. Anti-ADAM9 Antibody mAb-A

[0084] A representative anti-ADAM9 antibody, designated "mAb-A," was isolated from hybridoma cells that had been produced through immunization with cells expressing human ADAM9, with an ADAM9 polypeptide or a peptide epitope thereof. Antibody mAb-A was humanized. Representative anti-ADAM9 antibody mAb-A and its humanized/optimized variants are described in U.S. Pat. No. 11,242,402, which is hereby incorporated by reference herein.

[0085] Antibody mAb-A was found to be cross-reactive with ADAM9 of cynomolgus monkeys. The amino acid sequences of the VL and VH Domains of mAb-A are provided below. The **ADAM9-ADC** possesses all 3 of the CDR_{HS} of the VH Domain, all 3 of the CDR_{LS} of the VL Domain, and optionally the entire VH and VL Domains of humanized monoclonal antibody mAb-A ("hmAb-A").

A. Murine Anti-ADAM9 Antibody mAb-A

[0086] The amino acid sequence of the VL Domain of the murine anti-ADAM9 antibody mAb-A (**SEQ ID NO:14**) is shown below (CDR_L residues are shown underlined):

DIVLTQSPAS LAVSLGQRAT ISC**KASQSVD YDGDSYMN**WY QQIPGQPPKL LIY**AASDLES** GIPARFSGSG SGTDFTLNIH PVEEEDAATY YC**QQSHEDPF T**FGGGTKLEI K

[0087] The amino acid sequence of the **CDR_L1 Domain** of mAb-A is **(SEQ ID NO:15)**: KASQSVDYDGDSYMN.

[0088] The amino acid sequence of the **CDR_L2 Domain** of mAb-A is **(SEQ ID NO:16)**: AASDLES.

[0089] The amino acid sequence of the **CDR_L3 Domain** of mAb-A is **(SEQ ID NO:17)**: QQSHEDPFT.

[0090] The amino acid sequence of the VH Domain of anti-ADAM9 mAb-A **(SEQ ID NO:18)** is shown below (CDR_H residues are shown underlined):

QVQLQQPGAE LVKPGASVKL SCKASGYTFT **SYWMH**WVKQR PGQGLEWIG**E IIPINGHTNY NEKFKS**KATL TLDKSSSTAY MQLSSLASED SAVYYCAR**GG YYYYGSRDYF DY**WGQGTTLT VSS

[0091] The amino acid sequence of the **CDR_H1 Domain** of mAb-A is **(SEQ ID NO:19)**: SYWMH.

[0092] The amino acid sequence of the **CDR_H2 Domain** of mAb-A is **(SEQ ID NO:20)**: EIIPINGHTNYNEKFKS.

[0093] The amino acid sequence of the **CDR_H3 Domain** of mAb-A is **(SEQ ID NO:21)**: GGYYYYGSRDYFDY.

B. Humanized Anti-ADAM9 Antibody hmAb-A

[0094] The Variable Domains of the anti-ADAM9 antibody mAb-A were humanized to generate a humanized mAb-A ("hmAb-A"). In some instances, alternative humanized Variable Domains were generated to optimize binding activity and/or to remove antigenic epitopes and/or to remove potentially labile amino acid residues.

[0095] The amino acid sequence of the VL Domain of hmAb-A **(SEQ ID NO:22)** is shown below (CDR_L residues are shown underlined):

DIVMTQSPDS LAVSLGERAT ISCKASQSVD YSGDSYMNWY QQKPGQPPKL
LIYAASDLES GIPARFSGSG SGTDFTLTIS SLEPEDFATY YCQQSHEDPF
TFGQGTKLEI K

[0096] The amino acid sequence of a Light Chain of hmAb-A comprising the VL Domain of hmAb-A and a CL Kappa Domain (**SEQ ID NO:23**) is shown below:

DIVMTQSPDS LAVSLGERAT ISCKASQSVD YSGDSYMNWY QQKPGQPPKL
LIYAASDLES GIPARFSGSG SGTDFTLTIS SLEPEDFATY YCQQSHEDPF
TFGQGTKLEI KRTVAAPSVF IFPPSDEQLK SGTASVVCLL NNFYPREAKV
QWKVDNALQS GNSQESVTEQ DSKDSTYSLS STLTLSKADY EKHKVYACEV
THQGLSSPVT KSFNRGEC

[0097] In **SEQ ID NO:23**, amino acid residues 1-111 correspond to the VL Domain of hmAb-A (**SEQ ID NO:22**), and amino acid residues 112-218 correspond to the Light Chain kappa constant region (**SEQ ID NO:1**).

[0098] The amino acid sequence of the **CDR_L1 Domain** of hmAb-A is (**SEQ ID NO:24**): KASQSVDYSGDSYMN.

[0099] The amino acid sequence of the **CDR_L2 Domain** of hmAb-A is (**SEQ ID NO:25**): AASDLES.

[00100] The amino acid sequence of the **CDR_L3 Domain** of hmAb-A is (**SEQ ID NO:26**): QQSHEDPFT.

[00101] The amino acid sequence of the VH Domain of hmAb-A (**SEQ ID NO:27**) is shown below (CDR_H residues are shown underlined).

EVQLVESGGG LVKPGGSLRL SCAASGFTFS SYWMHWVRQA PGKGLEWVGE
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCARGG
YYYYPRQFL DYWGQGTTVT VSS

[00102] The amino acid sequence of a Heavy Chain comprising the VH Domain of hmAb-A and IgG1 CH1-H-CH2-CH3 Domains (**SEQ ID NO:28**) is shown below:

EVQLVESGGG LVKPGGSLRL SCAASGFTFS SYWMHWVRQA PGKGLEWVGE
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCARGG
YYYYPRQGFL DYWGQGTTVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC
LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG
TQTYICNVNH KPSNTKVDKR VEPKSCDKTH TCPPCPAPEL LGGPSVFLFP

PKPKDTLYIT REPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
PGX

wherein, X is a lysine (K) or is absent.

[00103] In **SEQ ID NO:28**, amino acids 1-123 correspond to the VH Domain of hmAb-A (**SEQ ID NO:27**), amino acid residues 124-221 correspond to the IgG1 CH1 Domain (**SEQ ID NO:3**), amino acid residues 222–236 correspond to the IgG1 Hinge Domain (**SEQ ID NO:6**), and amino acid residues 237–453 correspond to the IgG1 CH2-CH3 Domain (**SEQ ID NO:10**). The C-terminal residue "**X**" is a lysine (K) or is absent.

[00104] The amino acid sequence of the **CDR_H1 Domain** of hmAb-A is **(SEQ ID NO:29)**: SYWMH.

[00105] The amino acid sequence of the **CDR_H2 Domain** of hmAb-A is **(SEQ ID NO:30)**: EIIPIFGHTNYNEKFKS.

[00106] The amino acid sequence of the **CDR_H3 Domain** of hmAb-A is **(SEQ ID NO:31)**: GGYYYYPRQGFLDY.

III. Modification of the Fc Domain

[00107] The Fc Domain of the Fc Domain-containing molecules (e.g., antibodies and diabodies) may be either a complete Fc Domain (e.g., a complete IgG Fc Domain) or only a fragment of an Fc Domain. Optionally, the Fc Domain of the Fc Domain-containing molecules lacks the C-terminal lysine amino acid residue.

[00108] In traditional immune function, the interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody dependent cytotoxicity, mast cell degranulation, and phagocytosis to immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All of these interactions are initiated through the binding of the Fc Domain of antibodies or immune complexes to specialized cell surface receptors (singularly referred to as

an "Fc gamma receptor," "FcγR," and collectively as "FcγRs") found on the surfaces of multiple types of immune system cells (*e.g.*, B lymphocytes, follicular dendritic cells, natural killer cells, macrophages, neutrophils, eosinophils, basophils and mast cells). The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of the three Fc receptors: FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16). FcγRI (CD64), FcγRIIA (CD32A) and FcγRIII (CD16) are activating (*i.e.*, immune system enhancing) receptors; FcγRIIB (CD32B) is an inhibiting (*i.e.*, immune system dampening) receptor. In addition, interaction with the neonatal Fc Receptor (FcRn) mediates the recycling of IgG molecules from the endosome to the cell surface and release into the blood. The amino acid sequence of a represenative wild-type IgG1 CH2-CH3 Domains (**SEQ ID NO:10**), IgG2 (**SEQ ID NO:11**), IgG3 (**SEQ ID NO:12**), and IgG4 (**SEQ ID NO:13**) are presented above.

[00109] Modification of the Fc Domain may lead to an altered phenotype, for example altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function. Accordingly, in certain aspects, the Fc Domain of the Fc Domain-containing molecules may be an engineered variant Fc Domain. Although the Fc Domain of the Fc Domain-containing molecules may possess the ability to bind to one or more Fc receptors (*e.g.*, FcγR(s)), in particular such variant Fc Domain will have altered binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a), or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc Domain), *e.g.*, will have enhanced binding to an activating receptor and/or will have substantially reduced or no ability to bind to inhibitory receptor(s). Thus, the Fc Domain of the Fc Domain-containing molecules may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc Domain, or may comprise a variant CH2 and/or a variant CH3 sequence (that may include, for example, one or more insertions and/or one or more deletions with respect to the CH2 or CH3 domains of a complete Fc Domain). Such Fc Domains may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc Domains, or may comprise non-naturally occurring orientations of CH2 and/or CH3 Domains (such as, for example, two CH2 domains or two CH3 domains, or in the N-terminal to C-terminal direction, a CH3 Domain linked to a CH2 Domain, *etc.*).

[00110] In certain aspects, the Fc Domains of the binding molecules exhibit decreased (or substantially no) binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by the wild-type IgG1 Fc Domain (**SEQ ID NO:10**). In certain aspects, the binding molecules comprise an IgG Fc Domain that exhibits reduced ADCC effector function. In a such aspects, the CH2-CH3 Domains of binding molecules include any 1, 2, 3, or 4 of the substitutions: L234A, L235A, D265A, N297Q, and N297G. In another aspect, the CH2-CH3 Domains contain an N297Q substitution, an N297G substitution, L234A and L235A substitutions or a D265A substitution, as these mutations abolish FcR binding. Alternatively, a CH2-CH3 Domain of a naturally occurring Fc Domain that inherently exhibits decreased (or substantially no) binding to FcγRIIIA (CD16a) and/or reduced effector function (relative to the binding and effector function exhibited by the wild-type IgG1 Fc Domain (**SEQ ID NO:10**)) is utilized. In a specific aspect, the binding molecules comprise an IgG4 Fc Domain (**SEQ ID:NO:13**). When an IgG4 Fc Domain is utilized, the instant disclosure also encompasses the introduction of a stabilizing mutation, such as the Hinge Domain S228P substitution described herein (see, *e.g.*, **SEQ ID NO:9**).

[00111] The serum half-life of proteins comprising Fc Domains may be increased by increasing the binding affinity of the Fc Domain for FcRn. The term "half-life" as used herein means a pharmacokinetic property of a molecule that is a measure of the mean survival time of the molecules following their administration. Half-life can be expressed as the time required to eliminate fifty percent (50%) of a known quantity of the molecule from a subject's body (*e.g.*, a human patient or other mammal) or a specific compartment thereof, for example, as measured in serum, *i.e.*, circulating half-life, or in other tissues. In general, an increase in half-life results in an increase in mean residence time (MRT) in circulation for the molecule administered. Modifications capable of increasing the half-life of an Fc Domain-containing molecule are known in the art and include, for example M252Y, S254T, T256E, and combinations thereof. For example, see the modifications described in U.S. Patent Nos. 6,277,375; 7,083,784; 7,217,797; and 8,088,376; U.S. Publication Nos. 2002/0147311; 2007/0148164; and 2011/0081347.

[00112] In certain aspects, an IgG1 sequence for the CH2 and CH3 Domains of the Fc Region-containing molecules provided herein comprises the M252Y, S254T and T256E substitutions (shown underlined), so as to extend the serum half-life. The amino acid sequence of such molecule is (**SEQ ID NO:32**):

APELLGGPSV FLFPPKPKDT L**Y**I**T**R**E**PEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPG**X**

wherein **X** is a lysine (K) or is absent.

IV. ADAM9-ADC

[00113] The present disclosure relates to the above-described anti-ADAM9 antibody hmAb-A conjugated to a cytotoxic drug, an "**ADAM9-ADC**". Such **ADAM9-ADC** enhances the cytotoxicity of anti-ADAM9 therapy, particularly in the treatment of cancer. As indicated above, an **ADAM9-ADC** is represented by the formula:

$$\mathbf{Ab\text{-}(LM)_m\text{-}(D)_n,}$$

wherein:

Ab is an antibody that binds to ADAM9 that comprises a humanized Variable Heavy Chain (VH) Domain and a humanized Variable Light Chain (VL) Domain, or is an ADAM9-binding fragment thereof, and;

D is a cytotoxic drug moiety;

LM is a a Linker Molecule that covalently links **Ab** and **D**;

m is an integer between 1 and n and denotes the number of bonds or Linker Molecules of the **ADAM9-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic camptothecin moieties covalently linked to the **ADAM9-ADC**.

[00114] In certain aspects, an **ADAM9-ADC** comprises a naturally occurring Fc Domain of the IgG1 isotype. Such Fc Domain lacks the C-terminal lysine residue of a CH3 Domain. In

other aspects, an **ADAM9-ADC** comprises an IgG1 sequence for the CH2 and CH3 Domains comprising **SEQ ID NO:32**.

[00115] In specific aspects, an **ADAM9-ADC** will bind to a tumor cell expressing ADAM9 and will then be internalized into such cell through receptor-mediated endocytosis. Once inside a lysosome, an **ADAM9-ADC** may be be degraded so as to thereby cause the release of the cytotoxic camptothecin moiety inside the cell, resulting in cell death. As will be appreciated, the mechanism of action of cell death can vary based on the class of cytotoxic drug used. Neighboring cancer cells may also be killed when free drug is released into the tumor environment by the dying cell in a process known as the bystander effect (Panowski, S. *et al.* (2014) "*Site-Specific Antibody Drug Conjugates For Cancer Therapy*," mAbs 6(1):34-45; Kovtun, Y.V. *et al.* (2006) "*Antibody-Drug Conjugates Designed To Eradicate Tumors With Homogeneous And Heterogeneous Expression Of The Target Antigen*," Cancer Res. 66:3214-3221).

[00116] In aspects, the **Ab** of the **ADAM9-ADC** is an ADAM9 antibody as described above.

[00117] In aspects, **Ab** is a humanized ADAM9 antibody or ADAM9 binding fragment thereof that binds to ADAM9 and comprises: (i) the **CDR_L1** sequence KASQSVDYSGDSYMN (**SEQ ID NO:24**), the **CDR_L2** sequence AASDLES (**SEQ ID NO:25**) and the **CDR_L3** sequence QQSHEDPFT (**SEQ ID NO:26**) in its Variable Light Chain (VL) domain, and (ii) the **CDR_H1** sequence SYWMH (**SEQ ID NO:29**), the **CDR_H2** sequence EIIPIFGHTNYNEKFKS (**SEQ ID NO:30**) and the **CDR_H3** sequence GGYYYYPRQGFLDY (**SEQ ID NO:31**) in its Variable Heavy Chain (VH) domain.

[00118] In aspects, **Ab** comprises: (i) a humanized VL Domain comprising the amino acid sequence of **SEQ ID NO:22**, and (ii) a humanized VH Domain comprising the amino acid sequence of **SEQ ID NO:27.**

[00119] In aspects, **Ab** comprises: (i) a Light Chain comprising the amino acid sequence of **SEQ ID NO:23**, and (ii) a Heavy Chain comprising the amino acid sequence of **SEQ ID NO:28**.

[00120] In aspects, present disclosure provides an antibody **Ab** that has undergone glycan remodeling. Examples of glycan remodeling are described further below. In aspects, the **Ab** has undergone glycan remodeling and comprises an azidosugar. In aspects, the azidosugar is GalNAz (N-Azidoacetylgalactosamine), 6-azido-Gal or 6-azido-GalNAc.

A. Linker Molecules

[00121] The disclosure particularly contemplates **ADAM9-ADCs** that possess one or more Linker Molecule **LM** (*i.e.*, **m** is an integer from 2 through **n**, wherein n is an integer from 2 through 10), each of which Linker Molecule **LM** covalently links a camptothecin moiety **D** to the **Ab** of such **ADAM9-ADCs**.

[00122] The disclosure further provides **ADAM9-ADCs** whose **Ab** are linked to more than one Linker Molecule **LM**, wherein all such Linker Molecules are identical. The camptothecin moieties **D** that are covalently linked to the **Ab** of such **ADAM9-ADCs** may all be identical or may include 2, 3, 4, or more independently different camptothecin moieties **D**.

[00123] The disclosure further provides such **ADAM9-ADCs** whose **Ab** are linked to more than one Linker Molecule **LM**, wherein all such Linker Molecules are not identical and may independently differ. The camptothecin moieties **D** that are linked to the **Ab** of such **ADAM9-ADCs** may all be identical or may include 2, 3, 4, or more independently different campothecin moieties **D**.

[00124] Humanized VH and VL Domains of antibodies that bind to human ADAM9, and human antibody Constant Domains that may be included in an **ADAM9-ADC** are provided above. As stated above, an **ADAM9-ADC** additionally comprises at least one cytotoxic drug moiety, which is covalently linked to through an amino acid residue of such VH Domain or VL Domain and/or Constant Domain via a Linker Molecule attached to the side chain and the drug moiety. The Linker Molecule may be a non-peptide molecule, or a molecule that comprises a non-peptide portion and a peptide portion, or it may be a molecule that is composed solely of amino acid residues. The amino acid residues of any such Linker Molecules may contain naturally occurring or non-naturally occurring amino acid residues, including D-versions of naturally occurring amino acid residues, *p*-acetylphenylalanine, selenocysteine, *etc.* Optionally, or additionally, particular residues having a desired side chain (*e.g.*, a -CH_2-SH side chain, a-

CH_2-OH side chain, a -CH(CH_2)-SH side chain, a -CH_2-CH_2-S-CH_3 side chain; a -CH_2-C(O)-NH_2 side chain, a -CH_2-CH_2-C(O)-NH_2 side chain, a -CH_2-C(O)OH- side chain, a CH_2-CH_2-C(O)OH- side chain, a -CH_2-CH_2-CH_2-CH_2-NH_2 side chain, a -CH_2-CH_2-CH_2-NH-C(NH_2)$_2$ side chain, an imidazole side chain, a benzyl side chain, a phenol side chain, an indole side chain, *etc.*) may be engineered into an **ADAM9-ADC**.

[00125] In aspects, cytotoxic drug moieties may be conjugated to the **Ab** of the **ADAM9-ADC** of the disclosure by means known in the art (see, *e.g.*, WO2016053107; Yao, H. *et al.* (2016) "*Methods to Design and Synthesize Antibody-Drug Conjugates (ADC),*" Intl. J. Molec. Sci. 17(194):1-16); Behrens, C. R. *et al.* (2014) "*Methods For Site-Specific Drug Conjugation To Antibodies,*" mAbs 6(1):46-53; Bouchard, H. *et al.* (2014) "*Antibody-Drug Conjugates – A New Wave Of Cancer Drugs,*" Bioorganic & Medicinal Chem. Lett 24:5357-5363). The thiol group of a cysteine, the amino side group of lysine, glutamine or arginine, or the carboxyl group of glutamate or aspartate can be employed to conjugate the Linker Molecule-cytotoxic drug moiety (**LM-D**) to the **Ab** of the **ADAM9-ADC** of the disclosure. Native antibodies contain numerous lysine conjugation sites, and thus are capable of linking multiple conjugated molecules per antibody. Indeed, peptide mapping has determined that conjugation occurs on both the heavy and light chain at approximately 20 different lysine residues (40 lysines per mAb). Therefore, greater than one million different ADC species can be generated. Cysteine conjugation occurs after reduction of one to four inter-chain disulfide bonds, and the conjugation is thus limited in native VL and VH Domains to the eight exposed sulfhydryl groups. However, if desired, additional reactive (*e.g.*, lysine, cysteine, selenocysteine, *etc.*) residues may be engineered into an antibody (*e.g.*, within a VL Domain and/or a VH Domain and/or a Constant Domain). For example, one or more native amino acid residues may be substituted with a cysteine residue. An unnatural amino acid (e.g. *p*-acetylphenylalanine) may be genetically incorporated into an antibody using an amber stop codon suppressor tRNA/aaRS pair. (See, *e.g.*, Behrens CR, and Liu B. (2014) "*Methods For Site-Specific Drug Conjugation To Antibodies,*" mAbs 6(1):46-53. doi:10.4161/mabs.26632; Panowksi, S., *et al.* (2014) "*Site-Specific Antibody Drug Conjugates For Cancer Therapy,*" mAbs, 6(1), 34–45, doi:10.4161/mabs.27022; and WO 2008/070593). Alternatively, or additionally, enzymes (*e.g.*, a glycotransferase) may be used to conjugate the Linker Molecule-cytotoxic drug moiety (**LM-D**) to the **Ab** of the **ADAM9-ADC** of the disclosure. The glycotransferase platform attaches a

sugar moiety to a glycosylation site on an antibody (for example, position N297 of the Fc Domain of a human IgG antibody), which can then serve as the Linker Molecule (**LM**) of the present disclosure and conjugate the cytotoxic drug moiety (**D**) to the **Ab** of the **ADAM9-ADC** of the disclosure. Alternatively, a transglutaminase may be used to catalyze the formation of a covalent bond between a free amine group and a glutamine side chain.

[00126] In aspects, the Linker Molecule **LM** is attached to a glycan moiety attached to a side chain of the **Ab**. In aspects, the glycan moiety on the side chain is a naturally occurring glycan moiety. In aspects, the glycan moiety has undergone glycan remodeling prior to attachment to the Linker Molecule.

[00127] In aspects, glycan remodeling is performed by contacting the glycan moiety with an endoglycosidase and a glycosyl transferase. In aspects, the glycan remodeling adds an azidosugar to the end of the glycan moiety. In aspects, the azidosugar is GalNAz (N-Azidoacetylgalactosamine), 6-azido-Gal or 6-azido-GalNAc. In aspects, the Linker Molecule **LM** is attached to the azidosugar on the glycan moiety. In aspects, the Linker Molecule **LM** is attached to the azidosugar on the glycan moiety by reacting the azido group on the azidosugar to a reactive group on the **LM**.

[00128] In aspects, glycan remodeling is performed by contacting the glycan moiety with an endoglycosidase and an N-Acetylgalactosamine (GalNAc) transferase. In aspects, the glycan remodeling adds a N-Acetylgalactosamine (GalNAc) sugar to the end of the glycan moiety. In aspects, the GalNAc sugar comprises an azido group. In aspects, the Linker Molecule **LM** is attached to the GalNAc sugar on the glycan moiety. In aspects, the Linker Molecule **LM** is attached to the GalNAc sugar on the glycan moiety by reacting the azido group on the GalNAc to a reactive group on the **LM**.

[00129] In aspects, the glycan remodeling performed is the GlycoConnect™ process (Synaffix). In aspects, glycan remodeling is performed as described in US Patent Nos. US9,504,758, US10,745,488, US9,988,661, US10,858,641, US9,222,940, US10,239,807, and US11,358,921, each of which is hereby incorporated by reference herein.

[00130] In aspects, the Linker Molecule **LM** may be non-cleavable under physiologic conditions, for example composed of a hydrolytically stable moiety, for example, a thioether linker or a hindered disulfide linker. Hydrolytically stable linkers are substantially stable in water and do not react with water at useful pH values, including but not limited to, under physiological conditions for an extended period of time. In contrast, hydrolytically unstable or degradable linkers are degradable in water or in aqueous solutions, including for example, blood.

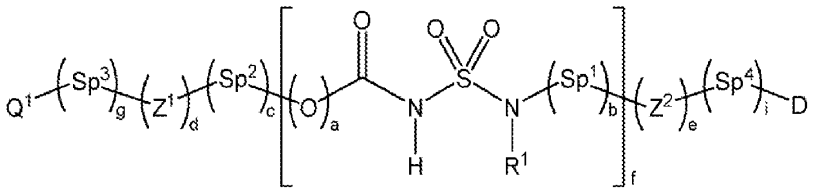
[00131] In alternative aspects, the Linker Molecule **LM** may be cleavable, or may contain a cleavable portion. Examples of such a cleavable portion includes an acid labile linker (*e.g.*, a 4-(4'-acetylpheonxy) butanoic acid linker which forms a hydrazine bond), a cleavable disulfide linker (that is cleaved in the reducing intracellular environment), and a protease cleavable linker. Acid-labile linkers are designed to be stable at pH levels encountered in the blood, but become unstable and degrade when the low pH environment in lysosomes is encountered. Protease-cleavable linkers are also designed to be stable in blood/plasma, but rapidly release free drug inside lysosomes in cancer cells upon cleavage by lysosomal enzymes (Panowski, S. *et al.* (2014) "*Site-Specific Antibody Drug Conjugates For Cancer Therapy*," mAbs 6(1):34-45). Alternatively, the Linker Molecule **LM** may be an enzyme-cleavable-substrate or contain an enzyme-cleavable-substrate, such as a cleavable peptide, (*e.g.*, a cleavable dipeptide such as a valine-alanine dipeptide para-aminobenzylalcohol linker (cAC10-mc-va-PABA), a valine-alanine dipeptide para-aminocarbamate (Val-Ala-PABC or VA-PABC) a valine-citrulline dipeptide para-aminobenzylalcohol linker (cAC10-mc-vc-PABA) or a valine-citrulline dipeptide para-aminocarbamate (Val-Cit-PABC or VC-PABC), which are selectively cleaved by lysosomal enzymes). Suitable cleavable linkers are known in the art, see, *e.g.*, de Groot, Franciscus M.H., *et al.* (2002) "*Design, Synthesis, and Biological Evaluation of a Dual Tumor-Specific Motive Containing Integrin-Targeted Plasmin-Cleavable Doxorubicin Prodrug*," Molecular Cancer Therapeutics, 1: 901-911; Dubowchik *et al.*, (2002) "Doxorubicin Immunoconjugates Containing Bivalent, Lysosomally-Cleavable Dipeptide Linkages." Bioorganic & Medicinal Chemistry Letters 12:1529-1532; US Patent Nos. 5,547,667; 6,214,345; 7,585,491; 7,754,681; 8,080,250; 8,461,117; and WO 02/083180.

[00132] In aspects, enzymatically unstable or degradable linkers can be employed. Such linkers are degraded by one or more enzymes. By way of example only, PEG and related polymers can include a degradable Linker Molecule(s) in the polymer backbone or in the linker group between the polymer backbone and one or more of the terminal functional groups of the polymer molecule. Such degradable Linker Molecule(s) include, but are not limited to, ester linkages formed by the reaction of PEG carboxylic acids or activated PEG carboxylic acids with alcohol groups on a biologically active agent, wherein such ester groups generally hydrolyze under physiological conditions to release the biologically active agent. Other hydrolytically degradable Linker Molecules include but are not limited to carbonate linkages; imine linkages resulting from reaction of an amine and an aldehyde; phosphate ester linkages formed by reacting an alcohol with a phosphate group; hydrazone linkages that are a reaction product of a hydrazide and an aldehyde; acetal linkages that are the reaction product of an aldehyde and an alcohol; orthoester linkages that are the reaction product of a formate and an alcohol; peptide linkages formed by an amine group, including but not limited to, at an end of a polymer such as PEG, and a carboxyl group of a peptide; and oligonucleotide linkages formed by a phosphoramidite group, including but not limited to, at the end of a polymer, and a 5' hydroxyl group of an oligonucleotide.

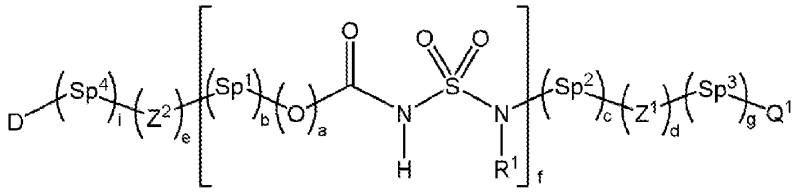
[00133] In aspects, the Linker Molecule **LM** comprises a peptidic linker. In aspects, the peptidic linker is a Valine-Alanine dipeptide linker. In aspects, the Linker Molecule comprises a cleavable linker. In aspects, the Linker Molecule comprises a Valine-Alanine (Val-Ala) amino acid linker. In aspects, the Val-Ala linker is a Val-Ala-PABC linker.

[00134] In aspects, the peptidic linker is a Valine-Citrulline dipeptide linker. In aspects, the Linker Molecule comprises a cleavable linker. In aspects, the Linker Molecule comprises a Valine-Citruline (Val-Cit) amino acid linker. In aspects, the Val-Cit linker is a Val-Cit-PABC linker.

[00135] In one aspect, the Linker Molecule **LM** can be, or can comprise, a cleavable Linker Molecule comprising formula (4a) or (4b), or a salt thereof:



4a



4b

wherein:

a is independently 0 or 1;

b is independently 0 or 1;

c is 0 or 1;

d is 0 or 1;

e is 0 or 1;

f is an integer in the range of 1 to 150;

g is 0 or 1;

i is 0 or 1;

D is a cytotoxic camptothecin moiety;

Q^1 is an alkenyl group, (hetero)cycloalkenyl group, bicyclo triazole group or cycloalkenyl group;

wherein Q^1 is attached to a functional group of the antibody;

wherein Sp^1, Sp^2, Sp^3 and Sp^4 are independently selected from the group consisting of linear or branched C_1-C_{200} alkylene groups, C_2-C_{200} alkenylene groups, C_2-C_{200} alkynylene groups, C_3-C_{200} cycloalkylene groups, C_5-C_{200} cycloalkenylene groups, C_8-C_{200} cycloalkynylene groups, C_7-C_{200} alkylarylene groups, C_7-C_{200} arylalkylene groups, C_8-C_{200} arylalkenylene groups and C_9-C_{200} arylalkynylene groups, the alkylene groups, alkenylene groups, alkynylene groups, cycloalkylene groups, cycloalkenylene groups, cycloalkynylene groups,

alkylarylene groups, arylalkylene groups, arylalkenylene groups and arylalkynylene groups being optionally substituted and optionally interrupted by one or more heteroatoms selected from the group of O, S and NR^3, wherein R^3 is independently selected from the group consisting of hydrogen, C_1 - C_{24} alkyl groups, C_2 - C_{24} alkenyl groups, C_2 - C_{24} alkynyl groups and C_3 - C_{24} cycloalkyl groups, the alkyl groups, alkenyl groups, alkynyl groups and cycloalkyl groups being optionally substituted;

Z^1 is a connecting group that connects Q^1 or Sp^3 to Sp^2, O or C(O) or $N(R^1)$;

Z^2 is a connecting group that connects D or Sp^4 to Sp^1, $N(R^1)$, O or C(O);

wherein Z^1 and Z^2 are independently selected from the group consisting of -O-, -S-, $-NR^2-$, -N=N-, -C(O)-, $-C(O)NR^2-$, -O-C(O)- , -O-C(O)-O-, $-O-C(O)-NR^2$, $-NR^2-C(O)-$, $-NR^2-C(O)-O-$, $-NR^2-C(O)-NR^2-$, -S-C(O)-, -S-C(O)-O-, $-S-C(O)-NR^2-$, -S(O)-, $-S(O)_2-$, $-O-S(O)_2-$, $-O-S(O)_2-O-$, $-O-S(O)_2-NR^2-$, -O-S(O)-, -O-S(O)-O-, $-O-S(O)-NR^2-$, $-O-NR^2-C(O)-$, $-O-NR^2-C(O)-O-$, $-O-NR^2-C(O)-NR^2-$, $-NR^2-O-C(O)-$, $-NR^2-O-C(O)-O-$, $-NR^2-O-C(O)-NR^2-$, $-O-NR^2-C(S)-$, $-O-NR^2-C(S)-O-$, $-O-NR^2-C(S)-NR^2-$, $-NR^2-O-C(S)-$, $-NR^2-O-C(S)-O-$, $-NR^2-O-C(S)-NR^2-$, -O-C(S)-, -O-C(S)-O-, $-O-C(S)-NR^2-$, $-NR^2-C(S)-$, $-NR^2-C(S)-O-$, $-NR^2-C(S)-NR^2-$, $-S-S(O)_2-$, $-S-S(O)_2-O-$, $-S-S(O)_2-NR^2-$, $-NR^2-O-S(O)-$, $-NR^2-O-S(O)-O-$, $-NR^2-O-S(O)-NR^2-$, $-NR^2-O-S(O)_2-$, $-NR^2-O-S(O)_2-O-$, $-NR^2-O-S(O)_2-NR^2-$, $-O-NR^2-S(O)-$, $-O-NR^2-S(O)-O-$, $-O-NR^2-S(O)-NR^2-$, $-O-NR^2-S(O)_2-O-$, $-O-NR^2-S(O)_2-NR^2-$, $-O-NR^2-S(O)_2-$, $-O-P(O)(R^2)_2-$, $-S-P(O)(R^2)_2-$, $-NR^2-P(O)(R^2)_2-$ and combinations of two or more thereof, wherein R^2 is independently selected from the group consisting of hydrogen, C_1 - C_{24} alkyl groups, C_2 - C_{24} alkenyl groups, C_2 - C_{24} alkynyl groups and C_3 - C_{24} cycloalkyl groups, the alkyl groups, alkenyl groups, alkynyl groups and cycloalkyl groups being optionally substituted; and

R^1 is selected from the group consisting of hydrogen, C_1 - C_{24} alkyl groups, C_3 - C_{24} cycloalkyl groups, C_2 - C_{24} (hetero)aryl groups, C_3 - C_{24} alkyl(hetero)aryl groups and C_3 - C_{24} (hetero)arylalkyl groups, the C_1 - C_{24} alkyl groups, C_3 - C_{24} cycloalkyl groups, C_2 - C_{24} (hetero)aryl groups, C_3 - C_{24} alkyl(hetero)aryl groups and C_3 - C_{24} (hetero)arylalkyl groups optionally substituted and optionally

interrupted by one or more heteroatoms selected from O, S and NR^3 wherein R^3 is independently selected from the group consisting of hydrogen and C_1 - C_4 alkyl groups; or

R^1 is D, $-[(Sp^1)_b(Z^2)_e-(Sp^4)_i-D]$ or $-[(Sp^2)_c-(Z^1)_d-(Sp^3)_g-Q^1]$, wherein Sp^1, Sp^2, Sp^3, Sp^4, Z^1, Z^2, D, Q^1, b, c, d, e, g and i are as defined above.

[00136] In some aspects Q1 is a triazole derivative of cyclooctyne.

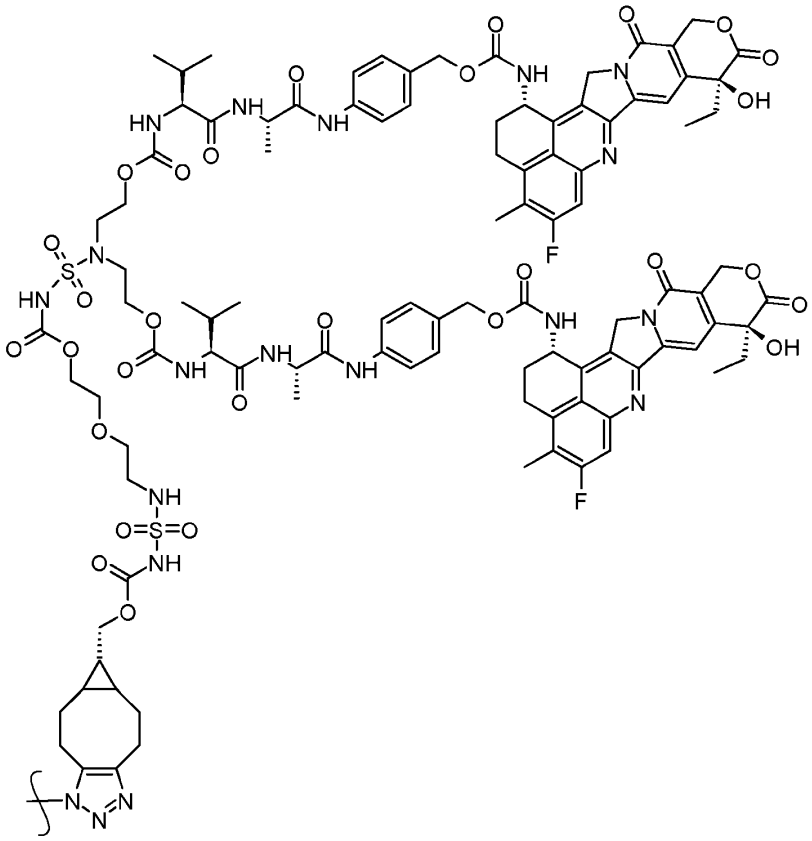
[00137] In some aspects, the Linker Molecule **LM** further comprises Sp^1, Sp^2, Sp^3 and Sp^4, if present, which are independently selected from the group consisting of linear or branched C_1-C_{20} alkylene groups, the alkylene groups being optionally substituted and optionally interrupted by one or more heteroatoms selected from the group consisting of O, S and NR^3, wherein R^3 is independently selected from the group consisting of hydrogen and C_1 - C_4 alkyl groups

[00138] In aspects, the cleavable linker is a linker as disclosed in US Patent No. US9,636,421, the disclosure of which is hereby incorporated by reference herein.

[00139] In aspects, the Linker Molecule **LM** comprises one or more polar spacers. In aspects, the polar spacer is a carbamoyl sulfamide. Examples of spacers that can be used are found in US Patent Nos. US9,636,421 and US10,792,369, which are hereby incorporated by reference herein.

[00140] In aspects, the **ADAM9-ADC** comprises two, three, four, five, six, seven, eight, nine or ten cytotoxic drug moieties, which may be the same, or may independently be the same or different from another cytotoxic drug moiety of the **ADAM9-ADC**. In one aspect, each such cytotoxic drug moiety is conjugated to the **Ab** of the **ADAM9-ADC** via a separate Linker Molecule. Alternatively, more than one cytotoxic drug moiety may be attached to the **Ab** of the **ADAM9-ADC** via the same Linker Molecule.

[00141] In aspects, the disclosure contemplates **ADAM9-ADCs** that comprise one or more Linker Molecule **LM** camptothecin moiety **D**, where the **LM** and **D** together comprise:

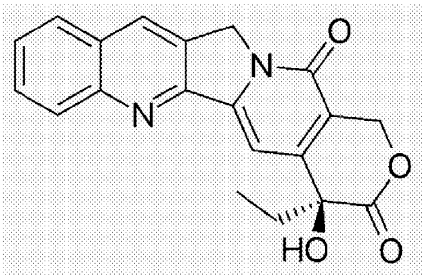


B. Exemplary Cytotoxic Drug Moieties

[00142] In aspects, the cytotoxic drug moiety of the **ADAM9-ADC** comprises a cytotoxin, a radioisotope, an immunomodulator, a cytokine, a lymphokine, a chemokine, a growth factor, a tumor necrosis factor, a hormone, a hormone antagonist, an enzyme, an oligonucleotide, a DNA molecule, an RNA molecule, an siRNA molecule, an RNAi molecule, a microRNA molecule, a photoactive therapeutic agent, an anti-angiogenic agent, a pro-apoptotic agent, a peptide, a lipid, a carbohydrate, a chelating agent, or a combination thereof.

1. Camptothecin Cytotoxic Drug Moieties

[00143] In aspects, the **ADAM9-ADC** can comprise a camptothecin cytotoxic drug moiety:



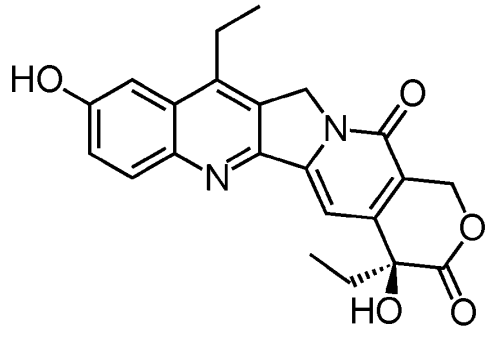
[00144] As used herein the term "camptothecin" means that class of compounds considered to be camptothecins, camptothecin analogs, camptothecin derivatives or camptothecin conjugates. These compounds are based on the characteristic five-ring backbone of camptothecin.

[00145] Camptothecin (CPT), a plant alkaloid, was found to have anticancer activity in the late 1950's. Camptothecin, whether substituted or unsubstituted, is believed to intervene in the mechanism of action of the nuclear enzyme topoisomerase I (topo I), arresting cells in the S phase. Without being bound by theory, it is believed that CPT accomplishes this by stabilizing the covalently linked complexes of DNA-topo I (termed cleavable complexes), thus halting the progression of replication forks. This collision of the replication fork with the cleavable complexes is believed to trigger the apoptotic pathway. Z. Darzynkiewicz et al., The Cell Cycle Effects of Camptothecin, 803 Annals of the New York Academy of Sciences 93 (1996). DNA strand breaks are also implicated in the cytotoxic effects of CPT. F. Traganos et al., Induction of Apoptosis by Camptothecin and Topotecan, 803 Annals of the New York Academy of Sciences 101 (1996).

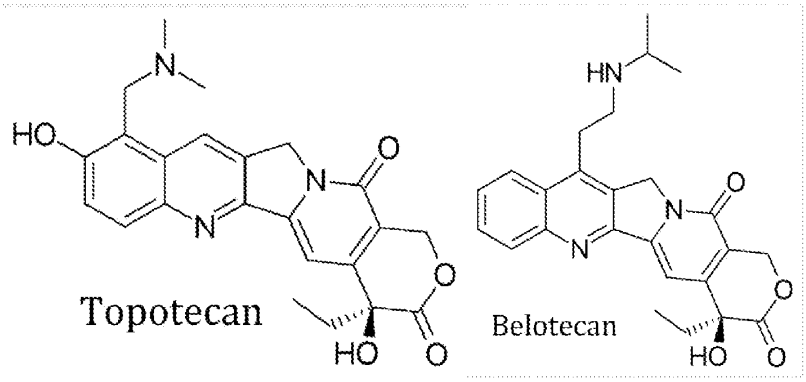
[00146] Examples of camptothecin include SN-38 (*S*-10-hydroxycamptothecin), irinotecan (CAMPTOSAR; 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxycamptothecin), topotecan (HYCAMPTIN; (S)-9-N,N-dimethylaminoethyl-10-hydroxycamptothecin), 9-aminocamptothecin (9-amino-20(S)-camptothecin), 9-nitrocamptothecin (also called rubitecan), lurtotecan (7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-camptothecin), exatecan, karenitecin, and a homocamptothecin. The structures and clinical information for some camptothecin compounds can be found in Garcia-Carbonero, et al., Clin. Cancer Res. (March 2002) 8: 641-661. Examples of camptothecin

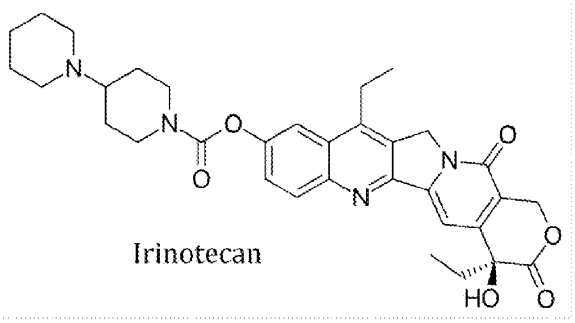
compounds can also be found in U.S. Pat. Nos. 4,604,463, 6,403,569, and 5,004,758, and in WO 2004/012661, WO 2003/101998, WO 2003/101996, WO 2003/101406, WO 2003/093274, WO 2003/086471, WO 01/76597, WO 01/64194, WO 00/70275, WO 00/53607, WO 99/17805, WO 99/17804, WO 99/05103, WO 98/35969, WO 97/28164, WO 97/25332, WO 97/16454, the contents of all of which are incorporated herein by reference.

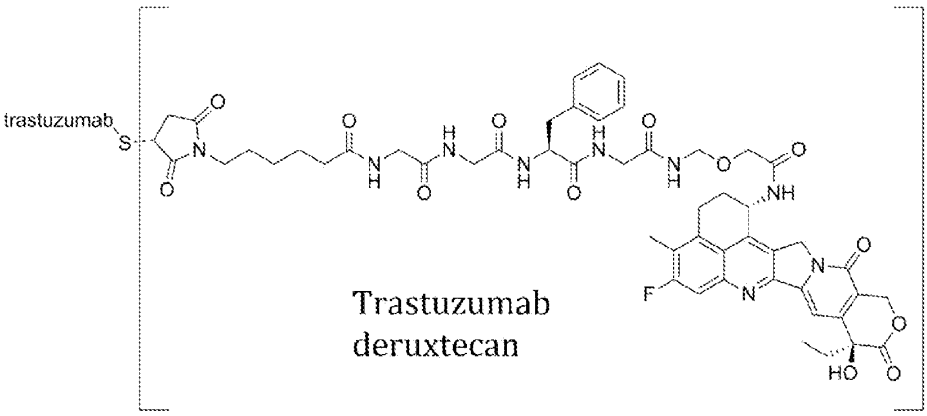
[00147] SN-38 (*S*-10-hydroxycamptothecin), Topotecan, irinotecan, belotecan, and trastuzumab deruxtecan are CPT analogues approved and are used in cancer chemotherapy today:



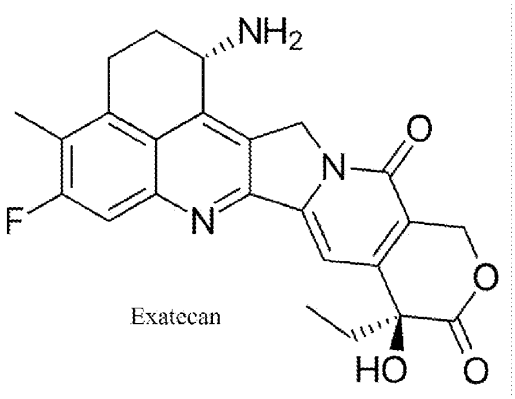
SN-38







[00148] In aspects, the camptothecin cytotoxic drug moiety is exatecan.



C. MGC028

[00149] In certain aspects, the **ADAM9-ADC** is MGC028. MGC028 comprises the Light Chain and Heavy Chain of anti-ADAM9 hmAb-A conjugated to an exatecan payload. The amino acid sequences of the **Ab**, the cytotoxic exatecan moiety **D**, and the Linker molecule, **LM**, in MGC028 are shown below:

the **Ab** comprises:

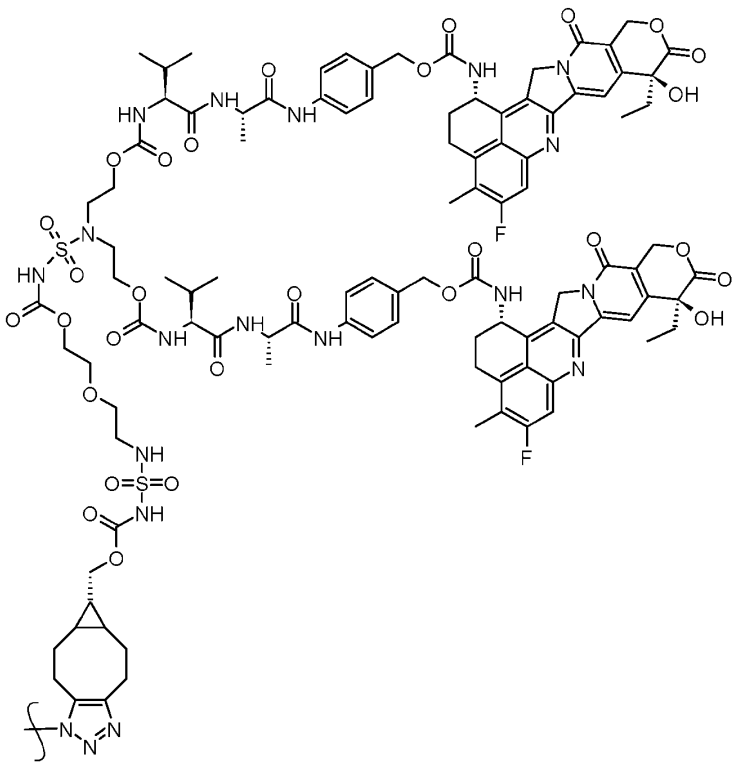
(i) a light chain comprising the amino acid sequence of **SEQ ID NO:23**; and

(ii) a heavy chain comprising the amino acid sequence of **SEQ ID NO:28**;

the **D** comprises exatecan; and

the **LM** comprises a Linker Molecule as described above.

[00150] In aspects, the Linker Molecule and exatecan together as conjugated to the **Ab** has the structure:



V. Methods of Production

[00151] The anti-ADAM9 antibody hmAb-A of the disclosure may be made recombinantly and expressed using any method known in the art for the production of recombinant proteins. For example, nucleic acids encoding the polypeptide chains of such binding molecules can be constructed, introduced into an expression vector, and expressed in suitable host cells. The binding molecules may be recombinantly produced in bacterial cells (e.g., *E. coli* cells), or eukaryotic cells (*e.g.*, CHO, 293E, COS, NS0 cells). In addition, the binding molecules can be expressed in a yeast cell such as *Pichia*, or *Saccharomyces*.

[00152] To produce the anti-ADAM9 antibody hmAb-A, one or more polynucleotides encoding the molecule may be constructed, introduced into an expression vector, and then expressed in suitable host cells. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the molecules (*See*, for example, the techniques described in Green, M.R. *et al.*, (2012), MOLECULAR CLONING, A LABORATORY MANUAL, 4th Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.* eds., 1998, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, NY). The expression vector(s) should have characteristics that permit replication of the vector in the host cell. The vector should also have promoter and signal sequences necessary for expression in the host cells. Such sequences are well known in the art. In addition to the nucleic acid sequence(s) encoding such binding molecules, the recombinant expression vectors may carry additional sequences, such as sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. Another method that may be employed is to express the gene sequence in plants (*e.g.*, tobacco) or a transgenic animal. Suitable methods useful for expressing such binding molecules recombinantly in plants or milk have been disclosed (see, for example, Peeters *et al.* (2001) "*Production Of Antibodies And Antibody Fragments In Plants*," Vaccine 19:2756; U.S. Patent No. 5,849,992; and Pollock *et al.* (1999) "*Transgenic Milk As A Method For The Production Of Recombinant Antibodies*," J. Immunol Methods 231:147-157).

[00153] Once an anti-ADAM9 antibody hmAb-A has been recombinantly expressed, it may be purified from inside or outside (such as from culture media) of the host cell by any method known in the art for purification of polypeptides or polyproteins. Methods for isolation

and purification commonly used for antibody purification (*e.g.*, antibody purification schemes based on antigen selectivity) may be used for the isolation and purification of such molecules and are not limited to any particular method. For example, by for example, column chromatography, filtration, ultrafiltration, salting out, solvent precipitation, solvent extraction, distillation, immunoprecipitation, SDS-polyacrylamide gel electrophoresis, isoelectric focusing, dialysis, and recrystallization. Chromatography includes, *e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen, sizing column chromatography, hydrophobic, gel filtration, reverse-phase, and adsorption (Marshak *et al.* (1996) STRATEGIES FOR PROTEIN PURIFICATION AND CHARACTERIZATION: A Laboratory Course Manual. (Eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

VI. Pharmaceutical Compositions

[00154] Pharmaceutical compositions for formulating ADAM9 ADCs as described herein include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) that can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of the **ADAM9-ADC** described herein, or a combination of such agents and a pharmaceutically acceptable carrier. Preferably, the pharmaceutical compositions comprise a prophylactically or therapeutically effective amount of the **ADAM9-ADC** and a pharmaceutically acceptable carrier. Also contemplated are such pharmaceutical compositions that additionally include a second therapeutic antibody (*e.g.*, tumor-specific monoclonal antibody) that is specific for a particular cancer antigen, and a pharmaceutically acceptable carrier.

[00155] As used herein, the term "pharmaceutically acceptable carrier" means a diluent, solvent, dispersion media, antibacterial and antifungal agents, excipient, or vehicle approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia as being suitable for administration to animals, and more particularly to humans. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly

for injectable solutions. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[00156] Generally, the ingredients of compositions are supplied either separately or mixed together in a dose form, for example, as a dry lyophilized powder or water-free concentrate, or as an aqueous solution in a hermetically sealed container such as a vial, ampoule or sachet indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection, saline or other diluent can be provided so that the ingredients may be mixed prior to administration.

VII. Pharmaceutical Kits

[00157] The disclosure also provides a pharmaceutical pack or kit comprising one or more containers containing a pharmaceutical composition or pharmaceutical compositions and instructional material (*e.g.*, a notice, package insert, instruction, *etc.*). Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical kit. The containers of such pharmaceutical kits may, for example, comprise one or more hermetically sealed vials, ampoules, sachets, *etc.*, indicating the quantity of active agent contained therein. Where the composition is to be administered by infusion, the container may be an infusion bottle, bag, *etc.* containing a sterile pharmaceutical-grade solution (*e.g.*, water, saline, a buffer, *etc.*). Where the compositions are to be administered by injection, the pharmaceutical kit may contain an ampoule of sterile water, saline or other diluent for injection, so as to facilitate the mixing of the components of the pharmaceutical kit for administration to a subject (*e.g.*, a human patient or other mammal). In aspects, a pharmaceutical pack or kit comprises an **ADAM9-ADC** pharmaceutical composition and instructional material.

[00158] In one aspect, an **ADAM9-ADC** of such kit is supplied as a dry sterilized lyophilized powder or water-free concentrate in a hermetically sealed container and can be

reconstituted, *e.g.*, with water, saline, or other diluent to the appropriate concentration for administration to a subject. In another aspect, an **ADAM9-ADC** of such kit is supplied as an aqueous solution in a hermetically sealed container and can be diluted, *e.g.*, with water, saline, or other diluent, to the appropriate concentration for administration to a subject. The kit can further comprise one or more other prophylactic and/or therapeutic agents useful for the treatment of cancer, in one or more containers; and/or the kit can further comprise one or more cytotoxic antibodies that bind one or more cancer antigens associated with cancer. In certain aspects, the other prophylactic or therapeutic agent is a chemotherapeutic agent. In other aspects, the prophylactic or therapeutic agent is a biological agent or hormonal therapeutic agent.

[00159] The included instructional material of the pharmaceutical kits may, for example, be of a content and format prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, and may indicate approval by the agency of the manufacture, sale or use of the pharmaceutical composition for human administration and/or for human therapy. The instructional material may, for example provide information relating to the contained dose of the pharmaceutical composition, modes of how it may be prepared (*e.g.*, reconstituted), and how it may be administered, etc. Such instructions may further provide information relating to the dose and administration of one or more pharmaceutical composition that are not provided in the kit.

VIII. Uses of ADAM9-ADCs

[00160] An **ADAM9-ADC** as described herein may be used to treat or prevent a variety of disorders, including cancer, including for example a cancer in which ADAM9 is expressed. Accordingly, the disclosure provides methods of treating cancer, such methods comprising administering an **ADAM9-ADC** to a subject in need thereof. In certain aspects, the disclosure provides methods of treating cancer, such methods comprising administering MGC028 to a subject in need thereof. As used herein, the term "subject" refers to a human (i.e., a human patient) or other mammal. Non-limiting dosing regimens for administering such therapy to a subject in need thereof are provided herein.

[00161] In aspects, the cancers that may be treated by the **ADAM9-ADCs** described herein include cancers selected from the group consisting of: bladder cancer, brain cancer (especially a glioma), breast cancer (especially a triple negative breast cancer (TNBC)), cervical cancer, colorectal cancer (especially an adenocarcinoma, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma, or squamous cell carcinoma), esophageal cancer, gallbladder cancer (especially a bile duct cancer or cholangiocarcinoma), gastric cancer (especially a gastroesophageal junction adenocarcinoma), head and neck cancer (especially a squamous cell carcinoma of the head and neck (SCCHN)), liver cancer (especially a hepatocellular carcinoma), lung cancer, non-small-cell lung cancer (NSCLC; especially a squamous cell carcinoma, adenocarcinoma, or large-cell undifferentiated carcinoma), myeloid cancer, oral cancer (especially a oral squamous cell carcinoma), ovarian cancer (especially a ovarian clear cell carcinoma), pancreatic cancer, prostate cancer, renal cancer (especially a renal cell carcinoma, renal adenocarcinoma or hypernephroma), thyroid cancer, testicular cancer, and uterine cancer.

[00162] In aspects, an **ADAM9-ADC** may be used in the treatment of bladder cancer, brain cancer (especially a glioma), breast cancer (especially a triple negative breast cancer (TNBC)), cervical cancer, colorectal cancer (especially an adenocarcinoma, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma, or squamous cell carcinoma), esophageal cancer, gallbladder cancer (especially a bile duct cancer or cholangiocarcinoma), gastric cancer (especially a gastroesophageal junction adenocarcinoma),, head and neck cancer (especially a squamous cell carcinoma of the head and neck (SCCHN)), liver cancer (especially a hepatocellular carcinoma), lung cancer, non-small-cell lung cancer (NSCLC; especially a squamous cell carcinoma, adenocarcinoma, or large-cell undifferentiated carcinoma), myeloid cancer, oral cancer (especially a oral squamous cell carcinoma), ovarian cancer (especially a ovarian clear cell carcinoma), pancreatic cancer, prostate cancer, renal cancer (especially a renal cell carcinoma, renal adenocarcinoma or hypernephroma), thyroid cancer, testicular cancer, and uterine cancer.

[00163] In apsects, an **ADAM9-ADC** may be used in the treatment of brain cancer, breast cancer, cervical cancer, colorectal cancer, gallbladder cancer, gastric cancer, head and neck cancer, lung cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer.

[00164] In aspects, an **ADAM9-ADC** may be used in the treatment of brain cancer.

[00165] In aspects, an **ADAM9-ADC** may be used in the treatment of glioma.

[00166] In aspects, an **ADAM9-ADC** may be used in the treatment of breast cancer.

[00167] In aspects, an **ADAM9-ADC** may be used in the treatment of triple negative breast cancer (TNBC).

[00168] In aspects, an **ADAM9-ADC** may be used in the treatment of cervical cancer.

[00169] In aspects, an **ADAM9-ADC** may be used in the treatment of colorectal cancer.

[00170] In aspects, an **ADAM9-ADC** may be used in the treatment of gallbladder cancer.

[00171] In aspects, an **ADAM9-ADC** may be used in the treatment of cholangiocarcinoma.

[00172] In aspects, an **ADAM9-ADC** may be used in the treatment of gastric cancer.

[00173] In aspects, an **ADAM9-ADC** may be used in the treatment of gastroesophageal junction adenocarcinoma.

[00174] In aspects, an **ADAM9-ADC** may be used in the treatment of head and neck cancer.

[00175] In aspects, an **ADAM9-ADC** may be used in the treatment of squamous cell carcinoma of the head and neck (SCCHN).

[00176] In aspects, an **ADAM9-ADC** may be used in the treatment of lung cancer.

[00177] In aspects, an **ADAM9-ADC** may be used in the treatment of non-small cell lung cancer (NSCLC).

[00178] In aspects, an **ADAM9-ADC** may be used in the treatment of oral cancer.

[00179] In aspects, an **ADAM9-ADC** may be used in the treatment of oral squamous cell carcinoma.

[00180] In aspects, an **ADAM9-ADC** may be used in the treatment of ovarian cancer.

[00181] In aspects, an **ADAM9-ADC** may be used in the treatment of ovarian clear cell carcinoma.

[00182] In aspects, an **ADAM9-ADC** may be used in the treatment of panreatic cancer.

[00183] In aspects, an **ADAM9-ADC** may be used in the treatment of prostate cancer.

[00184] In aspects, an **ADAM9-ADC** may be used in the treatment of renal cancer.

[00185] In aspects, an **ADAM9-ADC** may be used in the treatment of renal cell carcinoma.

IX. Methods of Administration

[00186] An **ADAM9-ADC** of the disclosure can be administered by a variety of methods to a subject, *e.g.*, a subject in need thereof, for example a human patient. For many applications, the route of administration is one of: intravenous injection or infusion (IV), subcutaneous injection (SC), intraperitoneally (IP), or intramuscular injection. It is also possible to use intra-articular delivery. Other modes of parenteral administration can also be used. Examples of such modes include: intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, and epidural and intrasternal injection.

[00187] An **ADAM9-ADC** of the disclosure can be administered as a weight-based dose or as a flat dose. The dose can also be selected to reduce or avoid production of antibodies against the administered molecules. Dosage regimens are adjusted to provide the desired response, *e.g.*, a therapeutic response or a combinatorial therapeutic effect. Generally, doses of an **ADAM9-ADC** (and optionally a further agent) can be used in order to provide a subject with the agent in bioavailable quantities. As used herein, the term "dose" refers to a specified amount of medication taken at one time. The term "dosage" refers to the administering of a specific amount, number, and frequency of doses over a specified period of time; the term dosage thus includes chronological features, such as duration and periodicity.

[00188] The term "weight-based dose" as used herein, refers to a discrete amount of a molecule to be administered per a unit of patient weight, for example milligrams of drug per kilograms of a subject's body weight (mg/kg body weight, abbreviated herein as "mg/kg"). The calculated dose will be administered based on the subject's body weight at baseline. The term "flat dose," as used herein, refers to a dose that is independent of the weight of the patient, and includes physically discrete units of a molecule that are suited as a unitary dose for the subjects to be treated; wherein each unit contains a predetermined quantity of a drug. Typically, a significant ($\geq$ 10%) change in body weight from baseline or established plateau weight will generally prompt recalculation of dose. Single or multiple doses may be given. Compositions comprising an **ADAM9-ADC** may be administered to a subject in need thereof via infusion.

[00189] The term "fractionated dose" as used herein, refers to two or more separate administrations of a molecule to be administered to achieve a particular desired dose. A fractionated dose provides that the desired dose can be fractionated into two or more separate administrations. The dose may be fractionated equally and/or unequally between such two or more administrations. In certain aspects, the fractionated dose can be two or more separate administrations within a cycle (*e.g.*, a 3-week or 4-week cycle).

EXAMPLES

[00190] Having now generally described the above aspects, the same will be more readily understood through reference to the following Examples. The following examples illustrate various methods for compositions in the diagnostic or treatment methods of the disclosure. The examples are intended to illustrate, but in no way limit, the scope of the appended claims.

Example 1
In vitro Cytotoxicity of MGC028

[00191] The ability of MGC028 to mediate cytotoxicity toward ADAM9-expressing A375.S2 human melanoma, Calu-3 human lung cancer, and HPAF-II human pancreatic cancer cells in vitro was investigated. MGC028 as investigated is the same ADC as described above. The negative control ADC (Nontargeting Control ADC) is a humanized control ADC that does not bind to either ADAM9 or any other human and murine protein. Human tumor cells were cultured in DMEM/F-12 + 10% FBS. Cells were washed with PBS and lifted using 0.05%

trypsin-EDTA. Antibodies and ADCs were diluted in a 9-point dose response curve (plus no antibody control well) with a final top concentration of 10 μg/ml (67 nM antibody concentration) and diluted 1:3 or 1:10, depending on cell line sensitivity. ADCs were made up at 5X final concentration and 20 μL are added to 96-well tissue culture treated plates. Suspended cells were plated at 5,000 cells/well and added to ADC wells at 80 μL/well (100 μL/well total volume).

[00192] Plates were incubated at 37°C for 7 days. Cell viability was measured using alamarBlue (Trek Diagnostics #00-100; 10 μL added to each well) and allowed to develop. Plates were read on a Gemini plate reader (Molecular Devices) according to specifications for alamarBlue.

[00193] Data was analyzed using Graph Pad Prism (4-parameter curve fit analysis) to determine IC_{50} values.

[00194] The cytotoxicity curves from these studies are presented in **FIGs. 1A-1C**. MGC028 mediated dose-dependent cytotoxicity toward the A375.S2 human melanoma line *in vitro*, with an IC_{50} = 99 pM (**FIG. 1A**), the Calu-3 human lung cancer line with an IC_{50} = 31 pM (**FIG. 1B**), and the HPAF-II human pancreatic cancer line with an IC_{50} = 83 (**FIG. 1C**). The negative control ADCs (Nontargeting Control ADC) were approximately 100-350-fold less active than MGC028, confirming the specificity of the cytotoxic activity of MGC028.

Example 2
ADCC Assay

[00195] The ability of unconjugated MGA027 (unconjugated ADAM9 antibody equivalent to the antibody used in conjugates), conjugated MGC028 (as described above), and conjugated negative control ADC (Nontargeting Control ADC as described above) to mediate antibody-dependent cellular cytotoxicity (ADCC) was assessed. The ADCC assay utilizes primary peripheral blood mononuclear cells (PBMCs) as a source of natural killer (NK) cells. The ADCC assay tests the ability of antibodies to bridge an interaction between the antigen-positive target cells and NK effector cells, and subsequent killing of the target cells by NK cells. Adherent target tumor cells grown in either F-12/DMEM containing 10% fetal bovine serum (FBS) or RPMI1640 containing 10% FBS were detached with 0.25% Trypsin-EDTA solution

and collected by centrifugation at 1000 rpm for 5 minutes. Collected tumor cells were rinsed once with phosphate buffered saline (PBS) then re-suspended in assay medium (RPMI1640 without phenol red + 5% FBS) and plated into 96-well U-bottom cell culture treated plate at 20,000 cells/well. Test antibodies were serially diluted and plated onto the cells in triplicate. Then 600,000 fresh PBMCs were added to the wells (30:1 effector: target ratio; E:T) and plates were incubated at 37° C/5% CO_2 overnight.

[00196] Following incubation, 15 µL of 10X lysis solution (Promega # G182A) were added to the maximum release control wells for 10 minutes to completely lyse the target cells, then the plates were centrifuged at 1200 rpm for 5 minutes. Fifty (50) µL of supernatant were transferred from each assay plate well to a clear flat bottom ELISA plate and 50 µL of lactate dehydrogenase (LDH) substrate solution (Promega # G1780) was added to each well. Plates were incubated for 5-10 minutes at room temperature in the dark, then 50 µL of Stop solution was added. The optical density was measured at 490 nm within 1 hour on a Emax plate reader (Molecular Devices). The percent cytotoxicity was calculated as described below and further analyzed using GraphPad Prism 5 software.

[00197] Specific cell lysis was calculated from optical density (OD) data using the following formula where maximum release (MR), antibody-independent cytotoxicity (AICC), and cell spontaneous release (SR) were incorporated:

Cytotoxicity (%) = 100 x (OD of Sample - OD of AICC) / (OD of MR - OD of SR)

[00198] The ADCC assay results are presented in **FIGs. 2A-2D**. MGA027 mediated ADCC toward the four ADAM9-expressing tumor cell lines tested. MGC028 did not mediate ADCC toward the four tumor cell lines.

Example 3
Surface Plasmon Resonance Assay

[00199] Binding of human and cynomolgus monkey ADAM9 to MGC028 or MGA027 was analyzed by surface plasmon resonance (SPR) in a BIAcore 3000 biosensor (GE, Healthcare). F(ab')2 fragment of goat anti-human IgG, Fc fragment specific (GAH Fc) was immobilized on the CM5 sensor chip according to the procedure recommended by the

manufacturer. Briefly, the carboxyl groups on the sensor chip surface were activated with an injection of a solution containing 0.2 M N-ethyl-N-(3dietylamino-propyl) carbodimide and 0.05 M N-hydroxy-succinimide. The mAb fragment (5 µg/mL) was injected over the activated CM5 surface in 10 mM sodium-acetate, pH 5.0, at a flow rate 5 µL/min, followed by 1 M ethanolamine for deactivation of remaining amine-reactive groups.

[00200] MGC028 or MGA027 (both as described above) were injected at a flow rate of 20 µL/min for 15 seconds to reach approximately 100 resonance units (RU) of captured mAb. The His-tagged human or cynomolgus monkey ADAM9 extracellular domain (ECD) was injected over the captured antibody for 120 seconds at a flow rate of 30 µL/min (in duplicate) in HBS-EP buffer at concentrations of 0, 6.25, 12.5, 25, 50 and 100 nM. Regeneration of the immobilized GAH Fc was performed by pulse injection of 10 mM glycine, pH 1.5.

[00201] Reference curves were obtained by injection of each dilution of ADAM9 over the treated surface with no immobilized protein. Binding curves at zero concentration were subtracted as a blank. Resulted ADAM9 binding curves were normalized to the same level of captured antibodies. Kinetic constants, k_a and k_d, were calculated by global fit of the association/dissociation (k_a/k_d) curves to the Langmuir 1:1 binding model (BIAevaluation software v4.1). The KD was calculated as k_a/k_d.

[00202] The antibodies MGC028 and MGA027 both demonstrated similar affinity binding to human and cynomolgus monkey ADAM9 in solution. (**FIG. 3A**). Kinetic constants are shown in **Table 1**. The K_D values determined for interaction with human ADAM9 were similar at 0.7 nM. The K_D values determined for interaction with cynomolgus monkey ADAM9 were also similar and ranged between 0.6 nM and 0.7 nM.

Table 1*

	MGA027		MGC028	
	Human	Cyno	Human	Cyno

k_a (1/M•s)	2.2 (2.2; 2.2) e6	9.9 (9.7; 10) e5	2.2 (2.2; 2.2) e6	1.0 (1.0; 1.0) e6
k_d (1/s)	1.6 (1.6; 1.6) e-3	6.4 (6.3; 6.5) e-4	1.5 (1.5; 1.5) e-3	6.4 (6.3; 6.4) e-4
K_D (nM)	0.7 (0.7; 0.7)	0.7 (0.7; 0.7)	0.7 (0.7; 0.7)	0.6 (0.6; 0.6)

*Average data from two independent experiments (shown in parenthesis)

[00203] SPR was used to analyze binding to human CD16A-His 158V and 158F alleles. Anti-penta-His tag mAb was immobilized on the SPR CM5 sensor chip according to the procedure recommended by the manufacturer. Briefly, the carboxyl groups on the sensor chip surface were activated with an injection of a solution containing 0.2 M N-ethyl-N-(3dietylamino-propyl) carbodimide and 0.05-M N-hydroxy-succinimide. The mAb (5 μg/mL) was injected over the activated CM5 surface in 10 mM sodium acetate, pH 5.0, at a flow rate 5 μL/min, followed by 1 M ethanolamine for deactivation of remaining amine-reactive groups.

[00204] The His-tagged human CD16A (Fc gamma RIIIA) extracellular domain proteins were injected at a flow rate of 20 μL/min for 10 seconds to reach approximately 120 RU of captured ligand. MGC028 or MGA027 was injected for 120 seconds at a flow rate of 30 μL/min (in duplicate) in HBS EP buffer at concentrations of 0, 62.5, 125, 250, 500, and 1000 nM. Regeneration of the immobilized anti-penta-His mAb surface was performed by pulse injection of 10 mM glycine, pH 1.5. The plot of equilibrium binding responses of Fc part of antibodies to captured human CD16A alleles versus mAb concentrations was fitted to Steady State affinity model to obtain K_D values.

[00205] The unconjugated antibody MGA027 bound to captured human CD16 alleles. MGA027 bound with higher affinity to the higher affinity CD16A 158V allele, compared to the lower affinity CD16A 158F allele, as expected. MGC028, the conjugated antibody, did not bind to either CD16A allele (**FIG. 3B**). Therefore, MGC028 would not be expected to mediate effector function through Fc gamma receptor CD16A, and this is consistent with the inability of MGC028 to mediate ADCC in vitro (**see Example 2**). The absence of effector function is

potentially advantageous for MGC028, as binding of an ADC to effector cells could reduce tumor localization, hinder internalization, and lead to off-target toxicities (McDonagh, Mol Cancer Ther 2008; Perez, Drug Discovery Today 2013).

Example 4
MGC028 Exhibits Potent *in vivo* Activity

[00206] In order to further demonstrate the antitumor activity of MGC028, the above-described MGC028 molecule was evaluated for *in vivo* toxicity in a CD-1 nude mouse model using different tumor cell lines. In brief, approximately 5 x 10^6 viable tumor cells suspended in 1:1 serum-free media and Matrigel Basement Membrane Matrix were subcutaneously inoculated into the flank of the CD-1 nude mice (Charles River Laboratories). When tumors had reached a mean volume ranging from approximately 145-200 mm^3, the mice were randomized and MGC028 or vehicle control were administered intravenously. Studies also included a nontargeting control ADC (as described above). In these studies, one dose of the MGC028, nontargeting control ADC, or vehicle control (formulation buffer (FB)) was administered once weekly (QW). Tumors were measured twice weekly by orthogonal measurements with electronic calipers, with tumor volumes calculated as: (length x $width^2$)/2. An animal was considered to have a Partial Regression ("PR") when tumor volume was reduced by 50% or greater when compared to the tumor volume at the day of first dose administration. A finding that the tumor volume of treated animals had decreased to $\leq$ 5 mm^3 during the study period was considered to denote a Complete Response ("CR"). The tumor volume (relative to vehicle control) was determined ("T/C"). Antitumor activity was evaluated according to National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

***In vivo* Activity Against HPAF-II Pancreatic Cancer Tumor Cells**

[00207] The results of this study with respect to subcutaneously inoculated HPAF-II pancreatic adenocarcinoma tumor cells are presented in **Table 2** and in **FIG. 4**, and show responsiveness against the HPAF-II tumor cells.

Table 2						
Treatment	**Dose (mg/kg Ab)**	**Schedule/Route**	**T/C-Day 35 (%)[a]**	**PR**	**CR**	**Antitumor Activity[b]**
MGC028	10	Single-Dose/IV	9	7/7	0/7	Highly Active
	6		9	7/7	0/7	Highly Active
	3		11	2/7	0/7	Active
	1		17	1/7	0/7	Active
Nontargeting Control ADC	10		101	0/7	0/7	Inactive
	6		78	0/7	0/7	Inactive
	3		94	0/7	0/7	Inactive
	1		69	0/7	0/7	Inactive

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1080 mm^3 on Day 35.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00208] Female CD-1 Nude (Homozygous) mice (n = 7/group) were implanted subcutaneously with HPAF-II (pancreatic adenocarcinoma) tumor cells (5×10^6 cells) suspended in serum free culture media/Matrigel (1:1) in a volume of 0.1 mL per mouse. When tumors reached approximately 160 mm^3 (188 ± 46 mm^3 mean tumor volume ± standard deviation) on Day 18, mice were randomized and treated intravenously with vehicle control (formulation buffer), targeting ADC (MGC028), or nontargeting control ADC on Day 18 (arrow) at the dose levels indicated for a total of one dose. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at all four dose levels following treatment with MGC028.

[00209] MGC028 reduced tumor volumes by 91% on Day 35 for the 10 mg/kg and 6 mg/kg dose levels, 89% at the 3 mg/kg dose level and 83% at the 1 mg/kg dose level. All dose levels tested were either highly active 10 mg/kg (9% T/C) and 6 mg/kg (9% T/C) or active 3 mg/kg (11% T/C) and 1 mg/kg (17% T/C) at each dose based on NCI standards. MGC028 induced partial regressions in 7/7, 7/7, 2/7, 1/7 at doses of 10 mg/kg, 6 mg/kg, 3 mg/kg, and 1 mg/kg, respectively, and no complete regressions. By comparison, the nontargeting control ADC group exhibited no partial or complete regressions at any of the 4 dose levels.

In vivo Activity Against Capan-1 Cancer Tumor Cells

[00210] The results of this study with respect to subcutaneously inoculated Capan-1 pancreatic adenocarcinoma tumor cells are presented in **Table 3** and in **FIG. 5**, and show responsiveness against the Capan-1 tumor cells.

Table 3

Treatment	Dose (mg/kg Ab)	Schedule/Route	T/C-Day 55 (%)[a]	PR	CR	Antitumor Activity[b]
MGC028	10	Single-Dose/IV	1	7/7	3/7	Highly Active
	6		2	7/7	2/7	Highly Active
	3		3	6/7	0/7	Highly Active
	1		11	4/7	0/7	Active
Nontargeting Control ADC	10		55	2/7	0/7	Inactive
	6		80	0/7	0/7	Inactive
	3		76	0/7	0/7	Inactive
	1		89	1/7	1/7	Inactive

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1618 mm^3 on Day 55.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00211] Female CD-1 Nude (Homozygous) mice (n = 7/group) were implanted subcutaneously with Capan-1 (pancreatic adenocarcinoma) tumor cells (5×10^6 cells) suspended in serum free culture media/Matrigel (1:1) in a volume of 0.1 mL per mouse. When tumors reached approximately 160 mm^3 (149 ± 47 mm^3 mean tumor volume ± standard deviation) on Day 16, mice were randomized and treated intravenously with vehicle control (formulation buffer), targeting ADC (MGC028), or nontargeting control ADC on Day 16 (arrow) at the dose levels indicated for a total of one dose. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at all four dose levels following treatment with MGC028.

[00212] MGC028 reduced tumor volumes by 99% on Day 55 for the 10 mg/kg, 98% for the 6 mg/kg, dose level, 97% at the 3 mg/kg dose level, and 89% at the 1 mg/kg dose level. The 10 mg/kg (1% T/C), 6 mg/kg (2% T/C), and 3 mg/kg (3% T/C) dose levels were highly active,

and the 1 mg/kg (11% T/C) dose level was active based on NCI standards. The nontargeting control ADC was inactive at all dose levels tested, 10 mg/kg (55% T/C), 6 mg/kg (80% T/C), 3 mg/kg (76% T/C), and 1 mg/kg (89% T/C). MGC028 induced partial regressions in 7/7 animals at 10 mg/kg and 6 mg/kg, 6/7 animals at 3 mg/kg, and 4/7 animals at 1 mg/kg dose levels. Complete regressions were observed at the 10 mg/kg dose level (3/7) and the 6 mg/kg dose level (2/7). By comparison, the nontargeting control ADC group exhibited partial regressions in 2/7 animals at the 10 mg/kg and 1/7 at the 1 mg/kg dose levels. Complete regressions were observed at the 1 mg/kg dose level in 1/7 animals. No complete regressions were seen at the higher dose levels of the nontargeting control ADC. Treatment with MGC028 at 10 mg/kg maintained antitumor control as a single-dose administration through the end of the study.

***In vivo* Activity Against Hs746T Cancer Tumor Cells**

[00213] The results of this study with respect to subcutaneously inoculated Hs746T gastric carcinoma tumor cells are presented in **Table 4** and in **FIG. 6**, and show responsiveness against the Hs746T tumor cells.

Table 4						
Treatment	**Dose (mg/kg Ab)**	**Schedule/Route**	**T/C-Day 35 (%)[a]**	**PR**	**CR**	**Antitumor Activity[b]**
MGC028	10	Single-Dose/IV	2	7/7	7/7	Highly Active
	6		2	7/7	7/7	Highly Active
	3		2	7/7	7/7	Highly Active
	1		1	7/7	7/7	Highly Active
Nontargeting Control ADC	10		4	6/7	6/7	Highly Active
	6		9	5/7	3/7	Highly Active
	3		73	0/7	0/7	Inactive
	1		109	0/7	0/7	Inactive

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1410 mm^3 on Day 35.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00214] Female CD-1 Nude (Homozygous) mice (n = 7/group) were implanted subcutaneously with Hs746T (gastric carcinoma) tumor cells (5×10^6 cells) suspended in serum free culture media/Matrigel (1:1) in a volume of 0.1 mL per mouse. When tumors reached approximately 160 mm^3 (152 ± 49 mm^3 mean tumor volume ± standard deviation) on Day 16, mice were randomized and treated intravenously with vehicle control (formulation buffer), targeting ADC (MGC028), or nontargeting control ADC on Day 16 (arrow) at the dose levels indicated for a total of one dose. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at all four dose levels following treatment with MGC028.

[00215] MGC028 reduced tumor volumes by 98% on Day 35 for the 10 mg/kg, 6 mg/kg, and 3 mg/kg dose levels, and 99% at the 1 mg/kg dose level. All dose levels tested were highly active 10 mg/kg (2% T/C), 6 mg/kg (2% T/C), 3 mg/kg (2% T/C), and 1 mg/kg (1% T/C) at each dose based on NCI standards. Although the nontargeting control ADC exhibited antitumor activity at 10 mg/kg and 6 mg/kg, there was no activity observed at 3 mg/kg and 1 mg/kg. The nontargeting control activity at the 10 mg/kg (4% T/C) and 6 mg/kg (9% T/C) were considered highly active based on NCI standards and inactive at the 3 mg/kg (73% T/C) and 1 mg/kg (109% T/C) dose levels. MGC028 induced partial regressions in 7/7 at all dose levels tested, and 7/7

complete regressions in all dose levels tested. By comparison, the nontargeting control ADC group exhibited partial regressions of 6/7, 5/7, at the 10 mg/kg and 6 mg/kg dose levels, respectively, and none at the 3 mg/kg and 1 mg/kg dose levels. Complete regressions were observed in 6/7, 3/7 at 10 mg/kg and 6 mg/kg, respectively, and none at 3 mg/kg and 1 mg/kg. MGC028 antitumor activity and complete tumor regressions persisted through the end of the study for all dose levels tested. Whereas the nontargeting control ADC tumors began to re-grow at the 10 mg/kg and 6 mg/kg dose levels.

In vivo Activity Against NCI-H1975 Cancer Tumor Cells

[00216] The results of this study with respect to subcutaneously inoculated NCI-H1975 lung adenocarcinoma tumor cells are presented in **Table 5** and in **FIG. 7**, and show responsiveness against the NCI-H1975 cells.

Table 5

Treatment	Dose (mg/kg Ab)	Schedule/Route	T/C-Day 43 (%)[a]	PR	CR	Antitumor Activity[b]
MGC028	10	Single-Dose/IV	5	6/6	2/6	Highly Active
	6		10	4/6	0/6	Highly Active
	3		4	6/6	3/6	Highly Active
	1		20	2/6	2/6	Active
Nontargeting Control ADC	10		5	5/6	3/6	Highly Active
	6		38	2/6	1/6	Active
	3		122	1/6	1/6	Inactive
	1		150	0/6	0/6	Inactive

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1122 mm^3 on Day 43.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00217] Female CD-1 Nude (Homozygous) mice (n = 6/group) were implanted subcutaneously with NCI-H1975 (lung adenocarcinoma) tumor cells (5×10^6 cells) suspended in serum free culture media/Matrigel (1:1) in a volume of 0.1 mL per mouse. When tumors reached approximately 160 mm^3 (203 ± 60 mm^3 mean tumor volume ± standard deviation) on Day 15, mice were randomized and treated intravenously with vehicle control (formulation

buffer), targeting ADC (MGC028), or nontargeting control ADC on Day 15 (arrow) at the dose levels indicated for a total of one dose. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at all four dose levels following treatment with MGC028.

[00218] MGC028 reduced tumor volumes by 95% on Day 43 for the 10 mg/kg dose level, 90% with 6 mg/kg, 96% with 3 mg/kg, and 80% at 1 mg/kg dose levels. Dose levels tested were highly active 10 mg/kg (5% T/C), 6 mg/kg (10% T/C), 3 mg/kg (4% T/C), and active at 1 mg/kg (20% T/C) at each dose based on NCI standards. Although the nontargeting control ADC exhibited antitumor activity at 10 mg/kg and to a lesser degree at 6 mg/kg, there was no activity observed at 3 mg/kg and 1 mg/kg, and the activity that was observed was limited and significantly less than that observed with targeted MGC028. The nontargeting control activity at the 10 mg/kg (5% T/C) was considered highly active based on NCI standards, active at 6 mg/kg (38% T/C) and inactive at the 3 mg/kg (122% T/C) and 1 mg/kg (150% T/C) dose levels. MGC028 induced partial regressions in 6/6, 4/6, 6/6, 2/6 at 10 mg/kg, 6 mg/kg, 3 mg/kg, and 1 mg/kg, respectively and complete regressions in 2/6, 3/6, 2/6 at 10 mg/kg, 3 mg/kg, and 1 mg/kg dose levels, respectively. By comparison, the nontargeting control ADC group exhibited partial regressions in 5/6, 2/6, 1/6, at the 10 mg/kg, 6 mg/kg, and 3 mg/kg dose levels, respectively. Complete regressions were observed in 3/6, 1/6, 1/6 at 10 mg/kg, 6 mg/kg, and 3 mg/kg, respectively.

In vivo Activity Against Calu-3 Cancer Tumor Cells

[00219] The results of this study with respect to subcutaneously inoculated Calu-3 NSCLC adenocarcinoma tumor cells are presented in **Table 6** and in **FIG. 8**, and show responsiveness against the Calu-3 tumor cells.

Table 6

Treatment	Dose (mg/kg Ab)	Schedule/Route	T/C-Day 48 (%)[a]	PR	CR	Antitumor Activity[b]
MGC028	10	Single-Dose/IV	3	7/7	5/7	Highly Active
	6		2	7/7	7/7	Highly Active
	3		4	6/7	6/7	Highly Active
	1		3	7/7	4/7	Highly Active

Table 6						
Treatment	**Dose (mg/kg Ab)**	**Schedule/Route**	**T/C-Day 48 (%)[a]**	**PR**	**CR**	**Antitumor Activity[b]**
Nontargeting Control ADC	10		80	0/7	0/7	Inactive
	6		92	0/7	0/7	Inactive
	3		79	0/7	0/7	Inactive
	1		77	1/7	0/7	Inactive

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1220 mm^3 on Day 48.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00220] Female CD-1 Nude (Homozygous) mice (n = 7/group) were implanted subcutaneously with Calu-3 (NSCLC adenocarcinoma) tumor cells (5×10^6 cells) suspended in serum free culture media/Matrigel (1:1) in a volume of 0.1 mL per mouse. When tumors reached approximately 160 mm^3 (144 ± 16 mm^3 mean tumor volume ± standard deviation) on Day 13, mice were randomized and treated intravenously with vehicle control (formulation buffer), targeting ADC (MGC028), or nontargeting control ADC on Day 13 (arrow) at the dose levels indicated for a total of one dose. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at all four dose levels following treatment with MGC028.

[00221] MGC028 reduced tumor volumes by 97% on Day 48 for the 10 mg/kg, 98% for the 6 mg/kg, dose level, 96% at the 3 mg/kg dose level, and 97% at the 1 mg/kg dose level. All dose levels of MGC028 tested, 10 mg/kg (3% T/C), 6 mg/kg (2% T/C), 3 mg/kg (4% T/C), and 1 mg/kg (3% T/C), were highly active based on NCI standards. The nontargeting control ADC was inactive at all dose levels tested, 10 mg/kg (80% T/C), 6 mg/kg (92% T/C), 3 mg/kg (79% T/C), and 1 mg/kg (77% T/C). MGC028 induced partial regressions in 7/7 animals at 10 mg/kg, 6 mg/kg, and 1 mg/kg, and 6/7 animals at the 3 mg/kg dose levels. Complete regressions were observed at the 10 mg/kg dose level (5/7), the 6 mg/kg dose level (7/7), the 3 mg/kg dose level (6/7), and the 1 mg/kg dose level (4/7). By comparison, the nontargeting control ADC group exhibited partial regressions in 1/7 animals at the 1 mg/kg dose level and no complete

regressions. Mice treated with MGC028 at 10, 6, 3, and 1 mg/kg maintained antitumor control as a single-dose administration through the end of the study for most animals.

***In vivo* Activity Against NCI-H1703 Cancer Tumor Cells**

[00222] The results of this study with respect to subcutaneously inoculated NCI-H1703 NSCLC squamous tumor cells are presented in **Table 7** and in **FIG. 9**, and show responsiveness against the NCI-H1703 tumor cells.

Table 7

Treatment	Dose (mg/kg Ab)	Schedule/Route	T/C-Day 99 (%)[a]	PR	CR	Antitumor Activity[b]
MGC028	10	Single-Dose/IV	4	4/5	0/5	Highly Active
	6		5	4/5	0/5	Highly Active
	3		54	0/5	0/5	Inactive
Nontargeting Control ADC	10		87	0/5	0/5	Inactive
	6		116	0/5	0/5	Inactive
	3		88	0/5	0/5	Inactive

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1554 mm^3 on Day 99.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00223] Female CD-1 Nude (Homozygous) mice (n = 5/group) were implanted subcutaneously with NCI-H1703 (NSCLC squamous) tumor cells (5×10^6 cells) suspended in serum free culture media/Matrigel (1:1) in a volume of 0.1 mL per mouse. When tumors reached approximately 160 mm^3 (195 ± 65 mm^3 mean tumor volume ± standard deviation) on Day 42, mice were randomized and treated intravenously with vehicle control (formulation buffer), targeting ADC (MGC028), or nontargeting control ADC on Day 42 (arrow) at the dose levels indicated for a total of one dose. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at all three dose levels following treatment with MGC028.

[00224] MGC028 reduced tumor volumes by 96% on Day 99 for the 10 mg/kg dose level, 95% at the 6 mg/kg dose level, and 46% at the 3 mg/kg dose level. The 10 mg/kg (4% T/C) and

6 mg/kg (5% T/C) dose levels were highly active based on NCI standards. The nontargeting control ADC was inactive at all dose levels tested, 10 mg/kg (87% T/C), 6 mg/kg (116% T/C), and 3 mg/kg (88% T/C). MGC028 induced partial regressions in 4/5 animals at the 10 and 6 mg/kg dose levels and no complete regressions. By comparison, the nontargeting control ADC group had no partial or complete regressions at any of the dose levels tested. Treatment with MGC028 at 10 mg/kg and 6 mg/kg maintained antitumor control as a single-dose administration through the end of the study.

In vivo Activity Against SW48 Cancer Tumor Cells

[00225] The results of this study with respect to subcutaneously inoculated SW48 colorectal adenocarcinoma tumor cells are presented in **Table 8** and in **FIG. 10**, and show responsiveness against the SW48 cells.

Table 8

Treatment	Dose (mg/kg Ab)	Schedule/Route	T/C-Day 41 (%)[a]	PR	CR	Antitumor Activity[b]
MGC028	10	Single-Dose/IV	16	1/7	1/7	Active
	6		18	2/7	1/7	Active
	3		31	0/7	0/7	Active
	1		69	0/7	0/7	Inactive
Nontargeting Control ADC	10		52	0/7	0/7	Inactive
	6		121	0/7	0/7	Inactive
	3		98	0/7	0/7	Inactive
	1		86	0/7	0/7	Inactive

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1133 mm^3 on Day 41.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00226] Female CD-1 Nude (Homozygous) mice (n = 7/group) were implanted subcutaneously with SW48 (colorectal adenocarcinoma) tumor cells (5×10^6 cells) suspended in serum free culture media/Matrigel (1:1) in a volume of 0.1 mL per mouse. When tumors reached approximately 160 mm^3 (146 ± 40 mm^3 mean tumor volume ± standard deviation) on Day 16, mice were randomized and treated intravenously with vehicle control (formulation

buffer), targeting ADC (MGC028), or nontargeting control ADC on Day 16 (arrow) at the dose levels indicated for a total of one dose. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at three dose levels following treatment with MGC028.

[00227] MGC028 reduced tumor volumes by 84% on Day 41 for the 10 mg/kg dose level, 82% with 6 mg/kg, and 69% with 3 mg/kg, dose levels. Dose levels were active at 10 mg/kg (16% T/C), 6 mg/kg (18% T/C), and 3 mg/kg (31% T/C) based on NCI standards. The nontargeting control was considered inactive at all doses tested based on NCI standards. MGC028 induced partial regressions in 1/7 and 2/7, at 10 mg/kg and 6 mg/kg, respectively and complete regressions in 1/7, at both the 10 mg/kg and 6 mg/kg dose levels. By comparison, the nontargeting control ADC group exhibited no partial regressions or complete regressions.

In vivo Activity Against Detroit 562 Cancer Tumor Cells

[00228] The results of this study with respect to subcutaneously inoculated Detroit 562 head and neck squamous cell carcinoma (SCCHN) tumor cells are presented in **Table 9** and in **FIG. 11**, and show responsiveness against the Detroit 562 tumor cells.

Table 9

Treatment	Dose (mg/kg Ab)	Schedule/Route	T/C-Day 42 (%)[a]	PR	CR	Antitumor Activity[b]
MGC028	10	Single-Dose/IV	8	5/7	0/7	Highly Active
	6		8	4/7	0/7	Highly Active
	3		21	3/7	0/7	Active
	1		31	3/7	0/7	Active
Nontargeting Control ADC	10		65	0/7	0/7	Inactive
	6		67	0/7	0/7	Inactive
	3		52	3/7	0/7	Inactive
	1		50	1/7	0/7	Inactive

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1825 mm^3 on Day 42.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C ≤ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable (≤ 5 mm^3) during the course of the study).

[00229] Female CD-1 Nude (Homozygous) mice (n = 7/group) were implanted subcutaneously with Detroit 562 (SCCHN) tumor cells (5×10^6 cells) suspended in serum free culture media/Matrigel (1:1) in a volume of 0.1 mL per mouse. When tumors reached approximately 160 mm^3 (185 ± 54 mm^3 mean tumor volume ± standard deviation) on Day 17, mice were randomized and treated intravenously with vehicle control (formulation buffer), targeting ADC (MGC028), or nontargeting control ADC on Day 17 (arrow) at the dose levels indicated for a total of one dose. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at all four dose levels following treatment with MGC028.

[00230] MGC028 reduced tumor volumes by 92% on Day 42 for the 10 mg/kg and 6 mg/kg, dose levels, 79% at the 3 mg/kg dose level, and 69% at the 1 mg/kg dose level. The 10 mg/kg (8% T/C) and 6 mg/kg (8% T/C) dose levels were highly active, and the 3 mg/kg (21% T/C) and 1 mg/kg (31% T/C) were active based on NCI standards. The nontargeting control ADC was inactive at all dose levels tested, 10 mg/kg (65% T/C), 6 mg/kg (67% T/C), 3 mg/kg (52% T/C), and 1 mg/kg (50% T/C). MGC028 induced partial regressions in 5/7, 4/7, 3/7, 3/7 at the 10 mg/kg, 6 mg/kg, 3 mg/kg, and 1 mg/kg dose levels respectively, and no complete regressions. By comparison, the nontargeting control ADC group exhibited no partial regressions at the 10 mg/kg and 6 mg/kg dose levels and 3/7 and 1/7 partial regressions, at the 3 mg/kg and 1 mg/kg dose levels and no complete regressions at all dose levels tested.

[00231] The results of these *in vivo* studies demonstrate that the MGC028 tested exhibited dose-dependent antitumor activity toward ADAM9-positive tumors in murine xenograft models of pancreatic, gastric, lung, colorectal cancer, and SCCHN.

In vivo Activity Against SW48 and NCI-H1975 Cancer Tumor Cells Requires Targeted Delivery of Exatecan

[00232] Female CD-1 Nude (Homozygous) mice (n = 6-7/group) were implanted subcutaneously with SW48 colorectal adenocarcinoma, or NCI-H1975 lung adenocarcinoma

tumor cells (5×10^6 cells) suspended in serum free culture media/Matrigel (1:1) in a volume of 0.1 mL per mouse. When tumors reached approximately 160 mm^3 (146 ± 40 mm^3 to 203 ± 60 mm^3 mean tumor volume ± standard deviation) on Day 15 or 16, mice were randomized and treated intravenously with vehicle control (formulation buffer), targeting ADC (MGC028), unconjugated precursor ADAM9-targeting antibody (MGA027), unconjugated exatecan, or the combination of MGA027 and unconjugated exatecan on Day 15 or 16 (arrow) at the dose levels indicated for a total of one dose. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible.

[00233] As shown in **FIGs. 12A and 12B**, MGC028 significantly reduced tumor volumes of both SW48 (**FIG. 12A**) and NCI-H1975 (**FIG. 12B**); however, no activity was observed with MGA027, unconjugated exatecan or the combination of MGA027 and unconjugated exatecan.

[00234] The results of these *in vivo* studies demonstrate that antitumor activity toward ADAM9-positive tumors in murine xenograft models of colorectal and lung cancer requires ADAM9-targeted delivery of exatecan to the tumor cells as provided by MGC028.

Example 5
MGC028 Exhibits *In Vivo* Activity in Patient-Derived Xenografts

[00235] In order to further demonstrate the antitumor activity of MGC028, the above-described MGC028 molecule was evaluated for *in vivo* toxicity in an Athymic Nude mouse model using different patient-derived tumor fragments. In brief, low passage tumor fragments were implanted into Athymic Nude-Foxn1^{nu} (Envigo, Charles River Laboratories) stock mice. When sufficient stock mice tumors reached 1000-1500 mm^3, tumors were harvested, and tumor fragments were re-implanted unilaterally on the left flank of pre-study mice. When tumors reached 150-300 mm^3, pre-study mice were randomized by tumor volume and treated with MGC028 or vehicle control (formulation buffer (FB)). The mice were assigned to each group and dosed intravenously by tail vein injection (approximately 100 µL). In these studies, MGC028 or vehicle control was administered once every two weeks for a total of two doses (Q2W x 2). Tumors were measured twice weekly by orthogonal measurements with electronic calipers, with tumor volumes calculated as: (width x 2) x length x 0.52. An animal was considered to have a Partial Regression ("PR") when tumor volume was reduced by 50% or

greater when compared to the tumor volume at the day of first dose administration. A finding that the tumor volume of treated animals had decreased to ≤ 5 mm^3 during the study period was considered to denote a Complete Response ("CR"). The tumor volume (relative to vehicle control) was determined ("T/C"). Antitumor activity was evaluated according to National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

***In vivo* Activity Against Cholangiocarcinoma Patient-Derived Tumors**

[00236] The results of this study with respect to subcutaneously inoculated cholangiocarcinoma patient-derived tumor fragments are presented in **Table 10** and in **FIG. 13**, and show responsiveness against the cholangiocarcinoma patient-derived tumor fragments.

Table 10

Treatment	Dose (mg/kg Ab)	Schedule/Route	T/C-Day 61 (%)[a]	PR	CR	Antitumor Activity[b]
MGC028	10	Q2W x 2/IV	2	3/3	1/3	Highly Active

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 2037 mm^3 on Day 61.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C < 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00237] Female Athymic Nude-Foxn1^{nu} mice (n = 3/group) were implanted subcutaneously with cholangiocarcinoma patient-derived tumor fragments from stock mice. When tumors reached an average of 150-300 mm^3 (205 ± 28 mm^3 mean tumor volume ± standard deviation), pre-study mice were randomized by tumor volume and treated with MGC028 or vehicle control (formulation buffer) on days 0 and 14 (arrows) for a total of two doses at the dose level indicated. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at the dose level tested following treatments with MGC028.

[00238] MGC028 reduced tumor volumes by 98% on Day 61 at the 10 mg/kg Q2W x 2 dose level and was highly active (2% T/C) based on NCI standards. MGC028 induced partial regressions in 3/3 mice and complete regressions in 1/3 mice, at this dose level.

In vivo Activity Against Pancreatic Cancer Patient-Derived Tumors

[00239] The results of this study with respect to subcutaneously inoculated pancreatic adenocarcinoma patient-derived tumor fragments are presented in **Table 11** and in **FIG. 14**, and show responsiveness against the pancreatic patient-derived tumor fragments.

Table 11

Treatment	Dose (mg/kg Ab)	Schedule/Route	T/C-Day 37 (%)[a]	PR	CR	Antitumor Activity[b]
MGC028	10	Q2W x 2/IV	6	3/3	0/3	Highly Active

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1299 mm^3 on Day 37.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00240] Female Athymic Nude-Foxn1^{nu} mice (n = 3/group) were implanted subcutaneously with pancreatic adenocarcinoma patient-derived tumor fragments from stock mice. When tumors reached an average of 150-300 mm^3 (285 ± 11 mm^3 mean tumor volume ± standard deviation), pre-study mice were randomized by tumor volume and treated with MGC028 or vehicle control (formulation buffer) on days 0 and 14 (arrows) for a total of two doses at the dose level indicated. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at the dose level tested following treatments with MGC028.

[00241] MGC028 reduced tumor volumes by 94% on Day 37 at the 10 mg/kg Q2W x 2 dose level and was highly active (6% T/C) based on NCI standards. MGC028 induced partial regressions in 3/3 mice and no complete regressions at this dose level.

In vivo Activity Against Pancreatic Cancer Patient-Derived Tumors

[00242] The results of this study with respect to subcutaneously inoculated pancreatic adenocarcinoma patient-derived tumor fragments are presented in **Table 12** and in **FIG. 15**, and show responsiveness against the pancreatic cancer patient-derived tumor fragments.

Table 12						
Treatment	**Dose (mg/kg Ab)**	**Schedule/Route**	**T/C-Day 34 (%)[a]**	**PR**	**CR**	**Antitumor Activity[b]**
MGC028	10	Q2W x 2/IV	3	3/3	0/3	Highly Active

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1835 mm^3 on Day 34.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C ≤ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable (≤ 5 mm^3) during the course of the study).

[00243] Female Athymic Nude-Foxn1^{nu} mice (n = 3/group) were implanted subcutaneously with pancreatic adenocarcinoma patient-derived tumor fragments from stock mice. When tumors reached an average of 150-300 mm^3 (248 ± 48 mm^3 mean tumor volume ± standard deviation), pre-study mice were randomized by tumor volume and treated with MGC028 or vehicle control (formulation buffer) on days 0 and 14 (arrows) for a total of two doses at the dose level indicated. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at the dose level tested following treatments with MGC028.

[00244] MGC028 reduced tumor volumes by 97% on Day 34 at the 10 mg/kg Q2W x 2 dose level and was highly active (3% T/C) based on NCI standards. MGC028 induced 3/3 partial regressions and no complete regressions at this dose level. Mice treated with MGC028 10 mg/kg Q2W x 2 maintained antitumor control through the end of the study.

In vivo Activity Against Lung Cancer Patient-Derived Tumors

[00245] The results of this study with respect to subcutaneously inoculated non-small cell lung cancer (NSCLC) adenocarcinoma patient-derived tumor fragments are presented in **Table 13** and in **FIG. 16**, and show responsiveness against the lung patient-derived tumor fragments.

Table 13						
Treatment	**Dose (mg/kg Ab)**	**Schedule/Route**	**T/C-Day 30 (%)[a]**	**PR**	**CR**	**Antitumor Activity[b]**
MGC028	10	Q2W x 2/IV	24	0/3	0/3	Active

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1914 mm^3 on Day 30.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C ≤ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable (≤ 5 mm^3) during the course of the study).

[00246] Female Athymic Nude-Foxn1^{nu} mice (n = 3/group) were implanted subcutaneously with NSCLC adenocarcinoma patient-derived tumor fragments from stock mice. When tumors reached an average of 150-300 mm^3 (208 ± 27 mm^3 mean tumor volume ± standard deviation), pre-study mice were randomized by tumor volume and treated with MGC028 or vehicle control (formulation buffer) on days 0 and 14 (arrows) for a total of two doses at the dose level indicated. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at the dose level tested following treatments with MGC028.

[00247] MGC028 reduced tumor volumes by 76% on Day 30 at the 10 mg/kg Q2W x 2 dose level and was active (24% T/C) based on NCI standards. Although antitumor activity was observed, MGC028 did not induce partial or complete regressions at this dose level.

In vivo Activity Against Lung Cancer Patient-Derived Tumors

[00248] The results of this study with respect to subcutaneously inoculated non-small cell lung cancer (NSCLC) adenocarcinoma patient-derived tumor fragments are presented in **Table 14** and in **FIG. 17**, and show responsiveness against the lung cancer patient-derived tumor fragments.

Table 14						
Treatment	**Dose (mg/kg Ab)**	**Schedule/Route**	**T/C-Day 48 (%)[a]**	**PR**	**CR**	**Antitumor Activity[b]**
MGC028	10	Q2W x 2/IV	6	3/3	0/3	Highly Active

c % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1172 mm^3 on Day 48

d Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C ≤ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable (≤ 5 mm^3) during the course of the study).

[00249] Female Athymic Nude-Foxn1^{nu} mice (n = 3/group) were implanted subcutaneously with NSCLC adenocarcinoma patient-derived tumor fragments from stock mice. When tumors reached an average of 150-300 mm^3 (248 ± 59 mm^3 mean tumor volume ± standard deviation), pre-study mice were randomized by tumor volume and treated with MGC028 or vehicle control (formulation buffer) on days 0 and 14 (arrows) for a total of two doses at the dose level indicated. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at the dose level tested following treatments with MGC028.

[00250] MGC028 reduced tumor volumes by 94% on Day 48 at the 10 mg/kg Q2W x 2 dose level and was highly active (6% T/C) based on NCI standards. MGC028 induced 3/3 partial regressions and no complete regressions at this dose level.

***In vivo* Activity Against Colorectal Cancer Patient-Derived Tumors**

[00251] The results of this study with respect to subcutaneously inoculated colorectal cancer patient-derived tumor fragments are presented in **Table 15** and in **FIG. 18** and show responsiveness against the colorectal cancer patient-derived tumor fragments.

Table 15						
Treatment	**Dose (mg/kg Ab)**	**Schedule/Route**	**T/C-Day 29 (%)[a]**	**PR**	**CR**	**Antitumor Activity[b]**
MGC028	10	Q2W x 2/IV	25	0/3	0/3	Active

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 1102 mm^3 on Day 29.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C ≤ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable (≤ 5 mm^3) during the course of the study).

[00252] Female Athymic Nude-Foxn1^{nu} mice (n = 3/group) were implanted subcutaneously with colorectal cancer patient-derived tumor fragments from stock mice. When tumors reached an average of 150-300 mm^3 (248 ± 93 mm^3 mean tumor volume ± standard deviation), pre-study mice were randomized by tumor volume and treated with MGC028 or vehicle control (formulation buffer) on days 0 and 14 (arrows) for a total of two doses at the dose level indicated. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at the dose level tested following treatments with MGC028.

[00253] MGC028 reduced tumor volumes by 75% on Day 29 at the 10 mg/kg Q2W x 2 dose level and was active (25% T/C) based on NCI standards. MGC028 did not induce partial or complete regressions at this dose level.

In vivo Activity Against Squamous Cell Carcinoma of the Head and Neck Patient-Derived Tumors

[00254] The results of this study with respect to subcutaneously inoculated squamous cell carcinoma of the head and neck (SCCHN) patient-derived tumor fragments are presented in **Table 16** and in **FIG. 19**, and show responsiveness against the SCCHN patient-derived tumor fragments.

Table 16						
Treatment	**Dose (mg/kg Ab)**	**Schedule/Route**	**T/C-Day 69 (%)[a]**	**PR**	**CR**	**Antitumor Activity[b]**
MGC028	10	Q2W x 2/IV	9	3/3	1/3	Highly Active

a % T/C = percentage of mean tumor size in treatment group (T) relative to mean tumor size of vehicle control group (C). Calculated when the vehicle tumor volume reached a mean of 841 mm^3 on Day 69.

b Antitumor Activity: Evaluated based on National Cancer Institute (NCI) standards; a T/C $\leq$ 42% is the minimum level of antitumor activity, while a T/C value of > 42% is inactive. A T/C < 10% is considered highly active.

Abbreviations: Ab: antibody; mg: milligram; kg: kilogram; IV: intravenous; PR: partial regression (tumor volume was reduced by 50% or greater from dosing day during study); CR: complete regression (defined as when no palpable tumor could be detected (0 mm^3) or when the tumor volume was non-measurable ($\leq$ 5 mm^3) during the course of the study).

[00255] Female Athymic Nude-Foxn1^{nu} mice (n = 3/group) were implanted subcutaneously with SCCHN patient-derived tumor fragments from stock mice. When tumors reached an average of 150-300 mm^3 (227 ± 24 mm^3 mean tumor volume ± standard deviation), pre-study mice were randomized by tumor volume and treated with MGC028 or vehicle control (formulation buffer) on days 0 and 14 (arrows) for a total of two doses at the dose level indicated. Tumor volume is shown as group mean ± standard error of the mean (SEM) with the upper SEM bar visible. Antitumor activity was observed at the dose level tested following treatments with MGC028.

[00256] MGC028 reduced tumor volumes by 91% on Day 69 at the 10 mg/kg Q2W x 2 dose level and was highly active (9% T/C) based on NCI standards. MGC028 induced 3/3 partial regressions and 1/3 complete regressions at this dose level. Mice treated with MGC028 10 mg/kg Q2W x 2 maintained antitumor control through the end of the study for most animals.

[00257] The results of these *in vivo* studies demonstrate that MGC028 exhibited antitumor activity toward ADAM9-positive tumors in murine patient-derived xenograft models of cholangiocarcinoma, pancreatic cancer, lung cancer, colorectal cancer, and SCCHN.

Example 6
MGC028 Plasma Stability Assessment

[00258] MGC028 is a recombinant IgG (Immunoglobulin G, MW 144.7 kDa) with two SYNtecan E™ linker-payloads conjugated to the heavy chain subunit. Each SYNtecan E™ molecule contains two enzymatically cleavable exatecan moieties, giving the ADC an average drug to antibody ratio (DAR) of four. MGC028 stability was examined by two different metrics, a change in DAR over the time by IC-LC-HRMS (Immunocapture-Liquid Chromatography-

High Resolution Mass Spectrometry) and measurement of the release of free exatecan by LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry). These analytical methods were able to detect < 1% release of free exatecan in plasma. As part of the study, three additional components were also examined, two linker-payload metabolites and SYNtecan E™.

[00259] MGC028 stability was assessed in human, mouse, or cynomolgus monkey K_2EDTA plasma under conditions of physiological temperature and pH. The stability samples were prepared by spiking MGC028 into mouse, human, or cynomolgus monkey plasma at a concentration of 500 µg/mL and incubated at 37°C. Samples for stability analyses were collected at timepoints of 0, 1, 4, 8, 24, 48, 72, and 168-hours and analyzed for DAR or release of exatecan.

[00260] Results indicate that MGC028 was stable in all three plasma types over the study period. The DAR level of MGC028 remained at four as observed up to 168 hours indicating the two SYNtecan E™ molecules remained attached to MGA027 (**Table 17, FIG. 20**). Lower masses, corresponding to partial or total loss of SYNtecan E were not detected by LC-HRMS.

Table 17: ADC stability in plasma presented as mean drug to antibody ratio (DAR) calculated at each timepoint.

Hours	DAR Mouse	DAR Human	DAR Cynomolgus
0	4	4	4
1	4	4	4
4	4	4	4
8	4	4	4
24	4	4	4
72	4	4	4
168	4	4	4

[00261] The LC-MS/MS assay was developed to analyze free exatecan in mouse, human or cynomolgus monkey plasma. The LLOQ for exatecan was 21 ng/mL. Free exatecan was detected in mouse plasma at a maximum of 92 ng/mL at 168 hours, less than 2% of the theoretical maximum (**FIGs. 21A-21B**). There was no detectable free exatecan above the LLOQ in either human or cynomolgus monkey plasma samples (**FIGs. 21C-21F**). Linker-payload metabolites and free SYNtecan E™ (LLOQ = 55 ng/mL) were not detected above 1% of the theoretical maximum release, and no apparent trends in metabolite concentration were observed over time.

Example 7
Tolerability Study of MGC028 in Cynomolgus Monkeys

[00262] To determine the tolerability of MGC028, a toxicology study was conducted in cynomolgus monkeys (Macaca fascicularis).

[00263] MGC028 was administered by a 15-minute intravenous (IV) infusion to a total of 6 cynomolgus monkeys. In the non-GLP study, doses of 22.5, and 55 mg/kg were administered to 3 animals each at a Q2W dose interval for a total of two doses (**Table 18**).

Table 18

Groups	Test Material	Dose Level (mg/kg)	No. of Males	No. of Females
1	MGC028	22.5	1	2
2	MGC028	55	1	2

[00264] This study evaluated animals for clinical observations, changes in body weight, food consumption, and hematology and clinical chemistry measurements. Animals were necropsied on study day 20 and evaluated for postmortem examinations, collection of bone marrow smears, and standard tissues were collected, weighed, and examined microscopically.

[00265] MGC028 administration of 22.2 or 55 mg/kg/dose at Q2W (study days 1 and 15) was well tolerated. All animals survived to the scheduled study termination. There were no MGC028-related effects on clinical observations or body weights. Food consumption was slightly decreased in both females at 55 mg/kg after dosing between Days 5 to 8 and Days 16 to 18 but did not impact body weight (**Table 19**).

Table 19: MGC028 non-GLP Cynomolgus Monkey Toxicology Study Results

	MGC028
Antibody	MGA027 with modification for linker
Payload	Exatecan: SYNtecan E
NON GLP TOX STUDIES	
REPEAT DOSE	**22.5, 55 mg/kg/dose**
Dose schedule	Q2W x 2 doses
Mortality	None
Body weight	No changes
Clinical observations	No observations
Skin erosion	No findings
Clinical Pathology	
Hematology	No findings
Clinical chemistry	No findings
Terminal Necropsy	
Gross Pathology	No findings
Organ Weights	No findings

Histopathology	
Bladder	No findings
Eye	No findings
Skin, Pigmentation	None
Skin, Lymphocytic Inflammation	No findings
Skin, Epidermal Hyperplasia	No findings
Skin, Single cell necrosis	No findings
Thymus	55 mg/kg: 2 out of 3 animals had minimally decreased lymphocyte cellularity in the thymus

[00266] There were no MGC028-related changes in hematology or clinical chemistry parameters.

[00267] There were no MGC028-related effects in the bone marrow. Myeloid:erythroid (M:E) ratios were well represented and demonstrated appropriate maturation to completion. Megakaryocytes were observed in adequate numbers. Cells from all lines appeared morphologically unremarkable.

[00268] The only MGC028-related microscopic change was non-adverse, minimally decreased lymphocyte cellularity in the thymus in two of three animals (one male and one female) at the 55 mg/kg dose level (**Table 19**). The findings in the thymus were non-adverse because substantial thymus tissue remained in animals, evidence of opportunistic infections was lacking, other components of the immune system (spleen, mesenteric lymph node, mandibular lymph nodes, gut associated immune system) were normal.

[00269] In summary, MGC028 was well tolerated in the non-GLP study up to and including the top dose of 55 mg/kg/dose. In comparison, IMGC936, another ADC targeted to ADAM9 but with a maytansinoid payload, had mortality, dermal, and ocular toxicity [Molecular Cancer Therapeutics, 21(7) July 2022] at dose levels 50% lower than what was administered in the MGC028 study.

Example 8
GLP Toxicology Study of MGC028 in Cynomolgus Monkeys

[00270] To evaluate the potential toxicity of MGC028, a GLP toxicology study was conducted in cynomolgus monkeys (Macaca fascicularis).

[00271] MGC028 was administered as an intravenous infusion once every 3 weeks on Days 1, 22, and 43 to cynomolgus monkeys. The study included four treatment groups and the animals received either the vehicle control (saline) or MGC028 administered once every three weeks, for a total of three doses (study days 1, 22, and 43), by a 15-minute intravenous (IV) infusion (**Table 20**). Group 1 animals received the vehicle control article and Groups 2 to 4 received MGC028 at 10, 30, or 60 mg/kg/dose. Main study animals (3/sex/group) were necropsied on study day 48, and the remaining animals in Groups 3 and 4 (2/sex/group) underwent an 11-week recovery period to evaluate the potential reversibility of any findings with recovery necropsy on study day 120.

Table 20: MGC028 Repeat-dose GLP Cynomolgus Monkey Toxicology Study Design

Group No.	Test Material	Dose Level (mg/kg/day)	Main Study Animals		Recovery Study Animals	
			No. of Males	No. of Females	No. of Males	No. of Females
1	Vehicle Control	0	3	3	-	-
2	MGC028	10	3	3	-	-
3	MGC028	30	3	3	2	2
4	MGC028	60	3	3	2	2

[00272] The following parameters and endpoints were evaluated in the GLP toxicology study: mortality, clinical observations, body weights, food consumption, veterinary physical examinations, ophthalmic examination, electrocardiology, heart rate, indirect blood pressure, visual respiratory rate, neural/behavior assessment, clinical pathology parameters (hematology, coagulation, clinical chemistry, and urinalysis), toxicokinetic assessment, anti-drug antibody formation, gross necropsy findings, organ weights, and a histopathologic examination of 61 different organs and tissues.

[00273] There were no unscheduled animal mortalities and no findings for food consumption, body weights, or clinical pathology parameters (clinical chemistry, coagulation, or urinalysis), ophthalmic examination, electrocardiology, blood pressure and heart rate, visual respiratory rate, neurological and musculoskeletal assessment (reaction to environmental stimuli, rectal temperature, ocular, motor reflex, and proprioceptive functions), gross necropsy or organ weights or microscopic assessment of female reproductive organs (females were sexually mature) performed in the GLP study (**Table 21**).

Table 21: MGC028 GLP Cynomolgus Monkey Toxicology Study Results	
	MGC028
Antibody	MGA027
Payload	Exatecan: SYNtecan E
GLP TOX STUDY	
Repeat Dose	**10, 30, 60 mg/kg/dose**
Dose schedule	Q3W x 3 doses
Mortality	None
Body Weight	No changes
Clinical Observations	No observations
Ophthalmic examination	No findings
Neural Assessment	No findings
ECGs, BP, Heart Rate, Respiration Rate	No findings
Clinical Pathology	
Hematology	Neutrophil Counts: transient mildly lower mean neutrophil counts were observed in females at ≥ 30 mg/kg/dose on Days 3, 24, and/or 47 (reflecting 2, 2, and 4 days post dose, respectively) that resolved by subsequent collection intervals on study days 8, 29, and 118. Reticulocyte Counts: transient mildly to moderately lower mean reticulocyte counts were observed in males at 60 mg/kg/dose and females at ≥ 30 mg/kg/dose on study days 8, 29, and/or 47 (reflecting 7, 7, and 4 days postdose, respectively) relative to concurrent control animals; that generally resolved by subsequent collection intervals on study days 21, and 42 , and 118 Erythrocyte Mass (red blood cell count [RBC], hemoglobin concentration, and/or hematocrit): minimally lower mean parameters of erythrocyte mass (red blood cell count [RBC], hemoglobin concentration, and/or hematocrit) in females at ≥ 30 mg/kg/dose at all collection intervals from study days 21 to Day 47 Red Blood Cell Distribution Width (RDW): minimally to mildly higher mean red blood cell distribution width (RDW), reflecting greater variation in erythrocyte size, was also observed in females at 60 mg/kg/dose at all collection intervals from study days 8 through 47, and in males at 60 mg/kg/dose from days 29 through 47, and females at 30 mg/kg/dose from days 42 through 47
Clinical chemistry	No findings
Coagulation	No findings
Urinalysis	No findings
Terminal Necropsy	
Gross Pathology	No findings
Organ Weights	No findings
Histopathology	
Bladder	No findings
Eye	No findings
Skin, Pigmentation	No findings
Skin, Lymphocytic Inflammation	No findings
Skin, Epidermal Hyperplasia	No findings
Skin, Single cell necrosis	No findings

Thymus	Mild to moderate decreased lymphoid cellularity observed in males at 60 mg/kg and moderate decreases in females at ≥10 mg/kg
Recovery Necropsy	
Gross Pathology	No findings
Organ Weights	No findings
Histopathology	
Bladder	No findings
Eye	No findings
Skin, Pigmentation	No findings
Skin, Lymphocytic Inflammation	No findings
Skin, Epidermal Hyperplasia	No findings
Skin, Single cell necrosis	No findings
Thymus	No findings above what was previously observed in control animals (showed reversibility)

[00274] MGC028 PK was linear between 30 mg/kg and 60 mg/kg/dose in cynomolgus monkey, but showed nonlinearity between 10 mg/kg and 30 mg/kg/dose likely due to target-dependent clearance as low doses and/or saturation of target specific clearance mechanisms at the higher dose level.

[00275] In the study, transient mild decreases in neutrophil counts were observed in females at ≥ 30 mg/kg/dose after each dose day that were resolved by subsequent collection intervals. Transient mildly to moderately lower mean reticulocyte count, relative to concurrent control means, were observed in males (60 mg/kg/dose) and females (≥ 30 mg/kg/dose) after each MGC028 dose. Relatively lower reticulocyte counts were indicative of a diminished or delayed erythropoietic response and were accompanied by minimally lower mean parameters of erythrocyte mass. MGC028-related microscopic findings pertained to the thymus, with decreased lymphoid cellularity consisting of thinning of the thymic cortex with decreased cortical-medullary ratio and increased number of apoptotic lymphocytes that was reversible by the end of the 11-week recovery period in the GLP study. Animals remained healthy for the duration of the study and therefore all findings were considered non-adverse.

[00276] In summary, MGC028 was well tolerated in the GLP study up to and including the top dose of 60 mg/kg/dose. In comparison, IMGC936, another ADC targeted to ADAM9 but with a maytansinoid payload, had mortality, dermal, and ocular toxicity [Molecular Cancer Therapeutics, 21(7) July 2022] at dose levels 50% lower than what was administered in the MGC028 study.

[00277] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While specific aspects are described herein, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the aspects.

WHAT IS CLAIMED IS:

Claim 1. An anti-ADAM9 antibody drug conjugate (**ADAM9-ADC**) that comprises the formula:

$$\textbf{Ab-(LM)}_{m}\textbf{-(D)}_{n},$$

wherein:

Ab is a humanized ADAM9 antibody or ADAM9 binding fragment thereof that binds to ADAM9 and comprises:

(i) the CDR_L1 sequence KASQSVDYSGDSYMN (**SEQ ID NO:24**), the CDR_L2 sequence AASDLES (**SEQ ID NO:25**) and the CDR_L3 sequence QQSHEDPFT (**SEQ ID NO:26**) in its Variable Light Chain (VL) domain, and

(ii) the CDR_H1 sequence SYWMH (**SEQ ID NO:29**), the CDR_H2 sequence EIIPIFGHTNYNEKFKS (**SEQ ID NO:30**) and the CDR_H3 sequence GGYYYYPRQGFLDY (**SEQ ID NO:31**) in its Variable Heavy Chain (VH) domain;

D is a camptothecin moiety;

LM is a Linker Molecule that covalently links **Ab** and **D**;

m is an integer between 1 and n and denotes the number of Linker Molecules of the **ADAM9-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic camptothecin moieties covalently linked to the **ADAM9-ADC** molecule.

Claim 2. The **ADAM9-ADC** of claim 1, wherein the **Ab** comprises:

(i) a humanized VL Domain comprising the amino acid sequence of **SEQ ID NO:22**, and

(ii) a humanized VH Domain comprising the amino acid sequence of **SEQ ID NO:27**.

Claim 3. The **ADAM9-ADC** of claim 1, wherein the **Ab** comprises:

(i) a Light Chain comprising the amino acid sequence of **SEQ ID NO:23**, and

(ii) a Heavy Chain comprising the amino acid sequence of **SEQ ID NO:28**.

Claim 4. The **ADAM9-ADC** of any one of claims 1-3, wherein the **Ab** is an antibody.

Claim 5. The **ADAM9-ADC** of any one of claims 1-4, wherein the **Ab** is an antigen binding fragment of an antibody.

Claim 6. The **ADAM9-ADC** of any one of claims 1-5, wherein the **Ab** comprises an Fc Domain of a human IgG.

Claim 7. The **ADAM9-ADC** of claim 6, wherein the human IgG is a human IgG1, IgG2, IgG3, or IgG4.

Claim 8. The **ADAM9-ADC** of any one of claims 6 or 7, wherein the Fc Domain is a variant Fc Domain that comprises:

(a) one or more amino acid modifications that reduce the affinity of the variant Fc Domain for an FcγR; and/or

(b) one or more amino acid modifications that enhance the serum half-life of the variant Fc Domain.

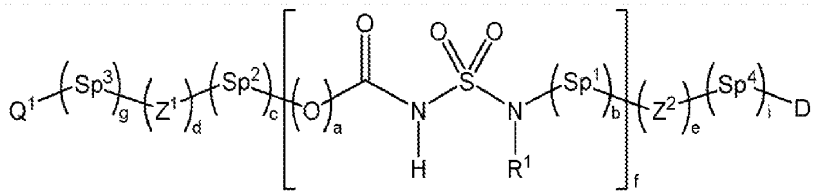
Claim 9. The **ADAM9-ADC** of claim 8, wherein the modifications that reduce the affinity of the variant Fc Domain for an FcγR comprise the substitution of L234A; L235A; or L234A and L235A, wherein the numbering is that of the EU index as in Kabat.

Claim 10. The **ADAM9-ADC** of any one of claims 8 or 9, wherein the modifications that that enhance the serum half-life of the variant Fc Domain comprise the substitution of M252Y; M252Y and S254T; M252Y and T256E; M252Y, S254T and T256E; or K288D and H435K, wherein the numbering is that of the EU index as in Kabat.

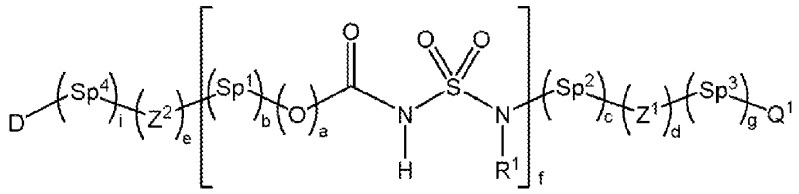
Claim 11. The **ADAM9-ADC** of claim 1, wherein the **LM** comprises a peptidic linker.

Claim 12. The **ADAM9-ADC** of claim 1, wherein the **LM** comprises a cleavable linker.

Claim 13. The **ADAM9-ADC** of claim 1, wherein the **LM** comprises the formula (4a) or (4b), or a salt thereof:



4a



4b

wherein:

a is independently 0 or 1;

b is independently 0 or 1;

c is 0 or 1;

d is 0 or 1;

e is 0 or 1;

f is an integer in the range of 1 to 150;

g is 0 or 1;

i is 0 or 1;

D is a cytotoxic camptothecin moiety;

Q^1 is an alkenyl group, (hetero)cycloalkenyl group, bicyclo triazole group or cycloalkenyl group;

wherein Q^1 is attached to a functional group of the antibody;

wherein Sp^1, Sp^2, Sp^3 and Sp^4 are independently selected from the group consisting of linear or branched C_1-C_{200} alkylene groups, C_2-C_{200} alkenylene groups, C_2-C_{200} alkynylene groups, C_3-C_{200} cycloalkylene groups, C_5-C_{200} cycloalkenylene groups, C_8-C_{200} cycloalkynylene groups, C_7-C_{200} alkylarylene

groups, C_7-C_{200} arylalkylene groups, C_8-C_{200} arylalkenylene groups and C_9-C_{200} arylalkynylene groups, the alkylene groups, alkenylene groups, alkynylene groups, cycloalkylene groups, cycloalkenylene groups, cycloalkynylene groups, alkylarylene groups, arylalkylene groups, arylalkenylene groups and arylalkynylene groups being optionally substituted and optionally interrupted by one or more heteroatoms selected from the group of O, S and NR^3, wherein R^3 is independently selected from the group consisting of hydrogen, C_1 - C_{24} alkyl groups, C_2 - C_{24} alkenyl groups, C_2 - C_{24} alkynyl groups and C_3 - C_{24} cycloalkyl groups, the alkyl groups, alkenyl groups, alkynyl groups and cycloalkyl groups being optionally substituted;

Z^1 is a connecting group that connects Q^1 or Sp^3 to Sp^2, O or C(O) or $N(R^1)$;
Z^2 is a connecting group that connects **D** or Sp^4 to Sp^1, $N(R^1)$, O or C(O);
wherein Z^1 and Z^2 are independently selected from the group consisting of -O-, -S-, -NR^2-, -N=N-, -C(O)-, -C(O)NR^2-, -O-C(O)- , -O-C(O)-O-, -O-C(O)-NR^2, -NR^2-C(O)-, -NR^2-C(O)-O-, -NR^2-C(O)-NR^2-, -S-C(O)-, -S-C(O)-O-, -S-C(O)-NR^2-, -S(O)-, -$S(O)_2$-, -O-$S(O)_2$-, -O-$S(O)_2$-O-, -O-$S(O)_2$-NR^2-, -O-S(O)-, -O-S(O)-O-, -O-S(O)-NR^2-, -O-NR^2-C(O)-, -O-NR^2-C(O)-O-, -O-NR^2-C(O)-NR^2-, -NR^2-O-C(O)-, -NR^2-O-C(O)-O-, -NR^2-O-C(O)-NR^2-, -O-NR^2-C(S)-, -O-NR^2-C(S)-O-, -O-NR^2-C(S)-NR^2-, -NR^2-O-C(S)-, -NR^2-O-C(S)-O-, -NR^2-O-C(S)-NR^2-, -O-C(S)-, -O-C(S)-O-, -O-C(S)-NR^2-, -NR^2-C(S)-, -NR^2-C(S)-O-, -NR^2-C(S)-NR^2-, -S-$S(O)_2$-, -S-$S(O)_2$-O-, -S-$S(O)_2$-NR^2-, -NR^2-O-S(O)-, -NR^2-O-S(O)-O-, -NR^2-O-S(O)-NR^2-, -NR^2-O-$S(O)_2$-, -NR^2-O-$S(O)_2$-O-, -NR^2-O-$S(O)_2$-NR^2-, -O-NR^2-S(O)-, -O-NR^2-S(O)-O-, -O-NR^2-S(O)-NR^2-, -O-NR^2-$S(O)_2$-O-, -O-NR^2-$S(O)_2$-NR^2-, -O-NR^2-$S(O)_2$-, -O-P(O)$(R^2)_2$-, -S-P(O)$(R^2)_2$-, -NR^2-P(O)$(R^2)_2$- and combinations of two or more thereof, wherein R^2 is independently selected from the group consisting of hydrogen, C_1 - C_{24} alkyl groups, C_2 - C_{24} alkenyl groups, C_2 - C_{24} alkynyl groups and C_3 - C_{24} cycloalkyl groups, the alkyl groups, alkenyl groups, alkynyl groups and cycloalkyl groups being optionally substituted; and

R^1 is selected from the group consisting of hydrogen, C_1 - C_{24} alkyl groups, C_3 - C_{24} cycloalkyl groups, C_2 - C_{24} (hetero)aryl groups, C_3 - C_{24} alkyl(hetero)aryl groups and C_3 - C_{24} (hetero)arylalkyl groups, the C_1 - C_{24} alkyl groups, C_3 - C_{24} cycloalkyl groups, C_2 - C_{24} (hetero)aryl groups, C_3 - C_{24} alkyl(hetero)aryl groups and C_3 - C_{24} (hetero)arylalkyl groups optionally substituted and optionally interrupted by one or more heteroatoms selected from O, S and NR^3 wherein R^3 is independently selected from the group consisting of hydrogen and C_1 - C_4 alkyl groups; or

R^1 is D, $-[(Sp^1)_b(Z^2)_e-(Sp^4)_i-D]$ or $-[(Sp^2)_c-(Z^1)_d-(Sp^3)_g-Q^1]$, wherein Sp^1, Sp^2, Sp^3, Sp^4, Z^1, Z^2, D, Q^1, b, c, d, e, g and i are as defined above.

Claim 14. The **ADAM9-ADC** of claim 13, wherein Sp^1, Sp^2, Sp^3 and Sp^4, if present, are independently selected from the group consisting of linear or branched C_1-C_{20} alkylene groups, the alkylene groups being optionally substituted and optionally interrupted by one or more heteroatoms selected from the group consisting of O, S and NR^3, wherein R^3 is independently selected from the group consisting of hydrogen and C_1 - C_4 alkyl groups.

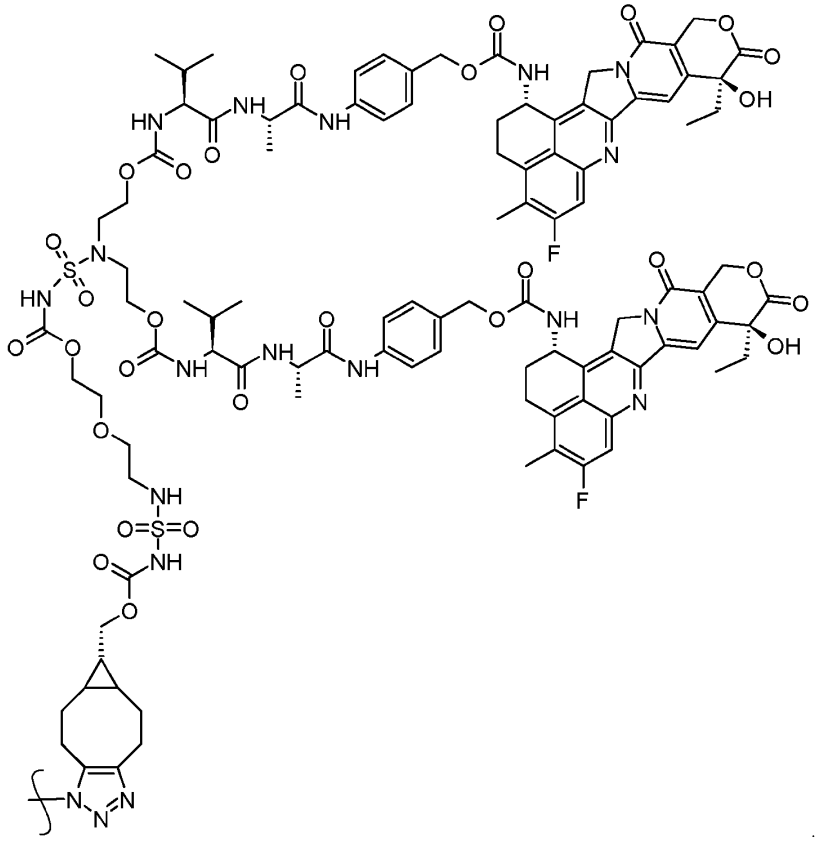
Claim 15. The **ADAM9-ADC** of any one of claims 1-14, where the **LM** comprises a valine-alanine (Val-Ala) amino acid linker.

Claim 16. The **ADAM9-ADC** of claim 15, wherein the Val-Ala linker is a Val-Ala-PABC linker.

Claim 17. The **ADAM9-ADC** of any one of claims 1-16, wherein the camptothecin moiety is selected from the group consisting of SN-38 (*S*-10-hydroxycamptothecin), topotecan (HYCAMPTIN; (S)-9-N,N-dimethylaminoethyl-10-hydroxycamptothecin), 9-aminocamptothecin (9-amino-20(S)-camptothecin), 9-nitrocamptothecin (also called rubitecan), lurtotecan (7-(4-methylpiperazinomethylene)-10,11-ethylenedioxy-20(S)-camptothecin), exatecan, belotecan, karenitecin, and a homocamptothecin.

Claim 18. The **ADAM9-ADC** of any one of claims 1-17, wherein the camptothecin moiety is exatecan.

Claim 19. The **ADAM9-ADC** of any one of claims 1-18, wherein **LM** and **D** together comprise:



Claim 20. An anti-ADAM9 antibody drug conjugate (**ADAM9-ADC**) that comprises the formula:

Ab-(LM)$_m$-(D)$_n$,

wherein:

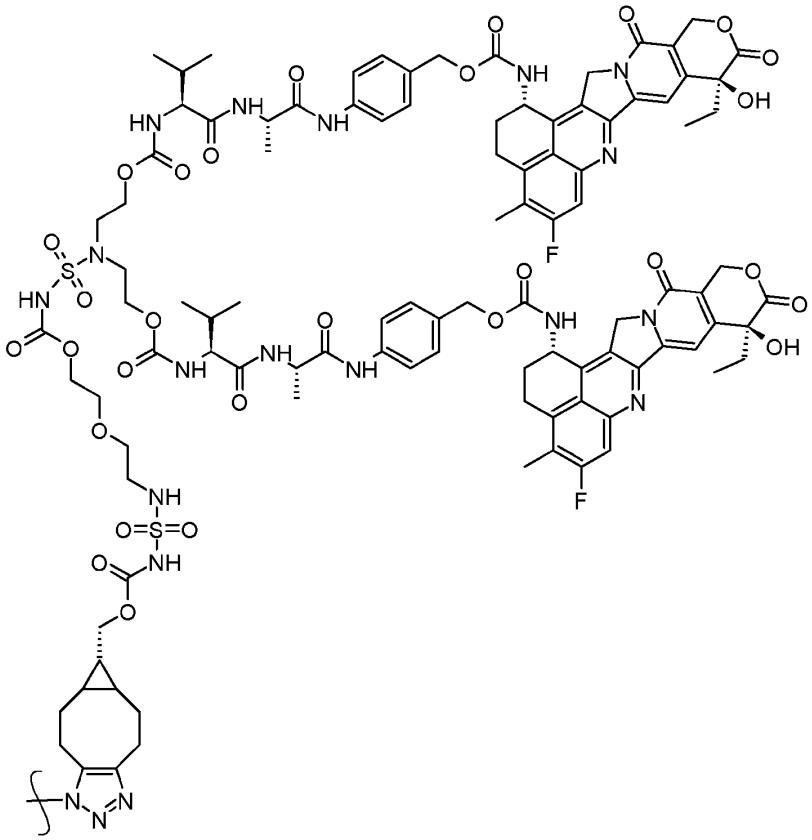
Ab is a humanized ADAM9 antibody or ADAM9 binding fragment thereof that binds to ADAM9 and comprises:

(i) the CDR$_L$1 sequence KASQSVDYSGDSYMN (**SEQ ID NO:24**), the CDR$_L$2 sequence AASDLES (**SEQ ID NO:25**) and the CDR$_L$3

sequence QQSHEDPFT (**SEQ ID NO:26**) in its Variable Light Chain (VL) domain, and

(ii) the CDR_H1 sequence SYWMH (**SEQ ID NO:29**), the CDR_H2 sequence EIIPIFGHTNYNEKFKS (**SEQ ID NO:30**) and the CDR_H3 sequence GGYYYYPRQGFLDY (**SEQ ID NO:31**) in its Variable Heavy Chain (VH) domain;

D and **LM** together comprise:



m is an integer between 0 and n and denotes the number of Linker Molecules of the **ADAM9-ADC**;

and

n is an integer between 1 and 10 and denotes the number of cytotoxic camptothecin moieties covalently linked to the **ADAM9-ADC** molecule.

Claim 21. The **ADAM9-ADC** of claim 20, wherein the **Ab** comprises:

(i) a humanized VL Domain comprising the amino acid sequence of **SEQ ID NO:22**, and

(ii) a humanized VH Domain comprising the amino acid sequence of **SEQ ID NO:27.**

Claim 22. The **ADAM9-ADC** of claim 20, wherein the **Ab** comprises:

(i) a Light Chain comprising the amino acid sequence of **SEQ ID NO:23**, and

(ii) a Heavy Chain comprising the amino acid sequence of **SEQ ID NO:28.**

Claim 23. A pharmaceutical composition that comprises an effective amount of the **ADAM9-ADC** of any of claims 1-22 and a pharmaceutically acceptable carrier, excipient or diluent.

Claim 24. Use of the **ADAM9-ADC** of any one of claims 1-22 or the pharmaceutical composition of claim 23 in the treatment of a disease or condition associated with or characterized by the expression of ADAM9.

Claim 25. A method of treating a disease or condition associated with or characterized by the expression of ADAM9 comprising administering the **ADAM9-ADC** of any one of claims 1-22 or the pharmaceutical composition of claim 23 to a subject.

Claim 26. The use of claim 24 or the method of claim 25, wherein the disease or condition associated with or characterized by the expression of ADAM9 is cancer.

Claim 27. The use or method of claim 26, wherein the cancer is selected from the group consisting of: brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gallbladder cancer, gastric cancer, head and neck cancer, liver cancer, lung cancer, myeloid cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, thyroid cancer, testicular cancer, and uterine cancer.

Claim 28. The use or method of claim 27, wherein the cancer is selected from the group

consisting of: brain cancer, bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gallbladder cancer, gastric cancer, head and neck cancer, liver cancer, lung cancer, myeloid cancer, oral cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, thyroid cancer, testicular cancer, and uterine cancer.

Claim 29. The use or method of claim 26, wherein the cancer is glioma.

Claim 30. The use or method of claim 26, wherein the cancer is breast cancer or triple negative breast cancer.

Claim 31. The use or method of claim 26, wherein the cancer is cervical cancer.

Claim 32. The use or method of claim 26, wherein the cancer is colorectal cancer.

Claim 33. The use or method of claim 26, wherein the cancer is cholangiocarcinoma.

Claim 34. The use or method of claim 26, wherein the cancer is gastric cancer or gastroesophageal junction adenocarcinoma.

Claim 35. The use or method of claim 26, wherein the cancer is squamous cell cancer of the head and neck.

Claim 36. The use or method of claim 26, wherein the cancer is non-small cell lung cancer.

Claim 37. The use or method of claim 26, wherein the cancer is oral cancer or oral squamous cell carcinoma.

Claim 38. The use or method of claim 26, wherein the cancer is ovarian cancer or ovarian clear cell carcinoma.

Claim 39. The use or method of claim 26, wherein the cancer is pancreatic cancer.

Claim 40. The use or method of claim 26, wherein the cancer is renal cell carcinoma.