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(54) Title: ADAM9-BINDING MOLECULES, AND METHODS OF USE THEREOF

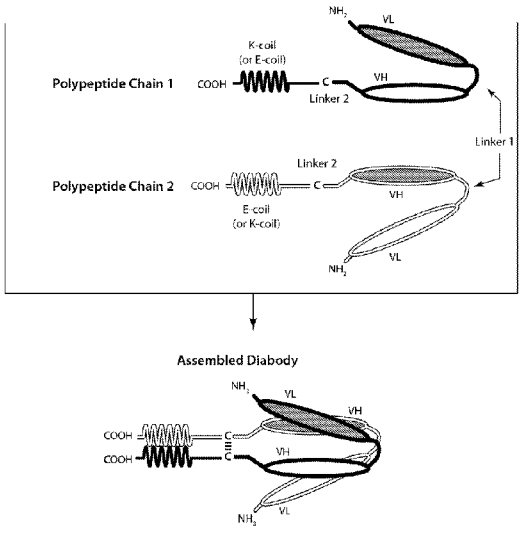

Figure 1

(57) Abstract: The present invention is directed to molecules, such as monospecific antibodies and bispecific, trispecific or multispecific binding molecules, including diabodies, BiTEs, and antibodies that are capable of specifically binding to "Disintegrin and Metalloproteinase Domain-containing Protein 9" ("ADAM9"). The invention particularly concerns such binding molecules that are capable of exhibiting high affinity binding to human and non- human ADAM9. The invention further particularly relates to such molecules that are thereby cross-reactive with human ADAM9 and the ADAM9 of a non-human primate (e.g., a cynomolgus monkey). The invention additionally pertains to all such ADAM9-binding molecules that comprise a Light Chain Variable (VL) Domain and/or a Heavy Chain Variable (VH) Domain that has been humanized and/or deimmunized so as to exhibit reduced immunogenicity upon administration of such ADAM9-binding molecule to a recipient subject. The invention is also directed to pharmaceutical compositions that contain any of such ADAM9-binding molecules, and to methods involving the use of any of such ADAM9-binding molecules in the treatment of cancer and other diseases and conditions.

WO 2018/119166 A1

[Continued on next page]

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TITLE OF THE INVENTION:

ADAM9-Binding Molecules, and Methods of Use Thereof

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Patent Appln. Serial No. 62/438,516 (filed on December 23, 2016; pending), which application is herein incorporated by reference in its entirety.

REFERENCE TO SEQUENCE LISTING

[0002] This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 *et seq.*, which are disclosed in computer-readable media (file name: 1301.0147PCT_Sequence_Listing_ST25.txt, created on November 29, 2017, and having a size of 175,962 bytes), which file is herein incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention is directed to molecules, such as monospecific antibodies and bispecific, trispecific or multispecific binding molecules, including diabodies, BiTEs, and antibodies that are capable of specifically binding to "Disintegrin and Metalloproteinase Domain-containing Protein 9" ("ADAM9"). The invention particularly concerns such binding molecules that are capable of exhibiting high affinity binding to human and non-human ADAM9. The invention further particularly relates to such molecules that are thereby cross-reactive with human ADAM9 and the ADAM9 of a non-human primate (*e.g.*, a cynomolgus monkey). The invention additionally pertains to all such ADAM9-binding molecules that comprise a Light Chain Variable (VL) Domain and/or a Heavy Chain Variable (VH) Domain that has been humanized and/or deimmunized so as to exhibit reduced immunogenicity upon administration of such ADAM9-binding molecule to a recipient subject. The invention is also directed to pharmaceutical compositions that contain any of such ADAM9-binding molecules, and to methods involving the use of any of such ADAM9-binding molecules in the treatment of cancer and other diseases and conditions.

BACKGROUND OF THE INVENTION

[0004] 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).

[0005] 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).

[0006] ADAM9 is a member of the ADAM family of molecule. 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).

[0007] 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.

[0008] 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).

[0009] 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).

[0010] 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.

[0011] However, despite all prior advances, a need remains for high affinity ADAM9-binding molecules that exhibit minimal binding to normal tissues and are capable of binding to human and non-human ADAM9 with similar high affinity. The present invention addresses this need and the need for improved therapeutics for cancer.

SUMMARY OF THE INVENTION

[0012] The present invention is directed to molecules, such as monospecific antibodies and bispecific, trispecific or multispecific binding molecules, including diabodies, BiTEs, and antibodies that are capable of specifically binding to "Disintegrin and Metalloproteinase Domain-containing Protein 9" ("ADAM9"). The invention particularly concerns such binding molecules that are capable of exhibiting high affinity binding to human and non-human ADAM9. The invention further particularly relates to such molecules that are thereby cross-reactive with human ADAM9 and the ADAM9 of a non-human primate (*e.g.*, a cynomolgus monkey). The invention additionally pertains to all such ADAM9-binding molecules that comprise a Light Chain Variable (VL) Domain and/or a Heavy Chain Variable (VH) Domain that have been humanized and/or deimmunized so as to exhibit reduced immunogenicity upon administration of such ADAM9-binding molecule to a recipient subject. The invention is also directed to pharmaceutical compositions that contain any of such ADAM9-binding molecules, and to methods involving the use of any of such ADAM9-binding molecules in the treatment of cancer and other diseases and conditions.

[0013] In detail, the invention provides an ADAM9-binding molecule that comprises an ADAM9-binding domain, wherein such ADAM9-binding domain comprises a Light Chain Variable (VL) Domain and a Heavy Chain Variable (VH) Domain, wherein such Heavy Chain Variable Domain comprises a CDR_H1 Domain, a CDR_H2 Domain and a

CDR_H3 Domain, and such Light Chain Variable Domain comprises a CDR_L1 Domain, a CDR_L2 Domain, and a CDR_L3 Domain, wherein:

(A) such CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain have the amino acid sequence of the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of a Heavy Chain Variable (VH) Domain of an optimized variant of **MAB-A**; and such CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain have the amino acid sequence of the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Light Chain Variable (VL) Domain of **MAB-A**; or

(B) such CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain have the amino acid sequence of the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of the Heavy Chain Variable (VH) Domain of **MAB-A**; and such CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain have the amino acid sequence of the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of a Light Chain Variable (VL) Domain of an optimized variant of **MAB-A**; or

(C) such CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain have the amino acid sequence of the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of a Heavy Chain Variable (VH) Domain of an optimized variant of **MAB-A**; and such CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain have the amino acid sequence of the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of a Light Chain Variable (VL) Domain of an optimized variant of **MAB-A**.

[0014] The invention particularly concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding domain possesses:

(A) (1) the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of the Heavy Chain Variable (VH) Domain of **MAB-A**; and

(2) the FR1, FR2, FR3 and FR4 of a VH Domain of a humanized variant of **MAB-A**; or

(B) (1) the CDR_L1 Domain, CDR_L2 Domain and CDR_L3 Domain of the Light Chain Variable (VL) Domain **MAB-A**; and

(2) the FR1, FR2, FR3 and FR4 of a VL Domain of a humanized variant of **MAB-A**; or

(C) (1) the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of a Heavy Chain Variable (VH) Domain of an optimized variant of **MAB-A**; and

(2) the FR1, FR2, FR3 and FR4 of the VH Domain of a humanized variant of **MAB-A**; or

(D) (1) the CDR_L1 Domain, CDR_L2 Domain and CDR_L3 Domain of a Light Chain Variable (VL) Domain of an optimized variant of **MAB-A**; and

(2) the FR1, FR2, FR3 and FR4 of the VL Domain of a humanized variant of **MAB-A**; or

(E) (1) the Heavy Chain Variable (VH) Domain of a humanized/optimized variant of **MAB-A**; and

(2) the VL Light Chain Variable (VL) Domain of a humanized/optimized variant of **MAB-A**.

[0015] The invention additionally concerns the embodiment of all such ADAM9-binding molecules, wherein such Heavy Chain Variable (VH) Domain of such optimized variant of **MAB-A** comprises the amino acid sequence of **SEQ ID NO:15**:

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWX_1H**WVRQA
PGKGLEWVG**E** **IIPIX_2GHTNY** **NEX_3F$X_4$$X_5$**RFTI SLDNSKNTLY
LQMGSLRAED TAVYYCAR**GG** **YYYY$X_6$$X_7$$X_8$$X_9$$X_{10}$$X_{11}$**
DYWGQGTTVT VSS

wherein: X_1, X_2, X_3, X_4, X_5, and X_6 are independently selected,

wherein: X_1 is M or I; X_2 is N or F;

X_3 is K or R; X_4 is K or Q;

X_5 is S or G, and X_6 is P, F, Y, W, I, L, V, T, G or D;

wherein: X_7, X_8, X_9, X_{10}, and X_{11} are selected such that:

when X_6 is P; X_7 is K or R; X_8 is F or M; X_9 is G; X_{10} is W or F; and X_{11} is M, L or K;

when X_6 is F, Y or W; X_7 is N or H; X_8 is S or K; X_9 is G or A; X_{10} is T or V; and X_{11} is M, L or K;

when X_6 is I, L or V; X_7 is G; X_8 is K; X_9 is G or A; X_{10} is V; and X_{11} is M, L or K;

when X_6 is T; X_7 is G; X_8 is K, M or N; X_9 is G; X_{10} is V or T; and X_{11} is L or M;

when X_6 is G; X_7 is G; X_8 is S; X_9 is G; X_{10} is V; and X_{11} is L;

when X_6 is D; X_7 is S; X_8 is N; X_9 is A; X_{10} is V; and X_{11} is L.

[0016] The invention additionally concerns the embodiment of all such ADAM9-binding molecules, wherein such CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of such Heavy Chain Variable (VH) Domain of such optimized variant of **MAB-A** respectively have the amino acid sequences of:

(1) **SEQ ID NO:47** ($SYWX_1H$)

wherein: X_1 is M or I;

(2) **SEQ ID NO:48** ($EIIPIX_2GHTNYNEX_3FX_4X_5$)

wherein: X_2, X_3, X_4, and X_5 are independently selected, and

wherein: X_2 is N or F; X_3 is K or R; X_4 is K or Q; and X_5 is S or G; and

(3) **SEQ ID NO:49** ($GGYYYYX_6X_7X_8X_9X_{10}X_{11}DY$)

wherein: X_6, is P, F, Y, W, I, L, V, T, G or D, and X_7, X_8, X_9, X_{10}, and X_{11} are selected such that:

(A) when X_6 is P:

X_7 is K or R; X_8 is F or M; X_9 is G;

X_{10} is W or F; and X_{11} is M, L or K;

(B) when X_6 is F, Y or W:

X_7 is N or H; X_8 is S or K; X_9 is G or A;

X_{10} is T or V; and X_{11} is M, L or K;

(C) when X_6 is I, L or V:

X_7 is G; X_8 is K; X_9 is G or A;

X_{10} is V; and X_{11} is M, L or K;

(D) when X_6 is T:

X_7 is G; X_8 is K, M or N; X_9 is G;

X_{10} is V or T; and X_{11} is L or M;

(E) when X_6 is G:

X_7 is G; X_8 is S; X_9 is G;

X_{10} is V; and X_{11} is L; and

(F) when X_6 is D:

X_7 is S; X_8 is N; X_9 is A;

X_{10} is V; and X_{11} is L.

[0017] The invention additionally concerns the embodiment of all such ADAM9-binding molecules, wherein such Heavy Chain Variable (VH) Domain of such optimized variant of **MAB-A** is selected from the group consisting of:

(1) **hMAB-A VH(1) (SEQ ID NO:16)**;

(2) **hMAB-A VH(2) (SEQ ID NO:17)**;

(3) **hMAB-A VH(3) (SEQ ID NO:18)**;

(4) **hMAB-A VH(4) (SEQ ID NO:19)**;

(5) **hMAB-A VH(2A) (SEQ ID NO:20)**;

(6) **hMAB-A VH(2B) (SEQ ID NO:21)**;

(7) **hMAB-A VH(2C) (SEQ ID NO:22)**;

(8) **hMAB-A VH(2D) (SEQ ID NO:23)**;

(9) **hMAB-A VH(2E) (SEQ ID NO:24)**;

(10) **hMAB-A VH(2F) (SEQ ID NO:25)**;

(11) **hMAB-A VH(2G) (SEQ ID NO:26)**;

(12) **hMAB-A VH(2H) (SEQ ID NO:27)**;

(13) **hMAB-A VH(2I) (SEQ ID NO:28)**; and

(14) **hMAB-A VH(2J) (SEQ ID NO:29)**.

[0018] The invention additionally concerns the embodiment of all such ADAM9-binding molecules, wherein such Light Chain Variable (VL) Domain comprises the amino acid sequence of **SEQ ID NO:53**:

DIVMTQSPDS LAVSLGERAT ISC**X_{12}ASQSVD**
YX_{13}GDSYX_{14}NWY QQKPGQPPKL LIY**AASDLES**
GIPARFSGSG SGTDFTLTIS SLEPEDFATY
YC**QQSX$_{15}$X$_{16}$X$_{17}$PF** **T**FGQGTKLEI K

wherein: **X_{12}**, **X_{13}**, **X_{14}**, **X_{15}**, **X_{16}**, and **X_{17}**, are independently selected, and

wherein: **X_{12}** is K or R; **X_{13}** is D or S;

X_{14} is M or L; **X_{15}** is H or Y;

X_{16} is E or S; and **X_{17}** is D or T.

[0019] The invention additionally concerns the embodiment of all such ADAM9-binding molecules, wherein such CDR_L1 Domain, CDR_L2 Domain and CDR_L3 Domain of such Light Chain Variable (VL) Domain of such optimized variant of **MAB-A** respectively have the amino acid sequences of:

(1) **SEQ ID NO:66** (X_{12}ASQSVDYX$_{13}$GDSYX$_{14}$N)

wherein: $\mathbf{X_{12}}$, $\mathbf{X_{13}}$, $\mathbf{X_{14}}$, are independently selected, and

wherein: $\mathbf{X_{12}}$ is K or R; $\mathbf{X_{13}}$ is D or S; and $\mathbf{X_{14}}$ is M or L;

(2) **SEQ ID NO:13** (AASDLES); and

(3) **SEQ ID NO:67** (QQSX$_{15}$X$_{16}$X$_{17}$PFT)

wherein: $\mathbf{X_{15}}$, $\mathbf{X_{16}}$, and $\mathbf{X_{17}}$, are independently selected, and

wherein: $\mathbf{X_{15}}$ is H or Y; $\mathbf{X_{16}}$ is E or S; and $\mathbf{X_{17}}$ is D or T.

[0020] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such Light Chain Variable (VL) Domain of such optimized variant of **MAB-A** is selected from the group consisting of:

(1) **hMAB-A VL(1) (SEQ ID NO:54)**;

(2) **hMAB-A VL(2) (SEQ ID NO:55)**;

(3) **hMAB-A VL(3) (SEQ ID NO:56)**;

(4) **hMAB-A VL(4) (SEQ ID NO:57)**;

(5) **hMAB-A VL(2A) (SEQ ID NO:20)**.

[0021] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein the ADAM9-binding domain comprises:

(A) (1) a CDR_H1 Domain that comprises the amino acid sequence SYWMH (**SEQ ID NO:8**);

(2) a CDR_H2 Domain that comprises the amino acid sequence EIIPIFGHTNYNEKFKS (**SEQ ID NO:35**); or

(3) a CDR_H3 Domain that comprises the amino acid sequence GGYYYYPRQGFLDY (**SEQ ID NO:45**);

or

(B) (1) a CDR_L1 Domain that comprises the amino acid sequence KASQSVDYSGDSYMN (**SEQ ID NO:62**);

(2) a CDR_L2 Domain that comprises the amino acid sequence AASDLES (**SEQ ID NO:13**); or

(3) a CDR_L3 Domain that comprises the amino acid sequence QQSHEDPFT (**SEQ ID NO:14**);

[0022] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein the ADAM9-binding domain comprises the CDR_H1 Domain that comprises the amino acid sequence SYWMH (**SEQ ID NO:8**), the CDR_H2 Domain that comprises the amino acid sequence EIIPIFGHTNYNEKFKS (**SEQ ID NO:35**), and the CDR_H3 Domain that comprises the amino acid sequence GGYYYYPRQGFLDY (**SEQ ID NO:45**).

[0023] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein the ADAM9-binding domain comprises the CDR_L1 Domain that comprises the amino acid sequence KASQSVDYSGDSYMN (**SEQ ID NO:62**), the CDR_L2 Domain that comprises the amino acid sequence AASDLES (**SEQ ID NO:13**), and the CDR_L3 Domain that cpomprises the amino acid sequence QQSHEDPFT (**SEQ ID NO:14**).

[0024] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding domain comprises:

(A) the Heavy Chain Variable (VH) Domain of **hMAB-A (2I.2) (SEQ ID NO:28)**; or

(B) the Light Chain Variable (VL) Domain of **hMAB-A (2I.2) (SEQ ID NO:55)**; or

(C) the Heavy Chain Variable (VH) Domain of **hMAB-A (2I.2) (SEQ ID NO:28)** and the Light Chain Variable (VL) Domain of **hMAB-A (2I.2) (SEQ ID NO:55)**.

[0025] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding domain comprises a CDR_H1 domain, a CDR_H2 domain, and a CDR_H3 domain and a CDR_L1 domain, a CDR_L2 domain, and a CDR_L3 domain having the sequences selected from the group consisting of:

(a) **SEQ ID NOs:8**, **35** and **10** and **SEQ ID NOs:62**, **13** and **14**, respectively

(b) **SEQ ID NOs:8**, **35** and **10** and **SEQ ID NOs:63**, **13** and **14**, respectively;

(c) **SEQ ID NOs:8**, **36** and **10** and **SEQ ID NOs:63**, **13** and **14**, respectively; and

(d) **SEQ ID NOs:34**, **36** and **10** and **SEQ ID NO:64**, **13** and **65**, respectively.

[0026] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding domain comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) having sequences that are at least 90%, at least 95%, or at least 99% identical to sequences selected from the group consisting of:

(a) **SEQ ID NO:17** and **SEQ ID NO:55**, respectively;

(b) **SEQ ID NO:17** and **SEQ ID NO:56**, respectively;

(c) **SEQ ID NO:18** and **SEQ ID NO:56**, respectively; and

(d) **SEQ ID NO:19** and **SEQ ID NO:57**, respectively.

[0027] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding domain comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) having the sequences selected from the group consisting of:

(a) **SEQ ID NO:17** and **SEQ ID NO:55**, respectively;

(b) **SEQ ID NO:17** and **SEQ ID NO:56**, respectively;

(c) **SEQ ID NO:18** and **SEQ ID NO:56**, respectively; and

(d) **SEQ ID NO:19** and **SEQ ID NO:57**, respectively.

[0028] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding domain has at least a 150-fold enhancement in binding affinity to cyno ADAM9 and retains high affinity binding to human ADAM9 as compared to **MAB-A**.

[0029] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding domain comprises a CDR_H1 domain, a CDR_H2 domain, and a CDR_H3 domain and a CDR_L1 domain, a CDR_L2 domain, and a CDR_L3 domain having the sequences selected from the group consisting of:

(a) **SEQ ID NOs:8**, **35** and **37** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(b) **SEQ ID NOs:8**, **35** and **38** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(c) **SEQ ID NOs:8**, **35** and **39** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(d) **SEQ ID NOs:8**, **35** and **40** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(e) **SEQ ID NOs:8**, **35** and **41** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(f) **SEQ ID NOs:8**, **35** and **42** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(g) **SEQ ID NOs:8**, **35** and **43** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(h) **SEQ ID NOs:8**, **35** and **44** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(i) **SEQ ID NOs:8**, **35** and **45** and **SEQ ID NOs:62**, **13** and **14**, respectively; and

(j) **SEQ ID NOs:8**, **35** and **46** and **SEQ ID NOs:62**, **13** and **14**, respectively.

[0030] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding domain comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) having sequences that are at least 90%, at least 95%, or at least 99% identical to sequences selected from the group consisting of:

(a) **SEQ ID NO:20** and **SEQ ID NO:55**, respectively;

(b) **SEQ ID NO:21** and **SEQ ID NO:55**, respectively;

(c) **SEQ ID NO:22** and **SEQ ID NO:55**, respectively;

(d) **SEQ ID NO:23** and **SEQ ID NO:55**, respectively;

(e) **SEQ ID NO:24** and **SEQ ID NO:55**, respectively;

(f) **SEQ ID NO:25** and **SEQ ID NO:55**, respectively;

(g) **SEQ ID NO:26** and **SEQ ID NO:55**, respectively;

(h) **SEQ ID NO:27** and **SEQ ID NO:55**, respectively;

(i) **SEQ ID NO:28** and **SEQ ID NO:55**, respectively; and

(j) **SEQ ID NO:29** and **SEQ ID NO:55**, respectively.

[0031] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding domain comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) having the sequences selected from the group consisting of:

(a) **SEQ ID NO:20** and **SEQ ID NO:55**, respectively;

(b) **SEQ ID NO:21** and **SEQ ID NO:55**, respectively;

(c) **SEQ ID NO:22** and **SEQ ID NO:55**, respectively;

(d) **SEQ ID NO:23** and **SEQ ID NO:55**, respectively;

(e) **SEQ ID NO:24** and **SEQ ID NO:55**, respectively;

(f) **SEQ ID NO:25** and **SEQ ID NO:55**, respectively;

(g) **SEQ ID NO:26** and **SEQ ID NO:55**, respectively;

(h) **SEQ ID NO:27** and **SEQ ID NO:55**, respectively;

(i) **SEQ ID NO:28** and **SEQ ID NO:55**, respectively; and

(j) **SEQ ID NO:29** and **SEQ ID NO:55**, respectively.

[0032] The invention additionally concerns the embodiment of all such ADAM9-binding molecules, wherein such molecule is a monospecific ADAM9-binding antibody or an ADAM9-binding fragment thereof, or wherein such molecule is a bispecific antibody.

[0033] The invention additionally concerns the embodiment of all such ADAM9-binding molecules, wherein such molecule is a diabody, such diabody being a covalently bonded complex that comprises two, three, four or five polypeptide chains.

[0034] The invention additionally concerns the embodiment of all such ADAM9-binding molecules, wherein such molecule is a trivalent binding molecule, such trivalent binding molecule being a covalently bonded complex that comprises three, four, five, or more polypeptide chains.

[0035] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such molecule comprises an Albumin-Binding Domain (ABD).

[0036] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such molecule comprises an Fc Region, and particularly the embodiment wherein such Fc Region is a variant Fc Region that comprises:

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

(b) one or more amino acid modification(s) that enhance(s) the serum half-life of such ADAM9-binding molecule.

[0037] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such one or more amino acid modification(s) that reduce(s) the affinity of the variant Fc Region for an FcγR comprise:

(A) L234A;

(B) L235A; or

(C) L234A and L235A;

wherein such numbering is that of the EU index as in Kabat.

[0038] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such one or more amino acid modification(s) that that enhance(s) the serum half-life of such ADAM9-binding molecule comprise:

(A) M252Y;

(B) M252Y and S254T;

(C) M252Y and T256E;

(D) M252Y, S254T and T256E; or

(E) K288D and H435K;

wherein such numbering is that of the EU index as in Kabat.

[0039] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such molecule is bispecific and comprises an epitope-binding site capable of immunospecific binding to an epitope of ADAM9 and an epitope-binding site capable of immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

[0040] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such molecule comprises two epitope-binding sites capable of immunospecific binding to epitope(s) of ADAM9 and two epitope-binding sites capable of immunospecific binding to epitope(s) of a molecule present on the surface of an effector cell.

[0041] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such molecule is trispecific and comprises:

(a) one epitope-binding site capable of immunospecific binding to an epitope of ADAM9;

(b) one epitope-binding site capable of immunospecific binding to an epitope of a first molecule present on the surface of an effector cell; and

(c) one epitope-binding site capable of immunospecific binding to an epitope of a second molecule present on the surface of an effector cell.

[0042] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such molecule is capable of simultaneously binding to ADAM9 and such molecule present on the surface of an effector cell.

[0043] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such molecule present on the surface of an effector cell is CD2, CD3, CD8, TCR, or NKG2D.

[0044] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such effector cell is a cytotoxic T-cell or a Natural Killer (NK) cell.

[0045] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such first molecule present on the surface of an effector cell is CD3 and such second molecule present on the surface of an effector cell is CD8

[0046] The invention additionally concerns the embodiment of such ADAM9-binding molecules, wherein such ADAM9-binding molecule mediates coordinated binding of a cell expressing ADAM9 and a cytotoxic T cell.

[0047] The invention additionally concerns a pharmaceutical composition that comprises an effective amount of any of the above-described ADAM9-binding molecules and a pharmaceutically acceptable carrier, excipient or diluent.

[0048] The invention additionally concerns the use of any of the above-described ADAM9-binding molecules, or the use of the above-described pharmaceutical composition in the treatment of a disease or condition associated with, or characterized by, the expression of ADAM9.

[0049] The invention particularly concerns such use wherein such disease or condition associated with, or characterized by, the expression of ADAM9 is cancer, and especially wherein such cancer is selected from the group consisting: bladder cancer, breast cancer, cervical cancer, colorectal cancer (especially an adenocarcinoma, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma, or squamous cell carcinoma), esophageal cancer, gastric cancer, head and neck cancer, liver cancer, non-small-cell lung cancer (especially a squamous cell carcinoma, adenocarcinoma, or large-cell undifferentiated carcinoma), myeloid cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, thyroid cancer, testicular cancer, and uterine cancer.

[0050] The invention additionally concerns a method for treating a disease or condition associated with, or characterized by, the expression of ADAM9 in a subject comprising administering to such subject an effective amount of any of the above-described ADAM9-binding molecules, or any of the above-described pharmaceutical compositions.

[0051] The invention particularly concerns such method wherein such disease or condition associated with, or characterized by, the expression of ADAM9 is cancer, and especially wherein such cancer is selected from the group consisting: bladder cancer, breast cancer, cervical cancer, colorectal cancer (especially an adenocarcinoma, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma, or squamous cell carcinoma), esophageal cancer, gastric cancer, head and neck cancer, liver cancer, non-small-cell lung cancer (especially a squamous cell carcinoma, adenocarcinoma, or large-cell undifferentiated carcinoma), myeloid cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, thyroid cancer, testicular cancer, and uterine cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] **Figure 1** provides a schematic of a representative covalently bonded diabody having two epitope-binding sites composed of two polypeptide chains, each having an E-coil or K-coil Heterodimer-Promoting Domain (alternative Heterodimer-Promoting Domains are provided below). A cysteine residue may be present in a linker and/or in the Heterodimer-Promoting Domain as shown in **Figure 3B**. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0053] **Figure 2** provides a schematic of a representative covalently bonded diabody molecule having two epitope-binding sites composed of two polypeptide chains, each having a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0054] **Figures 3A-3C** provide schematics showing representative covalently bonded tetravalent diabodies having four epitope-binding sites composed of two pairs of polypeptide chains (*i.e.*, four polypeptide chains in all). One polypeptide of each pair possesses a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern. The two pairs of polypeptide chains may be same. In such embodiments wherein the two pairs of polypeptide chains are the same and the VL and VH Domains recognize different epitopes (as shown in **Figures 3A-3B**), the resulting molecule possesses four epitope-binding sites and is bispecific and bivalent with respect to each bound epitope. In such embodiments wherein the VL and VH Domains recognize the same

epitope (*e.g.*, the same VL Domain CDRs and the same VH Domain CDRs are used on both chains) the resulting molecule possesses four epitope-binding sites and is monospecific and tetravalent with respect to a single epitope. Alternatively, the two pairs of polypeptides may be different. In such embodiments wherein the two pairs of polypeptide chains are different and the VL and VH Domains of each pair of polypeptides recognize different epitopes (as shown by the different shading and patterns in **Figure 3C**), the resulting molecule possesses four epitope-binding sites and is tetraspecific and monovalent with respect to each bound epitope. **Figure 3A** shows an Fc Region-containing diabody which contains a peptide Heterodimer-Promoting Domain comprising a cysteine residue. **Figure 3B** shows an Fc Region-containing diabody, which contains E-coil and K-coil Heterodimer-Promoting Domains comprising a cysteine residue and a linker (with an optional cysteine residue). **Figure 3C**, shows an Fc-Region-Containing diabody, which contains antibody CH1 and CL domains.

[0055] **Figures 4A-4B** provide schematics of a representative covalently bonded diabody molecule having two epitope-binding sites composed of three polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form all or part of an Fc Region. The polypeptide chains comprising the VL and VH Domain further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0056] **Figure 5** provides the schematics of a representative covalently bonded diabody molecule having four epitope-binding sites composed of five polypeptide chains. Two of the polypeptide chains possess a CH2 and CH3 Domain, such that the associated chains form an Fc Region that comprises all or part of an Fc Region. The polypeptide chains comprising the linked VL and VH Domains further comprise a Heterodimer-Promoting Domain. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0057] **Figures 6A-6F** provide schematics of representative Fc Region-containing trivalent binding molecules having three epitope-binding sites. **Figures 6A** and **6B**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains and a Fab-type binding domain having different domain orientations in which the diabody-type binding domains are N-terminal or C-terminal to an Fc Region. The molecules in **Figures 6A** and **6B** comprise four chains. **Figures 6C** and

6D, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains N-terminal to an Fc Region, and a Fab-type binding domain in which the light chain and heavy chain are linked via a polypeptide spacer, or an scFv-type binding domain. The trivalent binding molecules in **Figures 6E** and **6F**, respectively, illustrate schematically the domains of trivalent binding molecules comprising two diabody-type binding domains C-terminal to an Fc Region, and a Fab-type binding domain in which the light chain and heavy chain are linked via a polypeptide spacer, or an scFv-type binding domain. The trivalent binding molecules in **Figures 6C-6F** comprise three chains. VL and VH Domains that recognize the same epitope are shown using the same shading or fill pattern.

[0058] **Figures 7A-7C** present the results of an immunohistochemistry (IHC) studies and show the ability of **MAB-A** to specifically label a variety of non-small cell lung cancer types (**Figure 7A, Panels 1-8**), breast cancer cells, prostate cancer cells, gastric cancer cells (**Figure 7B, Panels 1-6**), and colon cancer cells (**Figure 7C, Panels 1-8**) while the isotype control failed to specifically label any of these cancer cell types (**Figures 7A-7C**).

[0059] **Figures 8A-8B** present the results of cell staining studies and show that **MAB-A** binds to human ADAM9, and to a lesser extent, cynomolgus monkey ADAM9, transiently expressed on the surface of 293-FT and CHO-K cells (**Figure 8A** and **Figure 8B**, respectively).

[0060] **Figures 9A-9B** depict the amino acid sequences of the murine anti-ADAM9-VH Domain aligned with several humanized/optimized variants of **MAB-A** (**Figure 9A, SEQ ID NOs:7, 16, 17, 18, 19, 21, 22, 23** and **28**) and the murine anti-ADAM9-VL Domain aligned with several humanized/optimized variants of **MAB-A** (**Figure 9B, SEQ ID NOs:11, 51, 52, 53** and **54**). Positions substituted within the CDRs during the initial optimization are underlined as follows: potential deamidation and isomeration sites are indicated with a single underline, lysine residues are indicated with double underline, additional labile residues are indicated with a double dashed underline.

[0061] **Figures 10A-10B** present the ELISA binding curves of the ten selected optimized h**MAB-A** clones comprising CDR_H3 variants, the parental h**MAB-A** (2.2), and an isotype control antibody. **Figure 10A** presents the binding curves for cynoADAM9 and **Figure 10B** presents the binding curves for huADAM9.

DETAILED DESCRIPTION OF THE INVENTION

[0062] The present invention is directed to molecules, such as monospecific antibodies and bispecific, trispecific or multispecific binding molecules, including diabodies, BiTEs, and antibodies that are capable of specifically binding to "Disintegrin and Metalloproteinase Domain-containing Protein 9" ("ADAM9"). The invention particularly concerns such binding molecules that are capable of exhibiting high affinity binding to human and non-human ADAM9. The invention further particularly relates to such molecules that are thereby cross-reactive with human ADAM9 and the ADAM9 of a non-human primate (*e.g.*, a cynomolgus monkey). The invention additionally pertains to all such ADAM9-binding molecules that comprise a Light Chain Variable (VL) Domain and/or a Heavy Chain Variable (VH) Domain that has been humanized and/or deimmunized so as to exhibit reduced immunogenicity upon administration of such ADAM9-binding molecule to a recipient subject. The invention is also directed to pharmaceutical compositions that contain any of such ADAM9-binding molecules, and to methods involving the use of any of such ADAM9-binding molecules in the treatment of cancer and other diseases and conditions.

I. Antibodies and Their Binding Domains

[0063] 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. 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. The last few decades have seen a revival of interest in the therapeutic potential of antibodies, and antibodies have become one of the leading classes of biotechnology-derived drugs (Chan, C.E. *et al.* (2009) "*The Use Of Antibodies In The*

Treatment Of Infectious Diseases," Singapore Med. J. 50(7):663-666). In addition to their use in diagnostics, antibodies have been shown to be useful as therapeutic agents. Over 200 antibody-based drugs have been approved for use or are under development.

[0064] Antibodies are capable of "**immunospecifically binding**" to a polypeptide or protein or a non-protein molecule 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**." 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 that viral epitope with greater affinity, avidity, more readily, and/or with greater duration than it immunospecifically binds to other viral epitopes or to 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" to a particular epitope does not necessarily require (although it can include) exclusive binding to that epitope. 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.

[0065] 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), single-chain (scFv), 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. The term 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. Cells used for immunization may be cultured for a period of time (*e.g.*, at least 24 hours) prior to their use as an immunogen. Cells may be used as immunogens by themselves or in combination with a non-denaturing adjuvant, such as Ribi (see, *e.g.*, Jennings, V.M. (1995) "*Review of Selected Adjuvants Used in Antibody Production*," ILAR J. 37(3):119-125). In general, cells should be kept intact and preferably viable when used as immunogens. Intact cells may allow antigens to be better detected than ruptured cells by the immunized animal. Use of denaturing or harsh adjuvants, *e.g.*, Freund's adjuvant, may rupture cells and therefore is discouraged. The immunogen may be administered multiple times at periodic intervals such as, bi weekly, or weekly, or may be administered in such a way as to maintain viability in the animal (*e.g.*, in a tissue recombinant). 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 embodiment, 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 of the invention 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.

[0066] Natural antibodies (such as natural 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 Region. 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). The Variable Domains of an IgG molecule consist of 1, 2, and most commonly 3, complementarity determining regions ("**CDR**", *i.e.*, **CDR1**, **CDR2** and **CDR3**, respectively), which contain the residues in contact with epitope, and non-CDR segments, referred to as **framework regions** ("**FR**"), which in general maintain the structure and determine the positioning of the CDR regions so as to permit such contacting (although certain framework residues may also contact the epitope). Thus, the VL and VH Domains typically have the structure: **n-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4-c** (where "n" denotes the N-terminus and "c" denotes the C-terminus). Polypeptides that are (or may serve as) the first, second, third, and fourth FR of the Light Chain of an antibody are herein respectively designated as: **FR_L1 Domain**, **FR_L2 Domain**, **FR_L3 Domain**, and **FR_L4 Domain**. Similarly, polypeptides that are (or may serve as) the first, second, third and fourth FR of the Heavy Chain of an antibody are herein respectively designated as: **FR_H1 Domain**, **FR_H2 Domain**, **FR_H3 Domain** and **FR_H4 Domain**. 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.

[0067] Accordingly, as used herein, the term "**epitope-binding fragment**" means a fragment of an antibody capable of immunospecifically binding to an epitope, and the term "**epitope-binding site**" refers to a portion of a molecule comprising an epitope-binding fragment. 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.

[0068] The invention particularly encompasses single-chain Variable Domain fragments ("**scFv**") comprising an anti-ADAM9-VL and/or VH Domain of the invention as well as multispecific binding molecules comprising such anti-ADAM9-VL and/or VH Domains. 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.

[0069] The invention also particularly encompasses the CDR_H1, CDR_H2, CDR_H3, CDR_L1, CDR_L2, and CDR_L3 Domains of humanized variants of the anti-ADAM9

antibodies of the invention, as well as VL Domains that contain any 1, 2, or 3 of such CDR_{LS} and VH Domains that contain any 1, 2, or 3 of such CDR_{HS}, as well as multispecific-binding molecules comprising the same. The term "**humanized**" antibody refers to a chimeric molecule having an epitope-binding site of an immunoglobulin from a non-human species and a remaining immunoglobulin structure that is based upon the structure and /or sequence of a human immunoglobulin. Humanized antibodies are generally prepared using recombinant techniques. The anti-ADAM9 antibodies of the present invention include humanized, chimeric or caninized variants of an antibody that is designated herein as "**MAB-A**." The polynucleotide sequences that encode the Variable Domains of **MAB-A** may be used for genetic manipulation to generate **MAB-A** derivatives possessing improved or altered characteristics (*e.g.*, affinity, cross-reactivity, specificity, *etc.*). 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) employing the actual humanizing or caninizing methodologies/techniques; and (4) transfecting and expressing the humanized antibody. See, for example, U.S. Patent 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.

[0070] The epitope-binding site may comprise either a complete Variable Domain fused to one or more Constant Domains or only the CDRs of such Variable Domain grafted to appropriate framework regions. Epitope-binding sites may be wild-type or may be modified by one or more amino acid substitutions, insertions or deletions. Such action partially or completely eliminates the ability of the Constant Region to serve as an immunogen in recipients (*e.g.*, human individuals), however, the possibility of an immune

response to the foreign Variable Domain remains (LoBuglio, A.F. *et al.* (1989) "*Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response*," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but on modifying the Variable Domains as well so as to reshape them as closely as possible to a form found in human immunoglobulins. It is known that the Variable Domains of both the Heavy and Light Chains of antibodies contain three CDRs which vary in response to the antigens in question and determine binding capability, flanked by the four framework regions, 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" by grafting CDRs derived from non-human antibody on the FRs present in the human antibody to be modified. Application of this approach to various antibodies has been reported by Sato, K. *et al.* (1993) Cancer Res 53:851-856. Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," Nature 332:323-327; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; Kettleborough, C. A. *et al.* (1991) "*Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation*," Protein Engineering 4:773-3783; Maeda, H. *et al.* (1991) "*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*," Human Antibodies Hybridoma 2:124-134; Gorman, S. D. *et al.* (1991) "*Reshaping A Therapeutic CD4 Antibody*," Proc. Natl. Acad. Sci. (U.S.A.) 88:4181-4185; Tempest, P.R. *et al.* (1991) "*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*," Bio/Technology 9:266-271; Co, M. S. *et al.* (1991) "*Humanized Antibodies For Antiviral Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 88:2869-2873; Carter, P. *et al.* (1992) "*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*," Proc. Natl. Acad. Sci. (U.S.A.) 89:4285-4289; and Co, M.S. *et al.* (1992) "*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*," J. Immunol. 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized murine antibody which contains all six of the CDRs present in the murine antibody). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, or six) that differ in sequence relative to the CDRs of the original antibody.

[0071] 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, Winter *et al.* (1991) "*Man-made Antibodies*," Nature 349:293-299; 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; Shaw *et al.* (1987) "*Characterization Of A Mouse/Human Chimeric Monoclonal Antibody (17-1A) To A Colon Cancer Tumor-Associated Antigen*," J. Immunol. 138:4534-4538; and Brown *et al.* (1987) "*Tumor-Specific Genetically Engineered Murine/Human Chimeric Monoclonal Antibody*," Cancer Res. 47:3577-3583). 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; Verhoeyen, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," Science 239:1534-1536; 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. Patent Nos. 6,180,377; 6,054,297; 5,997,867; and 5,866,692.

II. Fcγ Receptors (FcγRs)

[0072] The CH2 and CH3 Domains of the two Heavy Chains of an antibody interact to form an "**Fc Region**," 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 Region" is used to define the C-terminal region of an IgG Heavy Chain that comprises the CH2 and CH3 Domains of that chain. An Fc Region 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.

[0073] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG1 is (**SEQ ID NO:1**):

```
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.

[0074] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG2 is (**SEQ ID NO:2**):

```
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.

[0075] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG3 is (**SEQ ID NO:3**):

```
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.

[0076] The amino acid sequence of the CH2-CH3 Domain of an exemplary human IgG4 is (**SEQ ID NO:4**):

```
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.

[0077] 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) ("**Kabat**"), expressly incorporated herein by reference. The term "**the EU index as set forth in Kabat**" refers to the numbering of the Constant Domains of human IgG1 EU

antibody provided in Kabat. 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 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 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.

[0078] 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 invention 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 in the ADAM9-binding molecules of the invention. Specifically encompassed by the instant invention are ADAM9-binding molecules lacking the C-terminal residue of the CH3 Domain. Also specifically encompassed by the instant invention are such constructs comprising the C-terminal lysine residue of the CH3 Domain.

[0079] As stated above, the Fc Region of natural IgG antibodies is capable of binding to cellular Fc gamma Receptors (FcγRs). Such binding results in the transduction of activating or inhibitory signals to the immune system. The ability of such binding to result in diametrically opposing functions reflects structural differences among the different FcγRs, and in particular reflects whether the bound FcγR possesses an Immunoreceptor Tyrosine-Based Activation Motif ("**ITAM**") or an Immunoreceptor Tyrosine-Based Inhibitory Motif ("**ITIM**"). The recruitment of different cytoplasmic enzymes to these structures dictates the outcome of the FcγR-mediated cellular responses. ITAM-containing FcγRs include FcγRI, FcγRIIA, FcγRIIIA, and activate the immune system when bound to Fc Regions (*e.g.*, aggregated Fc Regions present in an immune complex). FcγRIIB is the only currently known natural ITIM-containing FcγR; it acts to dampen or inhibit the immune system when bound to aggregated Fc Regions. Human neutrophils express the FcγRIIA gene. FcγRIIA clustering via immune complexes or specific antibody cross-linking serves to aggregate ITAMs with receptor-associated kinases which facilitate ITAM phosphorylation. ITAM phosphorylation serves as a docking site for Syk kinase, the activation of which results in the activation of downstream substrates (*e.g.*, PI_3K). Cellular activation leads to release of pro-inflammatory mediators. The FcγRIIB gene is expressed on B lymphocytes; its extracellular domain is 96% identical to FcγRIIA and binds IgG complexes in an indistinguishable manner. The presence of an ITIM in the cytoplasmic domain of FcγRIIB defines this inhibitory subclass of FcγR. Recently the molecular basis of this inhibition was established. When co-ligated along with an activating FcγR, the ITIM in FcγRIIB becomes phosphorylated and attracts the SH2 domain of the inositol polyphosphate 5'-phosphatase (SHIP), which hydrolyzes phosphoinositol messengers released as a consequence of ITAM-containing FcγR- mediated tyrosine kinase activation, consequently preventing the influx of intracellular Ca^{++}. Thus, cross-linking of FcγRIIB dampens the activating response to FcγR ligation and inhibits cellular responsiveness. B-cell activation, B-cell proliferation and antibody secretion is thus aborted.

III. Bispecific Antibodies, Multispecific Diabodies and DART® Diabodies

[0080] The ability of an antibody to bind an epitope of an antigen depends upon the presence and amino acid sequence of the antibody's VL and VH Domains. Interaction of an antibody's Light Chain and Heavy Chain and, in particular, interaction of its VL and VH Domains forms one of the two epitope-binding sites of a natural antibody, such as an IgG. Natural antibodies are capable of binding to only one epitope species (*i.e.*, they are

monospecific), although they can bind multiple copies of that epitope species (*i.e.*, exhibiting bivalency or multivalency).

[0081] The functionality of antibodies can be enhanced by generating multispecific antibody-based molecules that can simultaneously bind two separate and distinct antigens (or different epitopes of the same antigen) and/or by generating antibody-based molecule having higher valency (i.e., more than two binding sites) for the same epitope and/or antigen.

[0082] In order to provide molecules having greater capability than natural antibodies, a wide variety of recombinant bispecific antibody formats have been developed (see, *e.g.*, PCT Publication Nos. WO 2008/003116, WO 2009/132876, WO 2008/003103, WO 2007/146968, WO 2009/018386, WO 2012/009544, WO 2013/070565), most of which use linker peptides either to fuse a further epitope-binding fragment (*e.g.*, an scFv, VL, VH, *etc.*) to, or within the antibody core (IgA, IgD, IgE, IgG or IgM), or to fuse multiple epitope-binding fragments (*e.g.*, two Fab fragments or scFvs). Alternative formats use linker peptides to fuse an epitope-binding fragment (*e.g.*, an scFv, VL, VH, *etc.*) to a dimerization domain such as the CH2-CH3 Domain or alternative polypeptides (see, *e.g.*, PCT Publication Nos. WO 2005/070966, WO 2006/107786A WO 2006/107617A, WO 2007/046893). PCT Publication Nos. WO 2013/174873, WO 2011/133886 and WO 2010/136172 disclose a trispecific antibody in which the CL and CH1 Domains are switched from their respective natural positions and the VL and VH Domains have been diversified (see, *e.g.*, PCT Publication Nos. WO 2008/027236; WO 2010/108127) to allow them to bind to more than one antigen. PCT Publication Nos. WO 2013/163427 and WO 2013/119903 disclose modifying the CH2 Domain to contain a fusion protein adduct comprising a binding domain. PCT Publication Nos. WO 2010/028797, WO 2010/028796 and WO 2010/028795 disclose recombinant antibodies whose Fc Regions have been replaced with additional VL and VH Domains, so as to form trivalent binding molecules. PCT Publication Nos. WO 2003/025018 and WO 2003/012069 disclose recombinant diabodies whose individual chains contain scFv Domains. PCT Publication Nos. WO 2013/006544 discloses multivalent Fab molecules that are synthesized as a single polypeptide chain and then subjected to proteolysis to yield heterodimeric structures. PCT Publication Nos. WO 2014/022540, WO 2013/003652, WO 2012/162583, WO 2012/156430, WO 2011/086091, WO 2008/024188, WO 2007/024715, WO 2007/075270, WO 1998/002463, WO 1992/022583 and WO 1991/003493 disclose adding additional binding domains or

functional groups to an antibody or an antibody portion (*e.g.*, adding a diabody to the antibody's light chain, or adding additional VL and VH Domains to the antibody's light and heavy chains, or adding a heterologous fusion protein or chaining multiple Fab Domains to one another).

[0083] The design of a diabody is based on the antibody derivative known as a single-chain Variable Domain fragment (**scFv**). Such molecules are made by linking Light and/ or Heavy Chain Variable Domains using a short linking peptide. Bird, R.E. *et al.* (1988) ("*Single-Chain Antigen-Binding Proteins*," Science 242:423-426) describes examples of linking peptides which bridge approximately 3.5 nm between the carboxy terminus of one Variable Domain and the amino terminus of the other Variable Domain. Linkers of other sequences have been designed and used (Bird *et al.* (1988) "*Single-Chain Antigen-Binding Proteins*," Science 242:423-426). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. 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.

[0084] The art has noted the capability to produce diabodies that differ from such natural antibodies in being capable of binding two or more different epitope species (*i.e.*, exhibiting bispecificity or multispecificity in addition to bivalency or multivalency) (see, *e.g.*, Holliger, P. *et al.* (1993) "'*Diabodies': Small Bivalent And Bispecific Antibody Fragments,*" Proc. Natl. Acad. Sci. (U.S.A.) 90:6444-6448; US 2004/0058400 (Hollinger *et al.*); US 2004/0220388 / WO 02/02781 (Mertens *et al.*); Alt *et al.* (1999) FEBS Lett. 454(1-2):90-94; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672; WO 02/02781 (Mertens *et al.*); Olafsen, T. *et al.* (2004) "*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*," Protein Eng. Des. Sel. 17(1):21-27; Wu, A. *et al.* (2001) "*Multimerization*

Of A Chimeric Anti-CD20 Single Chain Fv-Fv Fusion Protein Is Mediated Through Variable Domain Exchange," Protein Engineering 14(2):1025-1033; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain*," Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Baeuerle, P.A. *et al.* (2009) "*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*," Cancer Res. 69(12):4941-4944).

[0085] The provision of bispecific binding molecules (*e.g.*, non-monospecific diabodies) provides a significant advantage over antibodies, including but not limited to, a "trans" binding capability sufficient to co-ligate and/or co-localize different cells that express different epitopes and/or a "cis" binding capability sufficient to co-ligate and/or co-localize different molecules expressed by the same cell. Bispecific binding molecules (*e.g.*, non-monospecific diabodies) thus have wide-ranging applications including therapy and immunodiagnosis. Bispecificity allows for great flexibility in the design and engineering of the diabody in various applications, providing enhanced avidity to multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) *"Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris,"* Protein Eng. 10:1221-1225).

[0086] The ability to produce bispecific diabodies has led to their use (in "**trans**") to co-ligate two cells together, for example, by co-ligating receptors that are present on the surface of different cells (*e.g.*, cross-linking cytotoxic T-cells to tumor cells) (Staerz *et al.* (1985) *"Hybrid Antibodies Can Target Sites For Attack By T Cells,"* Nature 314:628-631; Holliger *et al.* (1996) *"Specific Killing Of* Lymphoma *Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody,"* Protein Eng. 9:299-305; and Marvin *et al.* (2005) "*Recombinant Approaches To IgG-Like Bispecific Antibodies*," Acta Pharmacol. Sin. 26:649-658). Alternatively (or additionally), bispecific (or tri- or multispecific) diabodies can be used (in "**cis**") to co-ligate molecules, such as receptors, *etc.*, that are present on the surface of the same cell. Co-ligation of different cells and/or receptors is useful to modulate effector functions and/or immune cell signaling. Multispecific molecules (*e.g.*, bispecific diabodies)

comprising epitope-binding sites may be directed to a surface determinant of any immune cell such as CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*, which are expressed on T lymphocytes, Natural Killer (NK) cells, Antigen-Presenting Cells or other mononuclear cells. In particular, epitope-binding sites directed to a cell surface receptor that is present on immune effector cells, are useful in the generation of multispecific binding molecules capable of mediating redirected cell killing.

[0087] However, the above advantages come at a salient cost. The formation of such non-monospecific diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to monospecific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-monospecific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (*i.e.*, so as to prevent homodimerization) (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) *"Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications,"* Prot. Engr. Des. Sel. 17:21-27; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Domain,*" Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; and Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity,*" J. Biol. Chem. 280(20):19665-19672).

[0088] However, the art has recognized that bispecific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional

monomers (see, *e.g.*, Lu, D. *et al.* (2005) "*A Fully Human* Recombinant *IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[0089] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-monospecific diabodies, termed **DART®** diabodies; see, *e.g.*, US Patent Nos. 9,296,816 and 9,284,375 and US Patent Publication Nos. 2015/0175697; 2014/0255407; 2014/0099318; 2013/0295121; WO 2012/018687; WO 2012/162068; 2010/0174053; WO 2010/080538; 2009/0060910; 2007-0004909; European Patent Publication Nos. EP 2714079; EP 2601216; EP 2376109; EP 2158221; EP 1868650; and PCT Publication Nos. WO 2012/162068; WO 2012/018687; WO 2010/080538; WO 2006/113665; and Sloan, D.D. *et al.* (2015) "*Targeting HIV Reservoir in Infected CD4 T Cells by Dual-Affinity Re-targeting Molecules (DARTs) that Bind HIV Envelope and Recruit Cytotoxic T Cells*," PLoS Pathog. 11(11):e1005233. doi: 10.1371/journal.ppat.1005233; Al Hussaini, M. *et al.* (2015) "*Targeting CD123 In AML Using A T-Cell Directed Dual-Affinity Re-Targeting (DART®) Platform*," Blood pii: blood-2014-05-575704; Chichili, G.R. *et al.* (2015) "*A CD3xCD123 Bispecific DART For Redirecting Host T Cells To Myelogenous Leukemia: Preclinical Activity And Safety In Nonhuman Primates*," Sci. Transl. Med. 7(289):289ra82; Moore, P.A. *et al.* (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*," Blood 117(17):4542-4551; Veri, M.C. *et al.* (2010) "*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcgamma Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold*," Arthritis Rheum. 62(7):1933-1943; and Johnson, S. *et al.* (2010) "*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And in vivo B-Cell Depletion*," J. Mol. Biol. 399(3):436-449). Such diabodies comprise two or more covalently complexed polypeptides and involve engineering one or more cysteine residues into each of the employed polypeptide species that permit disulfide bonds to form and thereby covalently bond one or more pairs of such polypeptide chains to one another. For example, the addition of a cysteine residue to the C-terminus of such constructs has been shown to allow disulfide bonding between the involved polypeptide chains, stabilizing the resulting diabody without interfering with the diabody's binding characteristics. Such molecules can be made to be bispecific (or multispecific) and thus may be made to co-ligate two or more molecules. Such co-ligation permits one to provide an enhanced immunotherapy. Additionally, because the individual polypeptide

chains of such molecules form a covalently bonded complex, the molecules exhibit far greater stability than diabodies involving non-covalently bonded polypeptide chains.

[0090] Recently, trivalent and multivalent molecules incorporating two diabody-type binding domains and one non-diabody-type domain and an Fc Region have been described (see, *e.g.*, PCT Publication Nos. WO 2015/184207 and WO 2015/184203). Such binding molecules may be utilized to generate monospecific, bispecific or trispecific molecules. The ability to bind three different epitopes provides enhanced capabilities.

[0091] Alternative constructs are known in the art for applications where a tetravalent molecule is desirable but an Fc is not required, including, but not limited to, tetravalent tandem antibodies, also referred to as "**TandAbs**" (see, *e.g.* US Patent Publication Nos. 2005-0079170, 2007-0031436, 2010-0099853, 2011-020667 2013-0189263; European Patent Publication Nos. EP 1078004, EP 2371866, EP 2361936 and EP 1293514; PCT Publication Nos. WO 1999/057150, WO 2003/025018, and WO 2013/013700) which are formed by the homo-dimerization of two identical polypeptide chains, each possessing a VH1, VL2, VH2, and VL2 Domain.

IV. ADAM9

[0092] A representative human ADAM9 polypeptide (NCBI Sequence NP_003807, including a 28 amino acid residue signal sequence, shown underlined) has the amino acid sequence (**SEQ ID NO:5**):

MGSGARFPSG TLRVRWLLLL GLVGPVLGAA RPGFQQTSHL SSYEIITPWR
LTRERREAPR PYSKQVSYVI QAEGKEHIIH LERNKDLLPE DFVVYTYNKE
GTLITDHPNI QNHCHYRGYV EGVHNSSIAL SDCFGLRGLL HLENASYGIE
PLQNSSHFEH IIYRMDDVYK EPLKCGVSNK DIEKETAKDE EEEPPSMTQL
LRRRRAVLPQ TRYVELFIVV DKERYDMMGR NQTAVREEMI LLANYLDSMY
IMLNIRIVLV GLEIWTNGNL INIVGGAGDV LGNFVQWREK FLITRRRHDS
AQLVLKKGFG GTAGMAFVGT VCSRSHAGGI NVFGQITVET FASIVAHELG
HNLGMNHDDG RDCSCGAKSC IMNSGASGSR NFSSCSAEDF EKLTLNKGGN
CLLNIPKPDE AYSAPSCGNK LVDAGEECDC GTPKECELDP CCEGSTCKLK
SFAECAYGDC CKDCRFLPGG TLCRGKTSEC DVPEYCNGSS QFCQPDVFIQ
NGYPCQNNKA YCYNGMCQYY DAQCQVIFGS KAKAAPKDCF IEVNSKGDRF
GNCGFSGNEY KKCATGNALC GKLQCENVQE IPVFGIVPAI IQTPSRGTKC
WGVDFQLGSD VPDPGMVNEG TKCGAGKICR NFQCVDASVL NYDCDVQKKC
HGHGVCNSNK NCHCENGWAP PNCETKGYGG SVDSGPTYNE MNTALRDGLL
VFFFLIVPLI VCAIFIFIKR DQLWRSYFRK KRSQTYESDG KNQANPSRQP

GSVPRHVSPV TPPREVPIYA NRFAVPTYAA KQPQQFPSRP PPPQPKVSSQ
GNLIPARPAP APPLYSSLT

Of the 819 amino acid residues of ADAM9 (**SEQ ID NO:5**), residues 1-28 are a signal sequence, residues 29-697 are the Extracellular Domain, residues 698-718 are the Transmembrane Domain, and residues 719-819 are the Intracellular Domain. Three structural domains are located within the Extracellular Domain: a Reprolysin (M12B) Family Zinc Metalloprotease Domain (at approximately residues 212-406); a Disintegrin Domain (at approximately residues 423-497); and an EGF-like Domain (at approximately residues 644-697). A number of post-translational modifications and isoforms have been identified and the protein is proteolytically cleaved in the trans-Golgi network before it reaches the plasma membrane to generate a mature protein. The removal of the pro-domain occurs via cleavage at two different sites. Processed most likely by a pro-protein convertase such as furin, at the boundary between the pro-domain and the catalytic domain (Arg-205/Ala-206). An additional upstream cleavage pro-protein convertase site (Arg-56/Glu-57) has an important role in the activation of ADAM9.

[0093] A representative cynomolgus monkey ADAM9 polypeptide (NCBI Sequence XM_005563126.2, including a possible 28 amino acid residue signal sequence, shown underlined) has the amino acid sequence (**SEQ ID NO:6**):

MGSGVGSPSG TLRVRWLLLL CLVGPVLGAA RPGFQQTSHL SSYEIITPWR
LTRERREAPR PYSKQVSYLI QAEGKEHIIH LERNKDLLPE DFVVYTYNKE
GTVITDHPNI QNHCHFRGYV EGVYNSSVAL SNCFGLRGLL HLENASYGIE
PLQNSSHFEH IIYRMDDVHK EPLKCGVSNK DIEKETTKDE EEEPPSMTQL
LRRRRAVLPQ TRYVELFIVV DKERYDMMGR NQTAVREEMI LLANYLDSMY
IMLNIRIVLV GLEIWTNGNL INIAGGAGDV LGNFVQWREK FLITRRRHDS
AQLVLKKGFG GTAGMAFVGT VCSRSHAGGI NVFGHITVET FASIVAHELG
HNLGMNHDDG RDCSCGAKSC IMNSGASGSR NFSSCSAEDF EKLTLNKGGN
CLLNIPKPDE AYSAPSCGNK LVDAGEECDC GTPKECELDP CCEGSTCKLK
SFAECAYGDC CKDCRFLPGG TLCRGKTSEC DVPEYCNGSS QFCQPDVFIQ
NGYPCQNNKA YCYNGMCQYY DAQCQVIFGS KAKAAPKDCF IEVNSKGDRF
GNCGFSGNEY KKCATGNALC GKLQCENVQE IPVFGIVPAI IQTPSRGTKC
WGVDFQLGSD VPDPGMVNEG TKCGADKICR NFQCVDASVL NYDCDIQKKC
HGHGVCNSNK NCHCENGWAP PNCETKGYGG SVDSGPTYNE MNTALRDGLL
VFFFLIVPLI VCAIFIFIKR DQLWRRYFRK KRSQTYESDG KNQANPSRQP
VSVPRHVSPV TPPREVPIYA NRFPVPTYAA KQPQQFPSRP PPPQPKVSSQ
GNLIPARPAP APPLYSSLT

The Reprolysin (M12B) Family Zinc Metalloprotease Domain of the protein is at approximately residues 212-406); the Disintegrin Domain of the protein is at approximately residues 423-497.

[0094] In certain embodiments, ADAM9-binding molecules of the invention (*e.g.*, scFvs, antibodies, bispecific diabodies, *etc.*) are characterized by any one, two, three, four, five, six, seven, or eight of the following criteria:

(1) the ability to immunospecifically bind human ADAM9 as endogenously expressed on the surface of a cancer cell;

(2) specifically binds human and non-human primate ADAM9 (*e.g.*, ADAM9 of cynomolgus monkey) with a similar binding affinity;

(3) specifically binds human ADAM9 with an equilibrium binding constant (K_D) of 4 nM or less;

(4) specifically binds non-human primate ADAM9 with an equilibrium binding constant (K_D) of 4 nM or less

(5) specifically binds human ADAM9 with an on rate (ka) of 5×10^5 $M^{-1}min^{-1}$ or more;

(6) specifically binds non-human primate ADAM9 with an on rate (ka) of 1×10^6 $M^{-1}min^{-1}$ or more;

(7) specifically binds human ADAM9 with an off rate (kd) of 1×10^{-3} min^{-1} or less;

(8) specifically binds non-human primate ADAM9 with an off rate (kd) of 9×10^{-4} min^{-1} or less;

(9) optimized to have at least 100-fold enhancement (*e.g.*, at least 100-fold, at least 150-fold, at least 200-fold, at least 250-fold, at least 300-fold, at least 350-fold, at least 400-fold, at least 450-fold, at least 500-fold, at least 550-fold, or at least 600-fold enhancement) in binding affinity (*e.g.*, as measured by BIACORE® analysis) to cyno ADAM9 and retains high affinity binding to human ADAM9 (*e.g.*, as measured by BIACORE® analysis) as compared to the chimeric or murine parental antibody.

[0095] As described herein, the binding constants of an ADAM9-binding molecule may be determined using surface plasmon resonance *e.g.*, via a BIACORE® analysis. Surface plasmon resonance data may be fitted to a 1:1 Langmuir binding model

(simultaneous ka kd) and an equilibrium binding constant K_D calculated from the ratio of rate constants kd/ka. Such binding constants may be determined for a monovalent ADAM9-binding molecule (*i.e.*, a molecule comprising a single ADAM9 epitope-binding site), a bivalent ADAM9-binding molecule (*i.e.*, a molecule comprising two ADAM9 epitope-binding sites), or ADAM9-binding molecules having higher valency (*e.g.*, a molecule comprising three, four, or more ADAM9 epitope-binding sites).

[0096] The present invention particularly encompasses ADAM9-binding molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, *etc.*) comprising anti-ADAM9 Light Chain Variable (VL) Domain(s) and anti-ADAM9 Heavy Chain Variable (VH) Domain(s) that immunospecifically bind to an epitope of a human ADAM9 polypeptide. Unless otherwise stated, all such ADAM9-binding molecules 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.

V. Murine Anti-Human ADAM9 Antibodies

[0097] A murine anti-ADAM9 antibody that blocks the target protein processing activity of ADAM9, is internalized and having anti-tumor activity was identified (see, *e.g.*, US Patent No. 8,361,475). This antibody, designated in US Patent Nos. 7,674,619 and 8,361,475 as an "anti-KID24" antibody produced by hybridoma clone ATCC PTA-5174, is designated herein as "**MAB-A**." **MAB-A** exhibits strong preferential binding to tumors over normal tissues (see, **Figures 7A-7C**). **MAB-A** exhibited little or no staining across a large panel of normal cell types (**Table 1**).

Table 1

Tissue	**MAB-A (1.25 µg/mL)**
Adrenal	Negative
Bladder	Negative
Bone Marrow	Negative
Breast	Negative
Cerebellum	Negative
Cerebrum	ND
Cervix	Negative

Table 1	
Tissue	**MAB-A (1.25 µg/mL)**
Colon	Negative
Esophagus	Smooth Muscle +/- to 1+ (gr c) <5%
Ovaduct	Negative
Heart	Negative
Kidney	Negative
Liver	Negative
Lung	Negative
Lymph Node	Negative
Ovary	Negative
Pancreas	Very rare (possible acinar) 1+ (c)
Parathyroid	Epithelium parenchymal cells 1+ (gr c) ,1% Cells (favor chief cells) 2+ (m,c)5% 1+ (m,c) 10% apical primarily
Pituitary	Posterior lobe cells (possibly neural cells and/or pituicytes 1+ (c>m) <5%
Placenta	Vascular lining cells within chorionic plate 1+ (gr c>m) Mesenchymal cells of chorionic plate 1-2+ (gr c) ,5%
Prostate	Glandular epithelium 2+ (gr c)5% and 1+ (gr c) 5%
Retina + Ciliary Body	Favor negative (pigmented epi layer 3-4+ (gr c) due to pigment not stained)
Submandibular Gland	Ductal epi +/- (c) 10%
Skeletal Muscle	Negative
Skin	Negative
Small Intestine	Negative
Spinal Cord	Neuropil 1+ (gr c) <1%
Spleen	Negative
Stomach	Negative
Testis	Seminiferous tubule 1+ (gr c) <5% Interstitial cells (possibly Leydig cells) 2-3+ (gr c) <5% and 1+ (gr c) 10%
Thyroid	Negative
Tonsil	Endo cells 2-3+ (c,m) <5% and 1+ (m,c) 15%

Table 1	
Tissue	**MAB-A (1.25 µg/mL)**
Ureter	Transitional epithelium 1+ (m,c) <5% and 1+ (m,c) 5%; Endo cells 1+ (c) <5%
Uterus	Negative
A498 Cell Pellet	2-3+ (m,c), 50%, 1+ (m,c) 45%

[0098] As shown in **Figures 8A-8B**, **MAB-A** binds human ADAM9 with high affinity, but binds non-human primate (*e.g.*, cynomolgus monkey) ADAM9 to a lesser extent.

[0099] The amino acid sequences of the VL and VH Domains of **MAB-A** are provided below. The VH and VL Domains of **MAB-A** were humanized and the CDRs optimized to improve affinity and/or to remove potential amino acid liabilities. The CDR_H3 was further optimized to enhance binding to non-human primate ADAM9 while maintaining its high affinity for human ADAM9.

[00100] The preferred anti-human ADAM9-binding molecules of the present invention possess the 1, 2 or all 3 of the CDR_Hs of a VH Domain and/or 1, 2 or all 3 of the CDR_Ls of the VL Domain of an optimized variant of **MAB-A**, and preferably further possess the humanized framework regions ("**FRs**") of the VH and/or VL Domains of humanized **MAB-A**. Other preferred anti-human ADAM9-binding molecules of the present invention possess the entire VH and/or VL Domains of a humanized/optimized variant of **MAB-A**. Such preferred anti-human ADAM9-binding molecules include antibodies, bispecific (or multispecific) antibodies, chimeric or humanized antibodies, BiTes, diabodies, *etc.*, as well as such binding molecules that additionally comprise a naturally occurring or a variant Fc Region.

[00101] The invention particularly relates to ADAM9-binding molecules comprising an ADAM9 binding domain that possess:

(A) (1) the three CDR_Hs of the VH Domain of **MAB-A**; and

(2) the four FRs of the VH Domain of a humanized variant of **MAB-A**; or

(B) (1) the three CDR_{L}s of the VL Domain of **MAB-A**; and

(2) the four FRs of the VL Domain of a humanized variant of **MAB-A**; or

(C) the three CDR_{H}s of the VH Domain of an optimized variant of **MAB-A**; and the three CDR_{L}s of the VL Domain of **MAB-A**; or

(D) the three CDR_{H}s of the VH Domain of **MAB-A**; and the three CDR_{L}s of the VL Domain of an optimized variant **MAB-A**; or

(E) the three CDR_{H}s of the VH Domain of an optimized variant of **MAB-A**; and the three CDR_{L}s of the VL Domain of an optimized **MAB-A**; or

(F) (1) the three CDR_{H}s of the VH Domain of an optimized variant of **MAB-A**; and

(2) the four FRs of the VH Domain of a humanized variant of **MAB-A**; or

(G) (1) the three CDR_{L}s of the VL Domain of an optimized variant of **MAB-A**; and

(2) the four FRs of the VL Domain of a humanized variant of **MAB-A**; or

(H) (1) the VH Domain of a humanized/optimized variant of **MAB-A**; and

(2) the VL Domain of a humanized/optimized variant of **MAB-A**. Murine Antibody "**MAB-A**"

[00102] The amino acid sequence of the VH Domain of the murine anti-ADAM9 antibody **MAB-A** is **SEQ ID NO:7** (the CDR_{H} residues are shown underlined):

QVQLQQPGAE LVKPGASVKL SCKASGYTFT **SYWMH**WVKQR PGQGLEWIG**E**
IIPINGHTNY NEKFKSKATL TLDKSSSTAY MQLSSLASED SAVYYCAR**GG**
YYYYGSRDYF DYWGQGTTLT VSS

[00103] The amino acid sequence of the **CDR_{H}1 Domain** of **MAB-A** is (**SEQ ID NO:8**): SYWMH.

[00104] The amino acid sequence of the **CDR_{H}2 Domain** of **MAB-A** is (**SEQ ID NO:9**): EIIPINGHTNYNEKFKS.

[00105] The amino acid sequence of the **CDR_{H}3 Domain** of **MAB-A** is (**SEQ ID NO:10**): GGYYYYGSRDYFDY.

[00106] The amino acid sequence of the VL Domain of the murine anti-ADAM9 antibody **MAB-A** is **SEQ ID NO:11** (the CDR_L residues are shown underlined):

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

[00107] The amino acid sequence of the **CDR_L1 Domain** of **MAB-A** is (**SEQ ID NO:12**): KASQSVDYDGDSYMN.

[00108] The amino acid sequence of the **CDR_L2 Domain** of **MAB-A** is (**SEQ ID NO:13**): AASDLES.

[00109] The amino acid sequence of the **CDR_L3 Domain** of **MAB-A** is (**SEQ ID NO:14**): QQSHEDPFT.

VI. Exemplary Humanized/Optimized Anti-ADAM9-VH and VL Domains

1. Variant VH Domains of MAB-A

[00110] The amino acid sequences of certain preferred humanized/optimized anti-ADAM9-VH Domains of **MAB-A** are variants of the ADAM9-VH Domain of **MAB-A** (**SEQ ID NO:7**) and are represented by **SEQ ID NO:15** (CDR_H residues are shown underlined):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWX_1H**WVRQA PGKGLEWVG**E** **IIPIX_2GHTNY** **NEX_3F$X_4$$X_5$**RFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG** **YYYY$X_6$$X_7$$X_8$$X_9$$X_{10}$$X_{11}$** **DY**WGQGTTVT VSS

wherein: X_1, X_2, X_3, X_4, X_5, and X_6 are independently selected,

wherein: X_1 is M or I; X_2 is N or F;

X_3 is K or R; X_4 is K or Q;

X_5 is S or G, and X_6 is P, F, Y, W, I, L, V, T, G or D;

wherein: X_7, X_8, X_9, X_{10}, and X_{11} are selected such that:

(A) when X_6 is P:
X_7 is K or R;
X_8 is F or M;
X_9 is G;
X_{10} is W or F; and
X_{11} is M, L or K;

(B) when X_6 is F, Y or W:
X_7 is N or H;
X_8 is S or K;
X_9 is G or A;
X_{10} is T or V; and
X_{11} is M, L or K;

(C) when X_6 is I, L or V:
X_7 is G;
X_8 is K;
X_9 is G or A;
X_{10} is V; and
X_{11} is M, L or K;

(D) when X_6 is T:
X_7 is G;
X_8 is K, M or N;
X_9 is G;
X_{10} is V or T; and
X_{11} is L or M;

(E) when X_6 is G:
X_7 is G;
X_8 is S;
X_9 is G;
X_{10} is V; and
X_{11} is L;

and

(F) when X_6 is D:
X_7 is S;
X_8 is N;
X_9 is A;
X_{10} is V; and
X_{11} is L.

[00111] The amino acid sequences of a preferred humanized anti-ADAM9 VH Domain of **MAB-A**: **hMAB-A VH(1) (SEQ ID NO:16)** and of the certain preferred humanized/optimized anti-ADAM9-VH Domains of **MAB-A**:

hMAB-A VH(2) (SEQ ID NO:17)
hMAB-A VH(3) (SEQ ID NO:18)
hMAB-A VH(4) (SEQ ID NO:19)
hMAB-A VH(2A) (SEQ ID NO:20)
hMAB-A VH(2B) (SEQ ID NO:21)
hMAB-A VH(2C) (SEQ ID NO:22)
hMAB-A VH(2D) (SEQ ID NO:23)
hMAB-A VH(2E) (SEQ ID NO:24)
hMAB-A VH(2F) (SEQ ID NO:25)
hMAB-A VH(2G) (SEQ ID NO:26)
hMAB-A VH(2H) (SEQ ID NO:27)
hMAB-A VH(2I) (SEQ ID NO:28)
and **hMAB-A VH(2J) (SEQ ID NO:29)**
are presented below (CDR_H residues are shown in single underline; differences relative to **hMAB-A VH(1) (SEQ ID NO:7)** are shown in double underline).

hMAB-A VH(1) (SEQ ID NO:16):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPINGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYGSRDYF DYWGQGTTVT VSS

hMAB-A VH(2) (SEQ ID NO:17):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYGSRDYF DYWGQGTTVT VSS

hMAB-A VH(3) (SEQ ID NO:18):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NERFQGRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYGSRDYF DYWGQGTTVT VSS

hMAB-A VH(4) (SEQ ID NO:19):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWIH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NERFQGRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYGSRDYF DYWGQGTTVT VSS

hMAB-A VH(2A) (SEQ ID NO:20):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYFNSGTL DYWGQGTTVT VSS

hMAB-A VH(2B) (SEQ ID NO:21):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYIGKGVL DYWGQGTTVT VSS

hMAB-A VH(2C) (SEQ ID NO:22):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYPRFGWL DYWGQGTTVT VSS

hMAB-A VH(2D) (SEQ ID NO:23):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYTGKGVL DYWGQGTTVT VSS

hMAB-A VH(2E) (SEQ ID NO:24):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYDSNAVL DYWGQGTTVT VSS

hMAB-A VH(2F) (SEQ ID NO:25):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYFHSGTL DYWGQGTTVT VSS

hMAB-A VH(2G) (SEQ ID NO:26):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYFNKAVL DYWGQGTTVT VSS

hMAB-A VH(2H) (SEQ ID NO:27):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYGGSGVL DYWGQGTTVT VSS

hMAB-A VH(2I) (SEQ ID NO:28):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYPRQGFL DYWGQGTTVT VSS

hMAB-A VH(2J) (SEQ ID NO:29):

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWMH**WVRQA PGKGLEWVG**E**
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCAR**GG**
YYYYYNSGTL DYWGQGTTVT VSS

[00112] Suitable human amino acid sequences for the FRs of a humanized and/or optimized anti-ADAM9-VH Domain of **MAB-A** are:

FR_H1 Domain (SEQ ID NO:30): EVQLVESGGGLVKPGGSLRLSCAASGFTFS

FR_H2 Domain (SEQ ID NO:31): WVRQAPGKGLEWVG

FR_H3 Domain (SEQ ID NO:32): RFTISLDNSKNTLYLQMGSLRAEDTAVYYCAR

FR_H4 Domain (SEQ ID NO:33): WGQGTTVTVSS

[00113] Suitable alternative amino acid sequences for the **CDR_H1 Domain** of an anti-ADAM9-VH Domain of **MAB-A** include:

SEQ ID NO:8: SYWMH

SEQ ID NO:34: SYWIH

[00114] Suitable alternative amino acid sequences for the **CDR_H2 Domain** of an anti-ADAM9-VH Domain of **MAB-A** include:

SEQ ID NO:9: EIIPINGHTNYNEKFKS

SEQ ID NO:35: EIIPIFGHTNYNEKFKS

SEQ ID NO:36: EIIPIFGHTNYNERFQG

[00115] Suitable alternative amino acid sequences for the **CDR_H3 Domain** of an anti-ADAM9-VH Domain of **MAB-A** include:

SEQ ID NO:10: GGYYYYGSRDYFDY

SEQ ID NO:37: GGYYYYFNSGTLDY

SEQ ID NO:38: GGYYYYIGKGVLDY

SEQ ID NO:39: GGYYYYPRFGWLDY

SEQ ID NO:40: GGYYYYTGKGVLDY

SEQ ID NO:41: GGYYYYDSNAVLDY

SEQ ID NO:42: GGYYYYFHSGTLDY

SEQ ID NO:43: GGYYYYFNKAVLDY

SEQ ID NO:44: GGYYYYGGSGVLDY

SEQ ID NO:45: GGYYYYPRQGFLDY

SEQ ID NO:46: GGYYYYYNSGTLDY

[00116] Accordingly, the present invention encompasses ADAM9 binding molecules having a VH domain comprising:

(1) a **CDR_H1 Domain** having the amino acid sequence:

SEQ ID NO:47: $SYWX_1H$

wherein: **X_1** is M or I;

(2) a **CDR_H2 Domain** having the amino acid sequence:

SEQ ID NO:48: $EIIPIX_2GHTNYNEX_3FX_4X_5$

wherein: X_2, X_3, X_4, and X_5 are independently selected, and

wherein: X_2 is N or F; X_3 is K or R;

X_4 is K or Q; and X_5 is S or G.

and

(3) a **CDR_H3 Domain** having the amino acid sequence:

SEQ ID NO:49: $GGYYYYX_6X_7X_8X_9X_{10}X_{11}DY$

wherein: X_6, is P, F, Y, W, I, L, V, T, G or D, and X_7, X_8, X_9, X_{10}, and X_{11} are selected such that:

(A) when X_6 is P:

X_7 is K or R;

X_8 is F or M;

X_9 is G;

X_{10} is W or F; and

X_{11} is M, L or K;

(B) when X_6 is F, Y or W:

X_7 is N or H;

X_8 is S or K;

X_9 is G or A;

X_{10} is T or V; and

X_{11} is M, L or K;

(C) when X_6 is I, L or V:

X_7 is G;

X_8 is K;

X_9 is G or A;

X_{10} is V; and

X_{11} is M, L or K;

(D) when X_6 is T:

X_7 is G;

X_8 is K, M or N;

X_9 is G;

X_{10} is V or T; and

X_{11} is L or M;

(E) when X_6 is G:

X_7 is G;

X_8 is S;

X_9 is G;

X_{10} is V; and

X_{11} is L;

and

(F) when X_6 is D:

X_7 is S;

X_8 is N;

X_9 is A;

X_{10} is V; and

X_{11} is L.

[00117] A first exemplary humanized/optimized IgG1 Heavy Chain of a derivative/variant of **MAB-A** contains the **hMAB-A VH (2)** Domain (**SEQ ID NO:17**), and has the amino acid sequence (**SEQ ID NO:50**):

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

[00118] A second exemplary humanized/optimized IgG1 Heavy Chain of a derivative/variant of **MAB-A** contains the **hMAB-A VH (2C)** Domain (**SEQ ID NO:22**), and has the amino acid sequence (**SEQ ID NO:51**):

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

[00119] A third exemplary humanized/optimized IgG1 Heavy Chain of a derivative/variant of **MAB-A** contains the **hMAB-A VH (2I)** Domain (**SEQ ID NO:28**), and has the amino acid sequence (**SEQ ID NO:52**):

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
PKPKDTLMIS RTPEVTCVVV 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.

[00120] As provided herein, the CH2-CH3 Domains of the Fc Region may be engineered for example, to reduce effector function. In certain embodiments, the CH2-CH3 Domains of the exemplary humanized/optimized IgG1 Heavy Chains of the invention comprise one or more subustitutions selected from: L234A and L235A.

[00121] Thus, a fourth exemplary humanized/optimized IgG1 Heavy Chain of a derivative/variant of **MAB-A** contains the h**MAB-A VH (2I)** Domain (**SEQ ID NO:28**), and further comprises the substitutions L234A, and L235A in the CH2-CH3 Domains of the Fc Region (**SEQ ID NO:106**), underlined below) and has the amino acid sequence (**SEQ ID NO:202):**

EVQLVESGGG LVKPGGSLRL SCAASGFTFS SYWMHWVRQA PGKGLEWVGE
IIPIFGHTNY NEKFKSRFTI SLDNSKNTLY LQMGSLRAED TAVYYCARGG
YYYYPRQGFL DYWGQGTTVT VSSASTKGPS VFPLAPSSKS TSGGTAALGC
LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVPSSSLG
TQTYICNVNH KPSNTKVDKR VEPKSCDKTH TCPPCPAPE**A** **A**GGPSVFLFP
PKPKDTLMIS RTPEVTCVVV DVSHEDPEVK FNWYVDGVEV HNAKTKPREE
QYNSTYRVVS VLTVLHQDWL NGKEYKCKVS NKALPAPIEK TISKAKGQPR
EPQVYTLPPS REEMTKNQVS LTCLVKGFYP SDIAVEWESN GQPENNYKTT
PPVLDSDGSF FLYSKLTVDK SRWQQGNVFS CSVMHEALHN HYTQKSLSLS
PG**X**

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

2. Variant VL Domains of MAB-A

[00122] The amino acid sequences of preferred humanized/optimized anti-ADAM9-VL Domains of **MAB-A** are variants of the ADAM9-VL Domain of **MAB-A** (**SEQ ID NO:11**) and are represented by **SEQ ID NO:53** (CDR_L residues are shown underlined):

DIVMTQSPDS LAVSLGERAT ISC**X_{12}ASQSVD** **YX_{13}GDSYX_{14}N**WY
QQKPGQPPKL LIY**AASDLES** GIPARFSGSG SGTDFTLTIS
SLEPEDFATY YC**QQSX$_{15}$X$_{16}$X$_{17}$PF** **T**FGQGTKLEI K

wherein: X_{12}, X_{13}, X_{14}, X_{15}, X_{16}, and X_{17}, are independently selected, and

wherein: X_{12} is K or R; X_{13} is D or S;
X_{14} is M or L; X_{15} is H or Y;
X_{16} is E or S; and X_{17} is D or T.

[00123] The amino acid sequences of a preferred humanized anti-ADAM9-VL Domain of **MAB-A: hMAB-A VL(1) (SEQ ID NO:54)**, and of certain preferred humanized/optimized anti-ADAM9-VL Domains of **MAB-A**: **hMAB-A VL(2) (SEQ ID**

NO:55), **hMAB-A VL(3) (SEQ ID NO:56)**, and **hMAB-A VL(4) (SEQ ID NO:57)**, are presented below (CDR_L residues are shown in single underline; differences relative to **hMAB-A VL(1) (SEQ ID NO:54)** are shown in double underline).

hMAB-A VL(1) (SEQ ID NO:54):

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

hMAB-A VL(2) (SEQ ID NO:55):

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

hMAB-A VL(3) (SEQ ID NO:56):

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

hMAB-A VL(4) (SEQ ID NO:57):

DIVMTQSPDS LAVSLGERAT ISCRASQSVD YSGDSYLNWY QQKPGQPPKL
LIYAASDLES GIPARFSGSG SGTDFTLTIS SLEPEDFATY YCQQSYSTPF
TFGQGTKLEI K

[00124] Accordingly, suitable human amino acid sequences for the FRs of a humanized and/or optimized anti-ADAM9-VL Domain of **MAB-A** are:

FR_L1 Domain (SEQ ID NO:58): DIVMTQSPDSLAVSLGERATISC

FR_L2 Domain (SEQ ID NO:59): WYQQKPGQPPKLLIY

FR_L3 Domain (SEQ ID NO:60): GIPARFSGSGSGTDFTLTISSLEPEDFATYYC

FR_L4 Domain (SEQ ID NO:61): FGQGTKLEIK

[00125] Suitable alternative amino acid sequences for the **CDR_L1 Domain** of an anti-ADAM9-VL Domain include:

SEQ ID NO:12: KASQSVDYDGDSYMN

SEQ ID NO:62: KASQSVDYSGDSYMN

SEQ ID NO:63: RASQSVDYSGDSYMN

SEQ ID NO:64: RASQSVDYSGDSYLN

[00126] Suitable alternative amino acid sequences for the **CDR_L3 Domain** of an anti-ADAM9-VL Domain include:

SEQ ID NO:14: QQSHEDPFT

SEQ ID NO:65: QQSYSTPFT

[00127] Accordingly, the present invention encompasses anti-ADAM9 antibody VL Domain comprising:

(1) a **CDR_L1 Domain** having the amino acid sequence:

SEQ ID NO:66: X_{12}ASQSVDYX_{13}GDSYX_{14}N

wherein: **X_{12}**, **X_{13}**, **X_{14}**, are independently selected, and

wherein: **X_{12}** is K or R; **X_{13}** is D or S; and **X_{14}** is M or L;

(2) a **CDR_L2 Domain** having the amino acid sequence:

SEQ ID NO:13: AASDLES

and

(3) a **CDR_L3 Domain** having the amino acid sequence:

SEQ ID NO:67: QQS$X_{15}X_{16}X_{17}$PFT

wherein: **X_{15}**, **X_{16}**, and **X_{17}**, are independently selected, and

wherein: **X_{15}** is H or Y; **X_{16}** is E or S; and **X_{17}** is D or T.

[00128] An exemplary humanized/optimized IgG1 Light Chain of a derivative/variant of **MAB-A** contains the **hMAB-A VL (2)** Domain (**SEQ ID NO:55**), and has the amino acid sequence (**SEQ ID NO:68**):

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

[00129] Thus, the present invention additionally expressly contemplates ADAM9-binding molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, *etc.*) that immunospecifically bind to an epitope of a human ADAM9 polypeptide, and that comprise any of the above-provided **MAB-A** CDR_H1, CDR_H2, CDR_H3, CDR_L1, CDR_L2, or CDR_L3, and particularly contemplates such ADAM9-binding molecules that comprise one of the above-provided **MAB-A** CDR_H1, one of the above-provided **MAB-A** CDR_H2, one of the above-provided **MAB-A** CDR_H3, one of the above-provided **MAB-A** CDR_L1, one of the above-provided **MAB-A** CDR_L2, and one of the above-provided **MAB-A** CDR_L3.

[00130] The invention further contemplates such ADAM9-binding molecules that further comprise any of the above-provided humanized **MAB-A** FR_H1, FR_H2, FR_H3, or FR_H4, FR_L1, FR_L2, FRL3, or FR_L4, and particularly contemplates such ADAM9-binding molecules that comprise FR_H1, FR_H2, FR_H3, and FR_H4, and/or that comprise FR_L1, FR_L2, FR_L3, FR_L4 and FR_H1.

[00131] In some embodiments, the ADAM9-binding molecules include a CDR_H1 domain, a CDR_H2 domain, and a CDR_H3 domain and a CDR_L1 domain, a CDR_L2 domain, and a CDR_L3 domain having the sequences selected from the group consisting of:

(a) **SEQ ID NOs:8**, **35** and **10** and **SEQ ID NOs:62**, **13**, and **14**, respectively;

(b) **SEQ ID NOs:8**, **35** and **10** and **SEQ ID NOs:63**, **13**, and **14**, respectively;

(c) **SEQ ID NOs:8**, **36** and **10** and **SEQ ID NOs:63**, **13** and **14**, respectively;

(d) **SEQ ID NOs:34**, **36** and **10** and **SEQ ID NO:64**, **13** and **65**, respectively

(e) **SEQ ID NOs:8**, **35** and **37** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(f) **SEQ ID NOs:8**, **35** and **38** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(g) **SEQ ID NOs:8**, **35** and **39** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(h) **SEQ ID NOs:8**, **35** and **40** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(i) **SEQ ID NOs:8**, **35** and **41** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(j) **SEQ ID NOs:8**, **35** and **42** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(k) **SEQ ID NOs:8**, **35** and **43** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(l) **SEQ ID NOs:8**, **35** and **44** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(m) **SEQ ID NOs:8**, **35** and **45** and **SEQ ID NOs:62**, **13** and **14**, respectively; and

(n) **SEQ ID NOs:8**, **35** and **46** and **SEQ ID NOs:62**, **13** and **14**, respectively.

[00132] In particular embodiments, the ADAM9-binding molecules include a CDR_H1 domain, a CDR_H2 domain, and a CDR_H3 domain and a CDR_L1 domain, a CDR_L2 domain, and a CDR_L3 domain having the sequences of **SEQ ID NOs:8, 35** and **45** and **SEQ ID NOs:62, 13** and **14**, respectively.

[00133] In some embodiments, the ADAM9-binding molecules of the invention include a heavy chain variable domain (VH) and a light chain variable domain (VL) having sequences that are at least 90%, at least 95%, at least 99%, or are 100% identical to the sequences as follows:

SEQ ID NO:17 and **SEQ ID NO:55**, respectively;

SEQ ID NO:17 and **SEQ ID NO:56**, respectively;
SEQ ID NO:18 and **SEQ ID NO:56**, respectively;
SEQ ID NO:19 and **SEQ ID NO:57**, respectively;
SEQ ID NO:20 and **SEQ ID NO:55**, respectively;
SEQ ID NO:21 and **SEQ ID NO:55**, respectively;
SEQ ID NO:22 and **SEQ ID NO:55**, respectively;
SEQ ID NO:23 and **SEQ ID NO:55**, respectively;
SEQ ID NO:24 and **SEQ ID NO:55**, respectively;
SEQ ID NO:25 and **SEQ ID NO:55**, respectively;
SEQ ID NO:26 and **SEQ ID NO:55**, respectively;
SEQ ID NO:27 and **SEQ ID NO:55**, respectively;
SEQ ID NO:28 and **SEQ ID NO:55**, respectively; and
SEQ ID NO:29 and **SEQ ID NO:55**, respectively.

[00134] By "substantially identical" or "identical" is meant a polypeptide exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably at least 80% or at least 85%, and more preferably at least 90%, at least 95% at least 99%, or even 100% identical at the amino acid level to the polypeptide sequence used for comparison.

[00135] Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e-3 and e-100 indicating a closely related sequence.

[00136] In particular embodiments, the ADAM9-binding molecules of the invention include a heavy chain variable domain (VH) and a light chain variable domain (VL) having

sequences that are at least 90%, at least 95%, at least 99%, or are 100% identical to the sequences of **SEQ ID NO:28** and **SEQ ID NO:55**, respectively.

[00137] In certain embodiments, the ADAM9-binding molecules of the invention comprise a heavy chain and a light chain sequence as follows:

SEQ ID NO:50 and **SEQ ID NO:68**, respectively;

SEQ ID NO:51 and **SEQ ID NO:68**, respectively;

SEQ ID NO:52 and **SEQ ID NO:68**, respectively; and

SEQ ID NO:202 and **SEQ ID NO:68**, respectively.

[00138] The present invention also expressly contemplates ADAM9-binding molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, *etc.*) that immunospecifically bind to an epitope of a human ADAM9 polypeptide, and that comprise any of the above-provided humanized/optimized anti-ADAM9 **MAB-A** VL or VH Domains. The present invention particularly contemplates such ADAM9-binding molecules that comprise any of the following combinations of humanized anti-ADAM9 VL or VH Domains:

hMAB-A VH / hMAB-A VL Combinations	
h**MAB-A** VH(1) / h**MAB-A** VL(1)	h**MAB-A** VH(2D) / h**MAB-A** VL(1)
h**MAB-A** VH(1) / h**MAB-A** VL(2)	h**MAB-A** VH(2D) / h**MAB-A** VL(2)
h**MAB-A** VH(1) / h**MAB-A** VL(3)	h**MAB-A** VH(2D) / h**MAB-A** VL(3)
h**MAB-A** VH(1) / h**MAB-A** VL(4)	h**MAB-A** VH(2D) / h**MAB-A** VL(4)
h**MAB-A** VH(2) / h**MAB-A** VL(1)	h**MAB-A** VH(2E) / h**MAB-A** VL(1)
h**MAB-A** VH(2) / h**MAB-A** VL(2)	h**MAB-A** VH(2E) / h**MAB-A** VL(2)
h**MAB-A** VH(2) / h**MAB-A** VL(3)	h**MAB-A** VH(2E) / h**MAB-A** VL(3)
h**MAB-A** VH(2) / h**MAB-A** VL(4)	h**MAB-A** VH(2E) / h**MAB-A** VL(4)
h**MAB-A** VH(3) / h**MAB-A** VL(1)	h**MAB-A** VH(2F) / h**MAB-A** VL(1)
h**MAB-A** VH(3) / h**MAB-A** VL(2)	h**MAB-A** VH(2F) / h**MAB-A** VL(2)
h**MAB-A** VH(3) / h**MAB-A** VL(3)	h**MAB-A** VH(2F) / h**MAB-A** VL(3)
h**MAB-A** VH(3) / h**MAB-A** VL(4)	h**MAB-A** VH(2F) / h**MAB-A** VL(4)
h**MAB-A** VH(4) / h**MAB-A** VL(1)	h**MAB-A** VH(2G) / h**MAB-A** VL(1)
h**MAB-A** VH(4) / h**MAB-A** VL(2)	h**MAB-A** VH(2G) / h**MAB-A** VL(2)
h**MAB-A** VH(4) / h**MAB-A** VL(3)	h**MAB-A** VH(2G) / h**MAB-A** VL(3)
h**MAB-A** VH(4) / h**MAB-A** VL(4)	h**MAB-A** VH(2G) / h**MAB-A** VL(4)
h**MAB-A** VH(2A) / h**MAB-A** VL(1)	h**MAB-A** VH(2H) / h**MAB-A** VL(1)
h**MAB-A** VH(2A) / h**MAB-A** VL(2)	h**MAB-A** VH(2H) / h**MAB-A** VL(2)
h**MAB-A** VH(2A) / h**MAB-A** VL(3)	h**MAB-A** VH(2H) / h**MAB-A** VL(3)
h**MAB-A** VH(2A) / h**MAB-A** VL(4)	h**MAB-A** VH(2H) / h**MAB-A** VL(4)

hMAB-A VH / hMAB-A VL Combinations	
h**MAB-A** VH(2B) / h**MAB-A** VL(1)	h**MAB-A** VH(2I) / h**MAB-A** VL(1)
h**MAB-A** VH(2B) / h**MAB-A** VL(2)	h**MAB-A** VH(2I) / h**MAB-A** VL(2)
h**MAB-A** VH(2B) / h**MAB-A** VL(3)	h**MAB-A** VH(2I) / h**MAB-A** VL(3)
h**MAB-A** VH(2B) / h**MAB-A** VL(4)	h**MAB-A** VH(2I) / h**MAB-A** VL(4)
h**MAB-A** VH(2C) / h**MAB-A** VL(1)	h**MAB-A** VH(2J) / h**MAB-A** VL(1)
h**MAB-A** VH(2C) / h**MAB-A** VL(2)	h**MAB-A** VH(2J) / h**MAB-A** VL(2)
h**MAB-A** VH(2C) / h**MAB-A** VL(3)	h**MAB-A** VH(2J) / h**MAB-A** VL(3)
h**MAB-A** VH(2C) / h**MAB-A** VL(4)	h**MAB-A** VH(2J) / h**MAB-A** VL(4)

[00139] The present invention specifically encompasses ADAM9-binding molecules comprising (i) a humanized/optimized anti-ADAM9-VL and/or VH Domain as provided above, and (ii) an Fc Region. In particular embodiments, the ADAM9-binding molecules of the present invention are monoclonal antibodies comprising (i) a humanized/optimized anti-ADAM9-VL and/or VH Domain as provided above, and (ii) an Fc Region. In other embodiments, the ADAM9-binding molecules of the present invention are selected from the group consisting of: monoclonal antibodies, multispecific antibodies, synthetic antibodies, chimeric antibodies, single-chain Fvs (scFv), single-chain antibodies, Fab fragments, F(ab') fragments, disulfide-linked bispecific Fvs (sdFv), BiTEs, diabodies, and trivalent binding molecules.

[00140] Although particular modifications to anti-ADAM9 VH and VL Domains are summarized above and compared in **Figures 9A-9B**, it is not necessary to modify all or most of these residues when engineering a humanized and/or optimized anti-ADAM9-VH or VL Domain of the invention. The present invention also encompasses minor variations of these VH and VL sequences including, for example, amino acid substitutions of the C-terminal and/or N-terminal amino acid residues which may be introduced to facilitate subcloning.

VII. Chimeric Antigen Receptors

[00141] The ADAM9-binding molecules of the present invention may be monospecific single-chain molecules, such as anti-ADAM9 single-chain variable fragments ("**anti-ADAM9-scFvs**") or anti-ADAM9 Chimeric Antigen Receptors ("**anti-ADAM9-CARs**"). As discussed above, scFvs are made by linking Light and Heavy Chain Variable Domains together via a short linking peptide. First-generation Chimeric Antigen Receptors ("**CARs**") typically comprise the intracellular domain from the CD3 ζ- chain, which is the primary transmitter of signals from endogenous T-cell Receptors ("**TCRs**"). Second-

generation CARs possess additional intracellular signaling domains from various costimulatory protein receptors (*e.g.*, CD28, 41BB, ICOS, *etc.*) fused to the cytoplasmic tail of the CAR in order to provide additional signals to the T-cell. Third-generation CARs combine multiple signaling domains, such as CD3ζ-CD28-41BB or CD3ζ-CD28-OX40, in order to further augment their potency (Tettamanti, S. *et al.* (2013) "*Targeting Of Acute Myeloid Leukaemia By Cytokine-Induced Killer Cells Redirected With A Novel CD123-Specific Chimeric* Antigen *Receptor*," Br. J. Haematol. 161:389-401; Gill, S. *et al.* (2014) "*Efficacy Against Human Acute Myeloid Leukemia And Myeloablation Of Normal Hematopoiesis In A Mouse Model Using Chimeric Antigen Receptor-Modified T Cells*," Blood 123(15): 2343-2354; Mardiros, A. *et al.* (2013) "*T Cells Expressing CD123-Specific Chimeric Antigen Receptors Exhibit Specific Cytolytic Effector Functions And Antitumor Effects Against Human Acute Myeloid Leukemia*," Blood 122:3138-3148; Pizzitola, I. *et al.* (2014) "*Chimeric Antigen Receptors Against CD33/CD123 Antigens Efficiently Target Primary Acute Myeloid Leukemia Cells in vivo*," Leukemia doi:10.1038/leu.2014.62). Chimeric Antigen receptors are discussed in US Patent Nos. 9,447,194; 9,266,960; 9,212,229; 9,074,000; 8,822,196; and 8,465,743, and in US Patent Publication Nos. 2016/0272718, 2016/0144026, 2016/0130357, 2016/0081314, 2016/0075784, 2016/0058857, 2016/0046729, 2016/0046700, 2016/0045551, 2016/0015750, 2015/0320799, 2015/0307623, 2015/0307564, 2015/0038684, 2014/0134142 and 2013/0280285.

[00142] The anti-ADAM9-CARs of the present invention comprise an anti-ADAM9-scFv fused to an intracellular domain of a receptor. The Light Chain Variable (VL) Domain and the Heavy Chain Variable (VH) Domain of the anti-ADAM9-scFv are selected from any of the humanized anti-ADAM9-VL and anti-ADAM9-VH Domains disclosed herein. Preferably, the VH Domain is selected from the group consisting of: **hMAB-A VH(1) (SEQ ID NO:16)**, **hMAB-A VH(2) (SEQ ID NO:17)**, **hMAB-A VH(3) (SEQ ID NO:18)**, **hMAB-A VH(4) (SEQ ID NO:19)**, **hMAB-A VH(2A) (SEQ ID NO:20)**, **hMAB-A VH(2B) (SEQ ID NO:21)**, **hMAB-A VH(2C) (SEQ ID NO:22)**, **hMAB-A VH(2D) (SEQ ID NO:23)**, **hMAB-A VH(2E) (SEQ ID NO:24)**, **hMAB-A VH(2F) (SEQ ID NO:25)**, **hMAB-A VH(2G) (SEQ ID NO:26)**, **hMAB-A VH(2H) (SEQ ID NO:27)**, **hMAB-A VH(2I) (SEQ ID NO:28)**, and **hMAB-A VH(2J) (SEQ ID NO:29)**, and the VL Domain is selected from the group consisting of: **hMAB-A VL(1) (SEQ ID NO:54)**, **hMAB-A VL(2) (SEQ ID NO:55)**, **hMAB-A VL(3) (SEQ ID NO:56)**, and **hMAB-A VL(4) (SEQ ID**

NO:57). Combinations of humanized/optimized anti-ADAM9-VL and anti-ADAM9-VH Domains and combinations of CDR_{HS} and CDR_{LS} that may be used to form such Chimeric Antigen Receptors are presented above.

[00143] The intracellular domain of the anti-ADAM9-CARs of the present invention is preferably selected from the intracellular domain of any of: 41BB-CD3ζ, b2c-CD3ζ, CD28, CD28-4-1BB-CD3ζ, CD28-CD3ζ, CD28-FcεRIγ, CD28mut-CD3ζ, CD28-OX40-CD3ζ, CD28-OX40-CD3ζ, CD3ζ, CD4-CD3ζ, CD4-FcεRIγ, CD8-CD3ζ, FcεRIγ, FcεRIγCAIX, Heregulin-CD3ζ, IL-13-CD3ζ, or Ly49H-CD3ζ (Tettamanti, S. *et al.* (2013) "*Targeting Of Acute Myeloid Leukaemia By Cytokine-Induced Killer Cells Redirected With A Novel CD123-Specific Chimeric* Antigen *Receptor*," Br. J. Haematol. 161:389-401; Gill, S. *et al.* (2014) "*Efficacy Against Human Acute Myeloid Leukemia And Myeloablation Of Normal Hematopoiesis In A Mouse Model Using Chimeric Antigen Receptor-Modified T Cells*," Blood 123(15): 2343-2354; Mardiros, A. *et al.* (2013) "*T Cells Expressing CD123-Specific Chimeric Antigen Receptors Exhibit Specific Cytolytic Effector Functions And Antitumor Effects Against Human Acute Myeloid Leukemia*," Blood 122:3138-3148; Pizzitola, I. *et al.* (2014) "*Chimeric Antigen Receptors Against CD33/CD123 Antigens Efficiently Target Primary Acute Myeloid Leukemia Cells in vivo*," Leukemia doi:10.1038/leu.2014.62).

VIII. Multispecific ADAM9-Binding Molecules

[00144] The present invention is also directed to multispecific (*e.g.*, bispecific, trispecific, *etc.*) ADAM9-binding molecules comprising an epitope-binding site (preferably comprising 1, 2 or all 3 of the CDR_{HS} of an anti-ADAM9-VH Domain of the invention and/or 1, 2 or all 3 of the CDR_{LS} of an anti-ADAM9-VL Domain of the invention, or such anti-ADAM9-VH Domain and/or such anti-ADAM9-VL Domain) and further comprising a second epitope-binding site that immunospecifically binds to a second epitope, where such second epitope is (i) a different epitope of ADAM9, or (ii) an epitope of a molecule that is not ADAM9. Such multispecific ADAM9-binding molecules preferably comprise a combination of epitope-binding sites that recognize a set of antigens unique to target cells or tissue type. In particular, the present invention relates to multispecific ADAM9-binding molecules that are capable of binding to an epitope of ADAM9 and an epitope of a molecule present on the surface of an effector cell, especially a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. For example, such ADAM9-binding molecules of the

present invention may be constructed to comprise an epitope-binding site that immunospecifically binds CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), or NKG2D.

[00145] One embodiment of the present invention relates to bispecific ADAM9-binding molecules that are capable of binding to a "**first epitope**" and a "**second epitope**," such epitopes not being identical to one another. Such bispecific molecules comprise "**VL1**" / "**VH1**" domains that are capable of binding to the first epitope, and "**VL2**" / "**VH2**" domains that are capable of binding to the second epitope. The notation "**VL1**" and "**VH1**" denote respectively, the Light Chain Variable Domain and Heavy Chain Variable Domain that bind the "first" epitope of such bispecific molecules. Similarly, the notation "**VL2**" and "**VH2**" denote respectively, the Light Chain Variable Domain and Heavy Chain Variable Domain that bind the "second" epitope of such bispecific molecules. It is irrelevant whether a particular epitope is designated as the first vs. the second epitope; such notation having relevance only with respect to the presence and orientation of domains of the polypeptide chains of the binding molecules of the present invention. In one embodiment, one of such epitopes is an epitope of human ADAM9 and the other is a different epitope of ADAM9, or is an epitope of a molecule that is not ADAM9. In particular embodiments, one of such epitopes is an epitope of human ADAM9 and the other is an epitope of a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In certain embodiments, a bispecific molecule comprises more than two epitope-binding sites. Such bispecific molecules will bind at least one epitope of ADAM9 and at least one epitope of a molecule that is not ADAM9, and may further bind additional epitopes of ADAM9 and/or additional epitopes of a molecule that is not ADAM9.

[00146] The present invention particularly relates to bispecific, trispecific and multispecific ADAM9-binding molecules (*e.g.*, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*) that possess epitope-binding fragments of antibodies (*e.g.*, VL and VH Domains) that enable them to be able to coordinately bind to at least one epitope of ADAM9 and at least one epitope of a second molecule that is not ADAM9. Selection of the VL and VH Domains of the polypeptide domains of such molecules is coordinated so that the polypeptides chains that make up such multispecific ADAM9-binding molecules assemble to form at least one functional epitope-binding site that is specific for at least one epitope of ADAM9 and at least one functional epitope-binding site that is specific for at

least one epitope of a molecule that is not ADAM9. Preferably, the multispecific ADAM9-binding molecules comprise 1, 2 or all 3 of the CDR_{HS} of an anti-ADAM9-VH Domain of the invention and/or 1, 2 or all 3 of the CDR_{LS} of an anti-ADAM9-VL Domain of the invention, or such anti-ADAM9-VH Domain and/or such anti-ADAM9-VL Domain, as provided herein.

A. Bispecific Antibodies

[00147] The present invention encompasses bispecific antibodies capable of simultaneously binding to an epitope of ADAM9 and an epitope of a molecule that is not ADAM9. In some embodiments, the bispecific antibody capable of simultaneously binding to ADAM9 and a second molecule that is not ADAM9 is produced using any of the methods described in PCT Publication Nos. WO 1998/002463, WO 2005/070966, WO 2006/107786 WO 2007/024715, WO 2007/075270, WO 2006/107617, WO 2007/046893, WO 2007/146968, WO 2008/003103, WO 2008/003116, WO 2008/027236, WO 2008/024188, WO 2009/132876, WO 2009/018386, WO 2010/028797, WO2010028796, WO 2010/028795, WO 2010/108127, WO 2010/136172, WO 2011/086091, WO 2011/133886, WO 2012/009544, WO 2013/003652, WO 2013/070565, WO 2012/162583, WO 2012/156430, WO 2013/174873, and WO 2014/022540, each of which is hereby incorporated herein by reference in its entirety.

B. Bispecific Diabodies Lacking Fc Regions

[00148] One embodiment of the present invention relates to bispecific diabodies that are capable of binding to a first epitope and a second epitope, wherein the first epitope is an epitope of human ADAM9 and the second is an epitope of a molecule that is not ADAM9, preferably a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. Such diabodies comprise, and most preferably are composed of, a first polypeptide chain and a second polypeptide chain, whose sequences permit the polypeptide chains to covalently bind to each other to form a covalently associated diabody that is capable of simultaneously binding to an epitope of ADAM9 and the second epitope.

[00149] The first polypeptide chain of such an embodiment of bispecific diabodies comprises, in the N-terminal to C-terminal direction: an N-terminus, the VL Domain of a monoclonal antibody capable of binding to either the first or second epitope (*i.e.*, either

$VL_{anti\text{-}ADAM9\text{-}VL}$ or $VL_{Epitope\ 2}$), a first intervening spacer peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding to either the second epitope (if such first polypeptide chain contains $VL_{anti\text{-}ADAM9\text{-}VL}$) or ADAM9 (if such first polypeptide chain contains $VL_{Epitope\ 2}$), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain and a C-terminus (**Figure 1**).

[00150] The second polypeptide chain of this embodiment of bispecific diabodies comprises, in the N-terminal to C-terminal direction: an N-terminus, a VL Domain of a monoclonal antibody capable of binding to either the first or second epitope (*i.e.*, either $VL_{anti\text{-}ADAM9\text{-}VL}$ or $VL_{Epitope\ 2}$, and being the VL Domain not selected for inclusion in the first polypeptide chain of the diabody), an intervening spacer peptide (Linker 1), a VH Domain of a monoclonal antibody capable of binding to either the second epitope (if such second polypeptide chain contains $VL_{anti\text{-}ADAM9\text{-}VL}$) or to ADAM9 (if such second polypeptide chain contains $VL_{Epitope\ 2}$), a second intervening spacer peptide (Linker 2) optionally containing a cysteine residue, a Heterodimer-Promoting Domain, and a C-terminus (**Figure 1**).

[00151] The VL Domain of the first polypeptide chain interacts with the VH Domain of the second polypeptide chain to form a first functional epitope-binding site that is specific for a first antigen (*i.e.*, either ADAM9 or a molecule that contains the second epitope). Likewise, the VL Domain of the second polypeptide chain interacts with the VH Domain of the first polypeptide chain in order to form a second functional epitope-binding site that is specific for a second antigen (*i.e.*, either the molecule that comprises the second epitope or ADAM9). Thus, the selection of the VL and VH Domains of the first and second polypeptide chains is coordinated, such that the two polypeptide chains of the diabody collectively comprise VL and VH Domains capable of binding to both an epitope of ADAM9 and to the second epitope (*i.e.*, they collectively comprise $VL_{anti\text{-}ADAM9\text{-}VL}/VH_{anti\text{-}ADAM9\text{-}VH}$ and $VL_{Epitope\ 2}/VH_{Epitope\ 2}$).

[00152] Most preferably, the length of the intervening spacer peptide (*i.e.*, "**Linker 1**," which separates such VL and VH Domains) is selected to substantially or completely prevent the VL and VH Domains of the polypeptide chain from binding to one another (for example consisting of from 0, 1, 2, 3, 4, 5, 6, 7, 8 or 9 intervening linker amino acid residues). Thus the VL and VH Domains of the first polypeptide chain are substantially or completely incapable of binding to one another. Likewise, the VL and VH Domains of the second polypeptide chain are substantially or completely incapable of binding to one

another. A preferred intervening spacer peptide (Linker 1) has the sequence (**SEQ ID NO:69**): GGGSGGGG.

[00153] The length and composition of the second intervening spacer peptide ("**Linker 2**") is selected based on the choice of one or more polypeptide domains that promote such dimerization (i.e., a "**Heterodimer-Promoting Domain**"). Typically, the second intervening spacer peptide (Linker 2) will comprise 3-20 amino acid residues. In particular, where the employed Heterodimer-Promoting Domain(s) do/does not comprise a cysteine residue a cysteine-containing second intervening spacer peptide (Linker 2) is utilized. A cysteine-containing second intervening spacer peptide (Linker 2) will contain 1, 2, 3 or more cysteines. A preferred cysteine-containing spacer peptide (Linker 2) has the sequence GGCGGG (**SEQ ID NO:70**). Alternatively, Linker 2 does not comprise a cysteine (*e.g.*, GGG, GGGS (**SEQ ID NO:71**), LGGGSG (**SEQ ID NO:72**), GGGSGGGSGGG (**SEQ ID NO:73**), ASTKG (**SEQ ID NO:74**), LEPKSS (**SEQ ID NO:75**), APSSS (**SEQ ID NO:76**), *etc.*) and a Cysteine-Containing Heterodimer-Promoting Domain, as described below is used. Optionally, both a cysteine-containing Linker 2 and a cysteine-containing Heterodimer-Promoting Domain are used.

[00154] The Heterodimer-Promoting Domains may be GVEPKSC (**SEQ ID NO:77**) or VEPKSC (**SEQ ID NO:78**) or AEPKSC (**SEQ ID NO:79**) on one polypeptide chain and GFNRGEC (**SEQ ID NO:80**) or FNRGEC (**SEQ ID NO:81**) on the other polypeptide chain (see, US Patent No. 9,296,816).

[00155] In a preferred embodiment, the Heterodimer-Promoting Domains will comprise tandemly repeated coil domains of opposing charge for example, "E-coil" helical domains (**SEQ ID NO:82**): EVAALEK-EVAALEK-EVAALEK-EVAALEK), whose glutamate residues will form a negative charge at pH 7, and "K-coil" domains (**SEQ ID NO:83**): KVAALKE-KVAALKE-KVAALKE-KVAALKE), whose lysine residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimer formation. Heterodimer-Promoting Domains that comprise modifications of the above-described E-coil and K-coil sequences so as to include one or more cysteine residues may be utilized. The presence of such cysteine residues permits the coil present on one polypeptide chain to become covalently bonded to a complementary coil present on another polypeptide chain,

thereby covalently bonding the polypeptide chains to one another and increasing the stability of the diabody. Examples of such particularly preferred are Heterodimer-Promoting Domains include a Modified E-Coil having the amino acid sequence EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:84**), and a modified K-coil having the amino acid sequence KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:85**).

[00156] As disclosed in PCT Publication No. WO 2012/018687, in order to improve the *in vivo* pharmacokinetic properties of diabodies, a diabody may be modified to contain a polypeptide portion of a serum-binding protein at one or more of the termini of the diabody. Most preferably, such polypeptide portion of a serum-binding protein will be installed at the C-terminus of a polypeptide chain of the diabody. Albumin is the most abundant protein in plasma and has a half-life of 19 days in humans. Albumin possesses several small molecule binding sites that permit it to non-covalently bind to other proteins and thereby extend their serum half-lives. The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 consists of 46 amino acid residues forming a stable three-helix bundle and has broad albumin-binding specificity (Johansson, M.U. *et al.* (2002) "*Structure, Specificity, And Mode Of Interaction For Bacterial Albumin-Binding Module*s," J. Biol. Chem. 277(10):8114-8120). Thus, a particularly preferred polypeptide portion of a serum-binding protein for improving the *in vivo* pharmacokinetic properties of a diabody is the Albumin-Binding Domain (ABD) from streptococcal protein G, and more preferably, the Albumin-Binding Domain 3 (ABD3) of Protein G of *Streptococcus* strain G148 (**SEQ ID NO:86**): LAEAKVLANR ELDKYGVSDY YKNLIDNAKS AEGVKALIDE ILAALP.

[00157] As disclosed in PCT Publication No. WO 2012/162068 (herein incorporated by reference), "**deimmunized**" variants of **SEQ ID NO:86** have the ability to attenuate or eliminate MHC class II binding. Based on combinational mutation results, the following combinations of substitutions are considered to be preferred substitutions for forming such a deimmunized ABD: 66D/70S +71A; 66S/70S +71A; 66S/70S +79A; 64A/65A/71A; 64A/65A/71A+66S; 64A/65A/71A+66D; 64A/65A/71A+66E; 64A/65A/79A+66S; 64A/65A/79A+66D; 64A/65A/79A+66E. Variant ABDs having the modifications L64A, I65A and D79A or the modifications N66S, T70S and D79A. Variant deimmunized ABD having the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLID_{66}NAKS_{70} A_{71}EGVKALIDE ILAALP (**SEQ ID NO:87**),

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKN**A**$_{64}$**A**$_{65}$NNAKT VEGVKALI**A**$_{79}$E ILAALP **(SEQ ID NO:88)**,

or the amino acid sequence:

LAEAKVLANR ELDKYGVSDY YKNLI**S**$_{66}$NAK**S**$_{70}$ VEGVKALI**A**$_{79}$E ILAALP **(SEQ ID NO:89)**,

are particularly preferred as such deimmunized ABD exhibit substantially wild-type binding while providing attenuated MHC class II binding. Thus, the first polypeptide chain of such a diabody having an ABD contains a third linker (Linker 3) preferably positioned C-terminally to the E-coil (or K-coil) Domain of such polypeptide chain so as to intervene between the E-coil (or K-coil) Domain and the ABD (which is preferably a deimmunized ABD). A preferred sequence for such Linker 3 is GGGS **(SEQ ID NO:71)**.

C. Multispecific Diabodies Containing Fc Regions

[00158] One embodiment of the present invention relates to multispecific diabodies capable of simultaneously binding to an epitope of ADAM9 and a second epitope (*i.e.*, a different epitope of ADAM9 or an epitope of a molecule that is not ADAM9) that comprise an Fc Region. The addition of an IgG CH2-CH3 Domain to one or both of the diabody polypeptide chains, such that the complexing of the diabody chains results in the formation of an Fc Region, increases the biological half-life and/or alters the valency of the diabody. Such diabodies comprise, two or more polypeptide chains whose sequences permit the polypeptide chains to covalently bind to each other to form a covalently associated diabody that is capable of simultaneously binding to an epitope of ADAM9 and the second epitope. Incorporating an IgG CH2-CH3 Domains onto both of the diabody polypeptides will permit a two-chain bispecific Fc-Region-containing diabody to form (**Figure 2**).

[00159] Alternatively, incorporating an IgG CH2-CH3 Domains onto only one of the diabody polypeptides will permit a more complex four-chain bispecific Fc Region-containing diabody to form (**Figures 3A-3C**). **Figure 3C** shows a representative four-chain diabody possessing the Constant Light (CL) Domain and the Constant Heavy CH1 Domain, however fragments of such domains as well as other polypeptides may alternatively be employed (see, *e.g.*, **Figures 3A** and **3B**, US Patent Publication Nos. 2013-0295121; 2010-0174053 and 2009-0060910; European Patent Publication Nos. EP 2714079; EP 2601216; EP 2376109; EP 2158221 and PCT Publication Nos. WO 2012/162068; WO 2012/018687;

WO 2010/080538). Thus, for example, in lieu of the CH1 Domain, one may employ a peptide having the amino acid sequence GVEPKSC (**SEQ ID NO:77**), VEPKSC (**SEQ ID NO:78**), or AEPKSC (**SEQ ID NO:79**), derived from the Hinge Region of a human IgG, and in lieu of the CL Domain, one may employ the C-terminal 6 amino acids of the human kappa light chain, GFNRGEC (**SEQ ID NO:80**) or FNRGEC (**SEQ ID NO:81**). A representative peptide containing four-chain diabody is shown in **Figure 3A**. Alternatively, or in addition, one may employ a peptide comprising tandem coil domains of opposing charge such as the "E-coil" helical domains (**SEQ ID NO:82**): EVAALEK-EVAALEK-EVAALEK-EVAALEK or **SEQ ID NO:84**): EVAACEK-EVAALEK-EVAALEK-EVAALEK); and the "K-coil" domains (**SEQ ID NO:83**): KVAALKE-KVAALKE-KVAALKE-KVAALKE or **SEQ ID NO:85**): KVAACKE-KVAALKE-KVAALKE-KVAALKE). A representative coil domain containing four-chain diabody is shown in **Figure 3B**.

[00160] The Fc Region-containing molecules of the present invention may include additional intervening spacer peptides (Linkers), generally such Linkers will be incorporated between a Heterodimer-Promoting Domain (*e.g.*, an E-coil or K-coil) and a CH2-CH3 Domain and/or between a CH2-CH3 Domain and a Variable Domain (*i.e.*, VH or VL). Typically, the additional Linkers will comprise 3-20 amino acid residues and may optionally contain all or a portion of an IgG Hinge Region (preferably a cysteine-containing portion of an IgG Hinge Region). Linkers that may be employed in the bispecific Fc Region-containing diabody molecules of the present invention include: GGGS (**SEQ ID NO:71**), LGGGSG (**SEQ ID NO:72**), GGGSGGGSGGG (**SEQ ID NO:73**), ASTKG (**SEQ ID NO:74**), LEPKSS (**SEQ ID NO:75**), APSSS (**SEQ ID NO:76**), APSSSPME (**SEQ ID NO:90**), VEPKSADKTHTCPPCP (**SEQ ID NO:91**), LEPKSADKTHTCPPC (**SEQ ID NO:92**), DKTHTCPPCP (**SEQ ID NO:93**), GGC, and GGG. LEPKSS (**SEQ ID NO:75**) may be used in lieu of GGG or GGC for ease of cloning. Additionally, the amino acids GGG, or LEPKSS (**SEQ ID NO:75**) may be immediately followed by DKTHTCPPCP (**SEQ ID NO:93**) to form the alternate linkers: GGGDKTHTCPPCP (**SEQ ID NO:94**); and LEPKSSDKTHTCPPCP (**SEQ ID NO:95**). Bispecific Fc Region-containing molecules of the present invention may incorporate an IgG Hinge Region in addition to or in place of a linker. Exemplary Hinge Regions include: EPKSCDKTHTCPPCP (**SEQ ID NO:96**) from IgG1, ERKCCVECPPCP (**SEQ ID NO:97**) from IgG2, ELKTPLGDTT HTCPRCPEPK

SCDTPPPCPR CPEPKSCDTP PPCPRCPEPK SCDTPPPCPR CP (**SEQ ID NO:206**) from IgG3, ESKYGPPCPSCP (**SEQ ID NO:98**) from IgG4, and ESKYGPPCPPCP (**SEQ ID NO:99**), which is an IgG4 hinge variant comprising a stabilizing S228P substitution (underlined) (as numbered by the EU index as set forth in Kabat) to reduce strand exchange.

[00161] As provided in **Figure 3A-3C**, Fc Region-containing diabodies of the invention may comprise four chains. The first and third polypeptide chains of such a diabody contain three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a Heterodimer-Promoting Domain, and (iv) a Domain containing a CH2-CH3 sequence. The second and fourth polypeptide chains contain: (i) a VL2-containing Domain, (ii) a VH1-containing Domain, and (iii) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the first/third polypeptide chains with the second/fourth polypeptide chains. The VL and/or VH Domains of the third and fourth polypeptide chains, and VL and/or VH Domains of the first and second polypeptide chains may be the same or different so as to permit tetravalent binding that is either monospecific, bispecific or tetraspecific. The notation "**VL3**" and "**VH3**" denote respectively, the Light Chain Variable Domain and Heavy Chain Variable Domain that bind a "third" epitope of such diabody. Similarly, the notation "**VL4**" and "**VH4**" denote respectively, the Light Chain Variable Domain and Heavy Chain Variable Domain that bind a "fourth" epitope of such diabody. The general structure of the polypeptide chains of a representative four-chain bispecific Fc Region-containing diabodies of invention is provided in **Table 2**:

Table 2

Bispecific	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-HPD-COOH
Tetraspecific	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VL3-VH4-HPD-CH2-CH3-COOH
	4th Chain	NH_2-VL4-VH3-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00162] In a specific embodiment, diabodies of the present invention are bispecific, tetravalent (*i.e.*, possess four epitope-binding sites), Fc-containing diabodies that are

composed of four total polypeptide chains (**Figures 3A-3C**). The bispecific, tetravalent, Fc-containing diabodies of the invention comprise two epitope-binding sites immunospecific for ADAM9 (which may be capable of binding to the same epitope of ADAM9 or to different epitopes of ADAM9), and two epitope-binding sites immunospecific for a second molecule (which may be capable of binding to the same epitope of the second molecule or to different epitopes of the second molecule). Preferably, the second molecule is a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell.

[00163] In a further embodiment, the Fc Region-containing diabodies of the present invention may comprise three polypeptide chains. The first polypeptide of such a diabody contains three domains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The second polypeptide of such a diabody contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's first polypeptide chain. The third polypeptide of such a diabody comprises a CH2-CH3 sequence. Thus, the first and second polypeptide chains of such a diabody associate together to form a VL1/VH1 epitope-binding site that is capable of binding to a first antigen (*i.e.*, either ADAM9 or a molecule that comprises a second epitope), as well as a VL2/VH2 epitope-binding site that is capable of binding to a second antigen (*i.e.*, either the molecule that contains the second epitope or ADAM9). The first and second polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective Third Domains. Notably, the first and third polypeptide chains complex with one another to form an Fc Region that is stabilized via a disulfide bond. Such bispecific diabodies have enhanced potency. **Figures 4A** and **4B** illustrate the structures of such diabodies. Such Fc-Region-containing diabodies may have either of two orientations (**Table 3**):

Table 3		
First Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	2nd Chain	NH_2-VL2-VH1-HPD-COOH
Second Orientation	3rd Chain	NH_2-CH2-CH3-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-HPD-COOH
	2nd Chain	NH_2-VL2-VH1-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00164] In a specific embodiment, diabodies of the present invention are bispecific, bivalent (*i.e.*, possess two epitope-binding sites), Fc-containing diabodies that are composed of three total polypeptide chains (**Figures 4A-4B**). The bispecific, bivalent Fc-containing diabodies of the invention comprise one epitope-binding site immunospecific for ADAM9, and one epitope-binding site immunospecific for a second molecule. Preferably, the second molecule is a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell.

[00165] In a further embodiment, the Fc Region-containing diabodies may comprise a total of five polypeptide chains. In a particular embodiment, two of said five polypeptide chains have the same amino acid sequence. The first polypeptide chain of such a diabody contains: (i) a VH1-containing domain, (ii) a CH1-containing domain, and (iii) a Domain containing a CH2-CH3 sequence. The first polypeptide chain may be the heavy chain of an antibody that contains a VH1 and a heavy chain constant region. The second and fifth polypeptide chains of such a diabody contain: (i) a VL1-containing domain, and (ii) a CL-containing domain. The second and/or fifth polypeptide chains of such a diabody may be light chains of an antibody that contains a VL1 complementary to the VH1 of the first/third polypeptide chain. The first, second and/or fifth polypeptide chains may be isolated from a naturally occurring antibody. Alternatively, they may be constructed recombinantly. The third polypeptide chain of such a diabody contains: (i) a VH1-containing domain, (ii) a CH1-containing domain, (iii) a Domain containing a CH2-CH3 sequence, (iv) a VL2-containing Domain, (v) a VH3-containing Domain and (vi) a Heterodimer-Promoting Domain, where the Heterodimer-Promoting Domains promote the dimerization of the third chain with the fourth chain. The fourth polypeptide of such diabodies contains: (i) a VL3-containing

Domain, (ii) a VH2-containing Domain and (iii) a Domain that promotes heterodimerization and covalent bonding with the diabody's third polypeptide chain.

[00166] Thus, the first and second, and the third and fifth, polypeptide chains of such diabodies associate together to form two VL1/VH1 epitope-binding sites capable of binding a first epitope. The third and fourth polypeptide chains of such diabodies associate together to form a VL2/VH2 epitope-binding site that is capable of binding to a second epitope, as well as a VL3/VH3 binding site that is capable of binding to a third epitope. The first and third polypeptides are bonded to one another through a disulfide bond involving cysteine residues in their respective constant regions. Notably, the first and third polypeptide chains complex with one another to form an Fc Region. Such multispecific diabodies have enhanced potency. **Figure 5** illustrates the structure of such diabodies. It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains may be the same or different so as to permit binding that is monospecific, bispecific or trispecific. As provided herein, these domains are preferably selected so as to bind an epitope of ADAM9, an epitope of second molecule and optionally an epitope of a third molecule.

[00167] The VL and VH Domains of the polypeptide chains are selected so as to form VL/VH binding sites specific for a desired epitope. The VL/VH binding sites formed by the association of the polypeptide chains may be the same or different so as to permit tetravalent binding that is monospecific, bispecific, trispecific or tetraspecific. In particular, the VL and VH Domains maybe selected such that a multivalent diabody may comprise two binding sites for a first epitope and two binding sites for a second epitope, or three binding sites for a first epitope and one binding site for a second epitope, or two binding sites for a first epitope, one binding site for a second epitope and one binding site for a third epitope (as depicted in **Figure 5**). The general structure of the polypeptide chains of representative five-chain Fc Region-containing diabodies of invention is provided in **Table 4**:

Table 4		
Bispecific (2x2)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL2-VH2-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL2-VH2-HPD-COOH
Bispecific (3x1)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL1-VH2-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL2-VH1-HPD-COOH
Trispecific (2x1x1)	2nd Chain	NH_2-VL1-CL-COOH
	1st Chain	NH_2-VH1-CH1-CH2-CH3-COOH
	3rd Chain	NH_2-VH1-CH1-CH2-CH3-VL2-VH3-HPD-COOH
	5nd Chain	NH_2-VL1-CL-COOH
	4th Chain	NH_2-VL3-VH2-HPD-COOH

HPD = Heterodimer-Promoting Domain

[00168] In a specific embodiment, diabodies of the present invention are bispecific, tetravalent (*i.e.*, possess four epitope-binding sites), Fc-containing diabodies that are composed of five total polypeptide chains having two epitope-binding sites immunospecific for ADAM9 (which may be capable of binding to the same epitope of ADAM9 or to different epitopes of ADAM9), and two epitope-binding sites specific for a second molecule (which may be capable of binding to the same epitope of the second molecule or to different epitopes of the second molecule). In another embodiment, the bispecific, tetravalent, Fc-containing diabodies of the invention comprise three epitope-binding sites immunospecific for ADAM9 (which may be capable of binding to the same epitope of ADAM9 or to two or three different epitopes of ADAM9), and one epitope-binding site specific for a second molecule. In another embodiment, the bispecific, tetravalent, Fc-containing diabodies of the invention comprise one epitope-binding site immunospecific for ADAM9, and three epitope-binding sites specific for a second molecule (which may be capable of binding to the same epitope of the second molecule or to two or three different epitopes of the second molecule). As provided above, the VL and VH domains may be selected to permit trispecific binding. Accordingly, the invention also encompasses trispecific, tetravalent, Fc-

containing diabodies. The trispecific, tetravalent, Fc-containing diabodies of the invention comprise two epitope-binding sites immunospecific for ADAM9, one epitope-binding site immunospecific for a second molecule, and one epitope-binding site immunospecific for a third molecule. In certain embodiments, the second molecule is a molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In certain embodiments, the second molecule is CD3 and the third molecule is CD8.

D. Trivalent Binding Molecules Containing Fc Regions

[00169] A further embodiment of the present invention relates to trivalent binding molecules comprising an Fc Region capable of simultaneously binding a first epitope, a second epitope and a third epitope, wherein at least one of such epitopes is not identical to another. Such trivalent binding molecules comprise three epitope-binding sites, two of which are Diabody-Type Binding Domains, which provide binding Site A and binding Site B, and one of which is a Fab-Type Binding Domain, or an scFv-Type Binding Domain, which provides binding Site C (see, *e.g.*, **Figures 6A-6F**, and PCT Publication Nos: WO 2015/184207; and WO 2015/184203). Such trivalent binding molecules thus comprise "**VL1**" / "**VH1**" domains that are capable of binding to the first epitope and "**VL2**" / "**VH2**" domains that are capable of binding to the second epitope and "**VL3**" and "**VH3**" domains that are capable of binding to the "third" epitope of such trivalent binding molecule. A "Diabody-Type Binding Domain" is the type of epitope-binding site present in a diabody, and especially, a DART® diabody, as described above. Each of a "Fab-Type Binding Domain" and an "scFv-Type Binding Domain" are epitope-binding sites that are formed by the interaction of the VL Domain of an immunoglobulin light chain and a complementing VH Domain of an immunoglobulin heavy chain. Fab-Type Binding Domains differ from Diabody-Type Binding Domains in that the two polypeptide chains that form a Fab-Type Binding Domain comprise only a single epitope-binding site, whereas the two polypeptide chains that form a Diabody-Type Binding Domain comprise at least two epitope-binding sites. Similarly, scFv-Type Binding Domains also differ from Diabody-Type Binding Domains in that they comprise only a single epitope-binding site. Thus, as used herein Fab-Type, and scFv-Type Binding Domains are distinct from Diabody-Type Binding Domains.

[00170] Typically, the trivalent binding molecules of the present invention will comprise four different polypeptide chains (see **Figures 6A-6B**), however, the molecules

may comprise fewer or greater numbers of polypeptide chains, for example by fusing such polypeptide chains to one another (*e.g.*, via a peptide bond) or by dividing such polypeptide chains to form additional polypeptide chains, or by associating fewer or additional polypeptide chains via disulfide bonds. **Figures 6C-6F** illustrate this aspect of the present invention by schematically depicting such molecules having three polypeptide chains. As provided in **Figures 6A-6F**, the trivalent binding molecules of the present invention may have alternative orientations in which the Diabody-Type Binding Domains are N-terminal (**Figures 6A**, **6C** and **6D**) or C-terminal (**Figures 6B**, **6E** and **6F**) to an Fc Region.

[00171] In certain embodiments, the first polypeptide chain of such trivalent binding molecules of the present invention contains: (i) a VL1-containing Domain, (ii) a VH2-containing Domain, (iii) a Heterodimer-Promoting Domain, and (iv) a Domain containing a CH2-CH3 sequence. The VL1 and VL2 Domains are located N-terminal or C-terminal to the CH2-CH3-containing domain as presented in **Table 4** (also see, **Figures 6A** and **6B**). The second polypeptide chain of such embodiments contains: (i) a VL2-containing Domain, (ii) a VH1-containing Domain, and (iii) a Heterodimer-Promoting Domain. The third polypeptide chain of such embodiments contains: (i) a VH3-containing Domain, (ii) a CH1-containing Domain and (iii) a Domain containing a CH2-CH3 sequence. The third polypeptide chain may be the heavy chain of an antibody that contains a VH3 and a heavy chain constant region, or a polypeptide that contains such domains. The fourth polypeptide of such embodiments contains: (i) a VL3-containing Domain and (ii) a CL-containing Domain. The fourth polypeptide chains may be a light chain of an antibody that contains a VL3 complementary to the VH3 of the third polypeptide chain, or a polypeptide that contains such domains. The third or fourth polypeptide chains may be isolated from naturally occurring antibodies. Alternatively, they may be constructed recombinantly, synthetically or by other means.

[00172] The Light Chain Variable Domain of the first and second polypeptide chains are separated from the Heavy Chain Variable Domains of such polypeptide chains by an intervening spacer peptide having a length that is too short to permit their VL1/VH2 (or their VL2/VH1) domains to associate together to form epitope-binding site capable of binding to either the first or second epitope. A preferred intervening spacer peptide (Linker 1) for this purpose has the sequence (**SEQ ID NO:69**): GGGSGGGG. Other Domains of the trivalent binding molecules may be separated by one or more intervening spacer peptides

(Linkers), optionally comprising a cysteine residue. In particular, as provided above, such Linkers will typically be incorporated between Variable Domains (*i.e.*, VH or VL) and peptide Heterodimer-Promoting Domains (*e.g.*, an E-coil or K-coil) and between such peptide Heterodimer-Promoting Domains (*e.g.*, an E-coil or K-coil) and CH2-CH3 Domains. Exemplary linkers useful for the generation of trivalent binding molecules are provided above and are also provided in PCT Application Nos: PCT/US15/33081; and PCT/US15/33076. Thus, the first and second polypeptide chains of such trivalent binding molecules associate together to form a VL1/VH1 binding site capable of binding a first epitope, as well as a VL2/VH2 binding site that is capable of binding to a second epitope. The third and fourth polypeptide chains of such trivalent binding molecules associate together to form a VL3/VH3 binding site that is capable of binding to a third epitope.

[00173] As described above, the trivalent binding molecules of the present invention may comprise three polypeptides. Trivalent binding molecules comprising three polypeptide chains may be obtained by linking the domains of the fourth polypeptide N-terminal to the VH3-containing Domain of the third polypeptide (*e.g.*, using an intervening spacer peptide (**Linker 4**)). Alternatively, a third polypeptide chain of a trivalent binding molecule of the invention containing the following domains is utilized: (i) a VL3-containing Domain, (ii) a VH3-containing Domain, and (iii) a Domain containing a CH2-CH3 sequence, wherein the VL3 and VH3 are spaced apart from one another by an intervening spacer peptide that is sufficiently long (at least 9 or more amino acid residues) so as to allow the association of these domains to form an epitope-binding site. One preferred intervening spacer peptide for this purpose has the sequence: GGGGSGGGGSGGGGS (**SEQ ID NO:100**).

[00174] It will be understood that the VL1/VH1, VL2/VH2, and VL3/VH3 Domains of such trivalent binding molecules may be different so as to permit binding that is monospecific, bispecific or trispecific. In particular, the VL and VH Domains may be selected such that a trivalent binding molecule comprises two binding sites for a first epitope and one binding sites for a second epitope, or one binding site for a first epitope and two binding sites for a second epitope, or one binding site for a first epitope, one binding site for a second epitope and one binding site for a third epitope.

[00175] However, as provided herein, these domains are preferably selected so as to bind an epitope of ADAM9, an epitope of second molecule, and an epitope of a third molecule. In certain embodiments, the second molecule is a molecule (*e.g.*, CD2, CD3,

CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In certain embodiments, the third molecule is CD8.

[00176] The general structure of the polypeptide chains of representative trivalent binding molecules of invention is provided in **Figures 6A-6F** and in **Table 5**:

Table 5

Four Chain 1st Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VH3-CH1-CH2-CH3-COOH
	2nd Chain	NH_2-VL3-CL-COOH
Four Chain 2nd Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-HPD-COOH
	3rd Chain	NH_2-VH3-CH1-CH2-CH3-COOH
	2nd Chain	NH_2-VL3-CL-COOH
Three Chain 1st Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-VL1-VH2-HPD-CH2-CH3-COOH
	3rd Chain	NH_2-VL3-VH3-HPD-CH2-CH3-COOH
Three Chain 2nd Orientation	2nd Chain	NH_2-VL2-VH1-HPD-COOH
	1st Chain	NH_2-CH2-CH3-VL1-VH2-HPD-COOH
	3rd Chain	NH_2-VL3-VH3-HPD-CH2-CH3-COOH

HPD = Heterodimer-Promoting Domain

[00177] One embodiment of the present invention relates to trivalent binding molecules that comprise two epitope-binding sites for ADAM9 and one epitope-binding site for a second molecule. The two epitope-binding sites for ADAM9 may bind the same epitope or different epitopes. Another embodiment of the present invention relates to trivalent binding molecules that comprise, one epitope-binding site for ADAM9 and two epitope-binding sites for a second molecule. The two epitope-binding sites for the second molecule may bind the same epitope or different epitopes of the second molecule. A further embodiment of the present invention relates to trispecific trivalent binding molecules that comprise, one epitope-binding site for ADAM9, one epitope-binding site for a second molecule, and one epitope-binding site for a third molecule. In certain embodiments, the second molecule is a

molecule (*e.g.*, CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), NKG2D, *etc.*) present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. In certain embodiments, the second molecule is CD3 and the third molecule is CD8. As provided above, such trivalent binding molecules may comprise three, four, five, or more polypeptide chains.]]

IX. **Constant Domains** and **Variant Fc Regions**

[00178] Provided herein are antibody "**Constant Domains**" useful in the generation of the ADAM9-binding molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, etc.) of the invention.

[00179] A preferred CL Domain is a human IgG CL Kappa Domain. The amino acid sequence of an exemplary human CL Kappa Domain is (**SEQ ID NO:101**):

RTVAAPSVFI FPPSDEQLKS GTASVVCLLN NFYPREAKVQ WKVDNALQSG
NSQESVTEQD SKDSTYSLSS TLTLSKADYE KHKVYACEVT HQGLSSPVTK
SFNRGEC

[00180] Alternatively, an exemplary CL Domain is a human IgG CL Lambda Domain. The amino acid sequence of an exemplary human CL Lambda Domain is (**SEQ ID NO:102**):

QPKAAPSVTL FPPSSEELQA NKATLVCLIS DFYPGAVTVA WKADSSPVKA
GVETTPSKQS NNKYAASSYL SLTPEQWKSH RSYSCQVTHE GSTVEKTVAP
TECS

[00181] As provided herein, the ADAM9-binding molecules of the invention may comprise an Fc Region. The Fc Region of such molecules of the invention may be of any isotype (*e.g.*, IgG1, IgG2, IgG3, or IgG4). The ADAM9-binding molecules of the invention may further comprise a CH1 Domain and/or a Hinge Region. When present, the CH1 Domain and/or Hinge Region may be of any isotype (*e.g.*, IgG1, IgG2, IgG3, or IgG4), and is preferably of the same isotype as the desired Fc Region.

[00182] An exemplary CH1 Domain is a human IgG1 CH1 Domain. The amino acid sequence of an exemplary human IgG1 CH1 Domain is (**SEQ ID NO:103**):

ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKRV

[00183] An exemplary CH1 Domain is a human IgG2 CH1 Domain. The amino acid sequence of an exemplary human IgG2 CH1 Domain is (**SEQ ID NO:104**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSNFGTQT YTCNVDHKPS NTKVDKTV

[00184] An exemplary CH1 Domain is a human IgG3 CH1 Domain. The amino acid sequence of an exemplary human IgG3 CH1 Domain is (**SEQ ID NO:207**):

ASTKGPSVFP LAPCSRSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YTCNVNHKPS NTKVDKRV

[00185] An exemplary CH1 Domain is a human IgG4 CH1 Domain. The amino acid sequence of an exemplary human IgG4 CH1 Domain is (**SEQ ID NO:105**):

ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV
HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRV

[00186] One exemplary Hinge Region is a human IgG1 Hinge Region. The amino acid sequence of an exemplary human IgG1 Hinge Region is (**SEQ ID NO:96**): EPKSCDKTHTCPPCP.

[00187] Another exemplary Hinge Region is a human IgG2 Hinge Region. The amino acid sequence of an exemplary human IgG2 Hinge Region is (**SEQ ID NO:97**): ERKCCVECPPCP.

[00188] Another exemplary Hinge Region is a human IgG4 Hinge Region. The amino acid sequence of an exemplary human IgG4 Hinge Region is (**SEQ ID NO:98**): ESKYGPPCPSCP. As described above, an IgG4 Hinge Region may comprise a stabilizing mutation, such as the S228P substitution. The amino acid sequence of an exemplary stabilized IgG4 Hinge Region is (**SEQ ID NO:99**): ESKYGPPCPPCP.

[00189] The Fc Region of the Fc Region-containing molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, *etc.*) of the present invention may be either a complete Fc Region (*e.g.*, a complete IgG Fc Region) or only a fragment of an Fc Region. Optionally, the Fc Region of the Fc Region-containing molecules of the present invention lacks the C-terminal lysine amino acid residue.

[00190] 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 Region of antibodies or immune complexes to specialized cell surface receptors on hematopoietic 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 exemplary wild-type IgG1 (**SEQ ID NO:1**), IgG2 (**SEQ ID NO:2**), IgG3 (**SEQ ID NO:3**), and IgG4 (**SEQ ID NO:4**) are presented above.

[00191] Modification of the Fc Region may lead to an altered phenotype, for example altered serum half-life, altered stability, altered susceptibility to cellular enzymes or altered effector function. It may therefore be desirable to modify an Fc Region-containing ADAM9-binding molecule of the present invention with respect to effector function, for example, so as to enhance the effectiveness of such molecule in treating cancer. Reduction or elimination of effector function is desirable in certain cases, for example in the case of antibodies whose mechanism of action involves blocking or antagonism, but not killing of the cells bearing a target antigen. Increased effector function is generally desirable when directed to undesirable cells, such as tumor and foreign cells, where the FcγRs are expressed at low levels, for example, tumor-specific B cells with low levels of FcγRIIB (*e.g.*, non-Hodgkin's lymphoma, CLL, and Burkitt's lymphoma). Molecules of the invention possessing such conferred or altered effector function activity are useful for the treatment and/or prevention of a disease, disorder or infection in which an enhanced efficacy of effector function activity is desired.

[00192] Accordingly, in certain embodiments, the Fc Region of the Fc Region-containing molecules of the present invention may be an engineered variant Fc Region. Although the Fc Region of the bispecific Fc Region-containing molecules of the present invention may possess the ability to bind to one or more Fc receptors (*e.g.*, FcγR(s)), more preferably such variant Fc Region 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 Region), *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 Region of the Fc Region-containing molecules of the present invention may include some or all of the CH2 Domain and/or some or all of the CH3 Domain of a complete Fc Region, 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 Region). Such Fc Regions may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc Regions, 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.*).

[00193] Fc Region modifications identified as altering effector function are known in the art, including modifications that increase binding to activating receptors (*e.g.*, FcγRIIA (CD16A) and reduce binding to inhibitory receptors (*e.g.*, FcγRIIB (CD32B) (see, *e.g.*, Stavenhagen, J.B. *et al.* (2007) "*Fc Optimization Of Therapeutic Antibodies Enhances Their Ability To Kill Tumor Cells In Vitro And Controls Tumor Expansion In Vivo Via Low-Affinity Activating Fcgamma Receptors*," Cancer Res. 57(18):8882-8890). **Table 6** lists exemplary single, double, triple, quadruple and quintuple substitutions (numbering is that of the EU index as in Kabat, and substitutions are relative to the amino acid sequence of **SEQ ID NO:1**) of exemplary modification that increase binding to activating receptors and/or reduce binding to inhibitory receptors.

Table 6
Variations of Preferred Activating Fc Regions

Single-Site Variations			
F243L	R292G	D270E	R292P
Y300L	P396L		
Double-Site Variations			
F243L and R292P	F243L and Y300L	F243L and P396L	R292P and Y300L
D270E and P396L	R292P and V305I	P396L and Q419H	P247L and N421K
R292P and P396L	Y300L and P396L	R255L and P396L	R292P and P305I
K392T and P396L			

Table 6 Variations of Preferred Activating Fc Regions	
Triple-Site Variations	
F243L, P247L and N421K	P247L, D270E and N421K
F243L, R292P and Y300L	R255L, D270E and P396L
F243L, R292P and V305I	D270E, G316D and R416G
F243L, R292P and P396L	D270E, K392T and P396L
F243L, Y300L and P396L	D270E, P396L and Q419H
V284M, R292L and K370N	R292P, Y300L and P396L
Quadruple-Site Variations	
L234F, F243L, R292P and Y300L	F243L, P247L, D270E and N421K
L234F, F243L, R292P and Y300L	F243L, R255L, D270E and P396L
L235I, F243L, R292P and Y300L	F243L, D270E, G316D and R416G
L235Q, F243L, R292P and Y300L	F243L, D270E, K392T and P396L
P247L, D270E, Y300L and N421K	F243L, R292P, Y300L, and P396L
R255L, D270E, R292G and P396L	F243L, R292P, V305I and P396L
R255L, D270E, Y300L and P396L	F243L, D270E, P396L and Q419H
D270E, G316D, P396L and R416G	
Quintuple-Site Variations	
L235V, F243L, R292P, Y300L and P396L	F243L, R292P, V305I, Y300L and P396L
L235P, F243L, R292P, Y300L and P396L	

[00194] Exemplary variants of human IgG1 Fc Regions with reduced binding to CD32B and/or increased binding to CD16A contain F243L, R292P, Y300L, V305I or P396L substitutions, wherein the numbering is that of the EU index as in Kabat. These amino acid substitutions may be present in a human IgG1 Fc Region in any combination. In one embodiment, the variant human IgG1 Fc Region contains a F243L, R292P and Y300L substitution. In another embodiment, the variant human IgG1 Fc Region contains a F243L, R292P, Y300L, V305I and P396L substitution.

[00195] In certain embodiments, it is preferred for the Fc Regions of ADAM9-binding molecules of the present invention to 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 Region (**SEQ ID NO:1**). In a specific embodiment, the ADAM9-binding molecules of the present invention comprise an IgG Fc Region that exhibits reduced ADCC effector function. In a preferred embodiment the CH2-CH3 Domains of such ADAM9-binding molecules include any 1, 2, 3, or 4 of the substitutions: L234A, L235A, D265A, N297Q, and N297G, wherein the numbering is that of the EU index as in Kabat. In another embodiment, 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 region 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 Region (**SEQ ID NO:1**)) is utilized. In a specific embodiment, the ADAM9-binding molecules of the present invention comprise an IgG2 Fc Region (**SEQ ID NO:2**) or an IgG4 Fc Region (**SEQ ID:NO:4**). When an IgG4 Fc Region is utilized, the instant invention also encompasses the introduction of a stabilizing mutation, such as the Hinge Region S228P substitution described above (see, *e.g.*, **SEQ ID NO:99**). Since the N297G, N297Q, L234A, L235A and D265A substitutions abolish effector function, in circumstances in which effector function is desired, these substitutions would preferably not be employed.

[00196] A preferred IgG1 sequence for the CH2 and CH3 Domains of the Fc Region-containing molecules of the present invention having reduced or abolished effector function will comprise the substitutions L234A/L235A (shown underlined) (**SEQ ID NO:106**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT 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.

[00197] A second preferred IgG1 sequence for the CH2 and CH3 Domains of the Fc Region-containing molecules of the present invention comprises an S442C substitution (shown underlined), so as to permit two CH3 domains to be covalently bonded to one another via a disulfide bond or to permit conjugation of a drug moiety. The amino acid sequence of such molecule is (**SEQ ID NO:107**):

APELLGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS L**C**LSPG**X**

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

[00198] A third preferred IgG1 sequence for the CH2 and CH3 Domains of the Fc Region-containing molecules of the present invention comprises the L234A/L235A substitutions (shown underlined) that reduce or abolish effector function and the S442C

substitution (shown underlined) that permits two CH3 domains to be covalently bonded to one another via a disulfide bond or conjugation of a drug moiety. The amino acid sequence of such molecule is (**SEQ ID NO:108**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLTCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS L**C**LSPG**X**

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

[00199] The serum half-life of proteins comprising Fc Regions may be increased by increasing the binding affinity of the Fc Region 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 (*e.g.*, a human patient or other mammal) body 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 administered molecule.

[00200] In some embodiments, the ADAM9-binding molecules of the present invention comprise a variant Fc Region that comprises at least one amino acid modification relative to a wild-type Fc Region, such that said molecule has an increased half-life (relative to a molecule comprising a wild-type Fc Region). In some embodiments, the ADAM9-binding molecules of the present invention comprise a variant IgG Fc Region, wherein said variant Fc Region comprises a half-live extending amino acid substitution at one or more positions selected from the group consisting of 238, 250, 252, 254, 256, 257, 256, 265, 272, 286, 288, 303, 305, 307, 308, 309, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424, 428, 433, 434, 435, and 436, wherein the numbering is that of the EU index as in Kabat. Numerous mutations capable of increasing the half-life of an Fc Region-containing molecule are known in the art and include, for example M252Y, S254T, T256E, and combinations thereof. For example, see the mutations described in U.S. Patent Nos. 6,277,375, 7,083,784; 7,217,797, 8,088,376; U.S. Publication Nos. 2002/0147311; 2007/0148164; and PCT Publication Nos. WO 98/23289; WO 2009/058492; and WO 2010/033279, which are herein incorporated by reference in their entireties. ADAM9-binding molecules with enhanced half-life also include those possessing variant Fc Regions

comprising substitutions at two or more of Fc Region residues 250, 252, 254, 256, 257, 288, 307, 308, 309, 311, 378, 428, 433, 434, 435 and 436. In particular, two or more substitutions selected from: T250Q, M252Y, S254T, T256E, K288D, T307Q, V308P, A378V, M428L, N434A, H435K, and Y436I, wherein the numbering is that of the EU index as in Kabat.

[00201] In a specific embodiment, an ADAM9-binding molecule of the present invention possesses a variant IgG Fc Region comprising the substitutions:

(A) M252Y, S254T and T256E;

(B) M252Y and S254T;

(C) M252Y and T256E;

(D) T250Q and M428L;

(E) T307Q and N434A;

(F) A378V and N434A;

(G) N434A and Y436I;

(H) V308P and N434A; or

(I) K288D and H435K.

[00202] In a preferred embodiment, an ADAM9-binding molecule of the present invention possesses a variant IgG Fc Region comprising any 1, 2, or 3 of the substitutions: M252Y, S254T and T256E. The invention further encompasses ADAM9-binding molecules possessing variant Fc Regions comprising:

(A) one or more mutations which alter effector function and/or FcγR; and

(B) one or more mutations which extend serum half-life.

[00203] A fourth preferred IgG1 sequence for the CH2 and CH3 Domains of the Fc Region-containing molecules of the present invention 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:200**):

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.

[00204] A fifth preferred IgG1 sequence for the CH2 and CH3 Domains of the Fc Region-containing molecules of the present invention comprises the L234A/L235A substitutions (shown underlined) that reduce or abolish effector function and 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:201**):

APE**AA**GGPSV 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.

[00205] A sixth preferred IgG1 sequence for the CH2 and CH3 Domains of the Fc Region-containing molecules of the present invention comprises the L234A/L235A substitutions (shown underlined) that reduce or abolish effector function and the M252Y, S254T and T256E substitutions (shown underlined), so as to extend the serum half-life and the S442C substitution (shown underlined), so as to permit two CH3 domains to be covalently bonded to one another via a disulfide bond or to permit conjugation of a drug moiety. The amino acid sequence of such molecule is (**SEQ ID NO:203**):

APE**AA**GGPSV 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 L**C**LSPG**X**

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

[00206] For certain antibodies, diabodies and trivalent binding molecules whose Fc Region-containing first and third polypeptide chains are not identical, it is desirable to reduce or prevent homodimerization from occurring between the CH2-CH3 Domains of two first polypeptide chains or between the CH2-CH3 Domains of two third polypeptide chains. The CH2 and/or CH3 Domains of such polypeptide chains need not be identical in sequence, and advantageously are modified to foster complexing between the two polypeptide chains. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a "knob", *e.g.,* tryptophan) can be introduced into the CH2 or CH3 Domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, "the hole"

(*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising CH2-CH3 Domains that forms an Fc Region to foster heterodimerization. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.*, Ridgway *et al.* (1996) *"'Knobs-Into-Holes' Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization,"* Protein Engr. 9:617-621; Atwell *et al.* (1997) *"Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library,"* J. Mol. Biol. 270: 26-35; and Xie *et al.* (2005) *"A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis,"* J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety).

[00207] A preferred knob is created by modifying an IgG Fc Region to contain the modification T366W. A preferred hole is created by modifying an IgG Fc Region to contain the modification T366S, L368A and Y407V. To aid in purifying the hole-bearing third polypeptide chain homodimer from the final bispecific heterodimeric Fc Region-containing molecule, the protein A binding site of the hole-bearing CH2 and CH3 Domains of the third polypeptide chain is preferably mutated by amino acid substitution at position 435 (H435R). Thus, the hole-bearing third polypeptide chain homodimer will not bind to protein A, whereas the bispecific heterodimer will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain. In an alternative embodiment, the hole-bearing third polypeptide chain may incorporate amino acid substitutions at positions 434 and 435 (N434A/N435K).

[00208] A preferred IgG amino acid sequence for the CH2 and CH3 Domains of the first polypeptide chain of an Fc Region-containing molecule of the present invention will have the "**knob-bearing**" sequence (**SEQ ID NO:109**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSL**W**CLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPG**X**

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

[00209] A preferred IgG amino acid sequence for the CH2 and CH3 Domains of the second polypeptide chain of an Fc Region-containing molecule of the present invention having two polypeptide chains (or the third polypeptide chain of an Fc Region-containing molecule having three, four, or five polypeptide chains) will have the "**hole-bearing**" sequence (**SEQ ID NO:110**):

APE**AA**GGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSL**S**C**A**VK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFL**V**SKL TVDKSRWQQG NVFSCSVMHE
ALHN**R**YTQKS LSLSPG**X**

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

[00210] As will be noted, the CH2-CH3 Domains of **SEQ ID NO:109** and **SEQ ID NO:110** include a substitution at position 234 with alanine and 235 with alanine, and thus form an Fc Region 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 Fc Region (**SEQ ID NO:1**). The invention also encompasses such CH2-CH3 Domains, which comprise the wild-type alanine residues, alternative and/or additional substitutions which modify effector function and/or FγR binding activity of the Fc region.

[00211] The invention also encompasses such CH2-CH3 Domains, which further comprise one or more half-live extending amino acid substitutions. In particular, the invention encompasses such hole-bearing and such knob-bearing CH2-CH3 Domains which further comprise the M252Y/S254T/T256E substitutions.

[00212] An exemplary knob-bearing CH2 and CH3 Domains comprising the L234A and L235A substitutions and further comprising the M252Y, S254T, and T256E substitutions is provided below (**SEQ ID NO:204**):

APE**AA**GGPSV FLFPPKPKDT L**Y**I**T**R**E**PEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSL**W**CLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPG**X**

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

[00213] An exemplary hole-bearing CH2 and CH3 Domains comprising the L234A and L235A substitutions and further comprising the M252Y, S254T, and T256E substitutions is provided below (**SEQ ID NO:205**):

APE**AA**GGPSV FLFPPKPKDT L**Y**I**T**R**E**PEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSL**S**C**A**VK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFL**V**SKL TVDKSRWQQG NVFSCSVMHE
ALHN**R**YTQKS LSLSPG**X**

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

[00214] It is preferred that the first polypeptide chain will have a "knob-bearing" CH2-CH3 sequence, such as that of **SEQ ID NO:109**. However, as will be recognized, a "hole-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:110** could be employed in the first polypeptide chain, in which case, a "knob-bearing" CH2-CH3 Domain (*e.g.*, **SEQ ID NO:109**) would be employed in the second polypeptide chain of an Fc Region-containing molecule of the present invention having two polypeptide chains (or in the third polypeptide chain of an Fc Region-containing molecule having three, four, or five polypeptide chains).

[00215] In other embodiments, the invention encompasses ADAM9-binding molecules comprising CH2 and/or CH3 Domains that have been engineered to favor heterodimerization over homodimerization using mutations known in the art, such as those disclosed in PCT Publication Nos. WO 2007/110205; WO 2011/143545; WO 2012/058768; and WO 2013/06867, all of which are incorporated herein by reference in their entirety.

X. Effector Cell Binding Capabilities

[00216] As provided herein, the ADAM9-binding molecules of the invention can be engineered to comprise a combination of epitope-binding sites that recognize a set of antigens unique to a target cell or tissue type. In particular, the present invention relates to multispecific ADAM9-binding molecules that are capable of binding to an epitope of ADAM9 and an epitope of a molecule present on the surface of an effector cell, such as a T lymphocyte, a natural killer (NK) cell or other mononuclear cell. For example, the ADAM9-binding molecules of the present invention may be construction to comprise an epitope-binding site that immunospecifically binds CD2, CD3, CD8, CD16, T-Cell Receptor (TCR), or NKG2D. The invention also relates to trispecific ADAM9-binding molecules that are capable of binding to an epitope of CD3 and an epitope of CD8 (see, *e.g.*, PCT Publication No. WO 2015/026894).

A. CD2 Binding Capabilities

[00217] In one embodiment, the bispecific, trispecific or multispecific ADAM9-binding molecules of the invention are capable of binding to an epitope of ADAM9 and an epitope of CD2. CD2 is a cell adhesion molecule found on the surface of T-cells and natural killer (NK) cells. CD2 enhances NK cell cytotoxicity, possibly as a promoter of NK cell nanotube formation (Mace, E.M. *et al.* (2014) "*Cell Biological Steps and Checkpoints in Accessing NK Cell Cytotoxicity*," Immunol. Cell. Biol. 92(3):245-255; Comerci, C.J. *et al.* (2012) "*CD2 Promotes Human Natural Killer Cell Membrane Nanotube Formation*," PLoS One 7(10):e47664:1-12). Molecules that specifically bind CD2 include the anti-CD2 antibody "**Lo-CD2a**."

[00218] The amino acid sequence of the VH Domain of Lo-CD2a (ATCC Accession No: 11423); **SEQ ID NO:111**) is shown below (CDR_H residues are shown underlined):

EVQLQQSGPE LQRPGASVKL SCKASGYIFT **EYYMY**WVKQR PKQGLELVG**R**
IDPEDGSIDY VEKFKKKATL TADTSSNTAY MQLSSLTSED TATYFCAR**GK**
FNYRFAYWGQ GTLVTVSS

[00219] The amino acid sequence of the VL Domain of Lo-CD2a (ATCC Accession No: 11423; **SEQ ID NO:112**) is shown below (CDR_L residues are shown underlined):

DVVLTQTPPT LLATIGQSVS ISC**RSSQSLL HSSGNTYLN**W LLQRTGQSPQ
PLIY**LVSKLE S**GVPNRFSGS GSGTDFTLKI SGVEAEDLGV YYC**MQFTHYP**
YTFGAGTKLE LK

B. CD3 Binding Capabilities

[00220] In one embodiment, the bispecific, trispecific or multispecific ADAM9-binding molecules of the invention are capable of binding to an epitope of ADAM9 and an epitope of CD3. CD3 is a T-cell co-receptor composed of four distinct chains (Wucherpfennig, K.W. *et al.* (2010) "*Structural Biology Of The T-Cell* Receptor*: Insights Into Receptor Assembly, Ligand Recognition, And Initiation Of Signaling*," Cold Spring Harb. Perspect. Biol. 2(4):a005140; pages 1-14). In mammals, the complex contains a CD3γ chain, a CD3δ chain, and two CD3ε chains. These chains associate with a molecule known as the T-Cell Receptor (TCR) in order to generate an activation signal in T lymphocytes. In the absence of CD3, TCRs do not assemble properly and are degraded (Thomas, S. *et al.* (2010) "*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*," Immunology 129(2):170–177). CD3 is found bound to the membranes of all mature T-cells, and in virtually no other cell type (see, Janeway, C.A. *et al.* (2005) In:

IMMUNOBIOLOGY: THE IMMUNE SYSTEM IN HEALTH AND DISEASE," 6th ed. Garland Science Publishing, NY, pp. 214- 216; Sun, Z. J. *et al.* (2001) "*Mechanisms Contributing To T Cell Receptor Signaling And Assembly Revealed By The Solution Structure Of An Ectodomain Fragment Of The CD3ε:γ Heterodimer*," Cell 105(7):913-923; Kuhns, M.S. *et al.* (2006) "*Deconstructing The Form And Function Of The TCR/CD3 Complex*," Immunity. 2006 Feb;24(2):133-139). Molecules that specifically binds CD3 include the anti-CD3 antibodies "**CD3 mAb-1**" and "**OKT3**." The anti-CD3 antibody CD3 mAb-1 is capable of binding non-human primates (*e.g.*, cynomolgus monkey).

[00221] The amino acid sequence of the VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:113**) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **TYAMN**WVRQA PGKGLEWVG**R**
IRSKYNNYAT **YYADSVKD**RF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS **WFAY**WGQGTL VTVSS

[00222] The amino acid sequence of an alternative VH Domain of **CD3 mAb-1 VH(2)** (**SEQ ID NO:114**) is shown below (CDR_H residues are shown in single underline; differences relative to the VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:92**) are shown in double underline).

EVQLVESGGG LVQPGGSLRL SCAASGFTF***N*** **TYAMN**WVRQA PGKGLEWV***A***R
IRSKYNNYAT **YYADSVKD**RF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS **WFAY**WGQGTL VTVSS

[00223] The amino acid sequence of the VL Domain of CD3 mAb-1 (**SEQ ID NO:115**) is shown below (CDR_L residues are shown underlined):

QAVVTQEPSL TVSPGGTVTL TC**RSSTGAVT** **TSNYAN**WVQQ KPGQAPRGLI
G**GTNKRAP**WT PARFSGSLLG GKAALTITGA QAEDEADYYC **ALWYSNLWV**F
GGGTKLTVLG

[00224] The VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:**) may be used with the VL Domain of CD3 mAb-1 (**SEQ ID NO:**) to form a functional CD3-binding molecule in accordance with the present invention. Likewise, the VH Domain of **CD3 mAb-1 VH(2)** (**SEQ ID NO:**) may be used with the VL Domain of CD3 mAb-1 (**SEQ ID NO:**) to form a functional CD3-binding molecule in accordance with the present invention.

[00225] As discussed below, in order to illustrate the present invention, bispecific ADAM9 x CD3-binding molecules were produced. In some of the ADAM9 x CD3 constructs, a variant of CD3 mAb-1 was employed. The variant "**CD3 mAb-1 (D65G)**,"

comprises the VL Domain of CD3 mAb-1 (**SEQ ID NO:115**) and a VH CD3 mAb-1 Domain having a D65G substitution (Kabat position 65, corresponding to residue 68 of **SEQ ID NO:113)**.

[00226] The amino acid sequence of the VH Domain of **CD3 mAb-1 (D65G)** (**SEQ ID NO:116**) is shown below (CDR_H residues are shown underlined, the substituted position (D65G) is shown in double underline):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **TYAMN**WVRQA PGKGLEWVG**R**
IRSKYNNYAT **YYADSVKG**RF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYVS **WFAY**WGQGTL VTVSS

[00227] Alternatively, an affinity variant of CD3 mAb-1 may be incorporated. Variants include a low affinity variant designated "**CD3 mAb-1 Low**" and a variant having a faster off rate designated "**CD3 mAb-1 Fast.**" The VL Domain of CD mAb1 (**SEQ ID NO:115**) is common to CD3 mAb-1 Low and CD3 mAb1 Fast and is provided above. The amino acid sequences of the VH Domains of each of CD3 mAb-1 Low and CD3 mAb-1 Fast are provided below.

[00228] The amino acid sequence of the VH Domain of anti-human CD3 mAb-1 Low (**SEQ ID NO:117**) is shown below (CDR_H residues are shown underlined; differences relative to the VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:113**) are shown in double underline):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **TYAMN**WVRQA PGKGLEWVG**R**
IRSKYNNYAT **YYADSVKG**RF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
HGNFGNSYV*T* **WFAY**WGQGTL VTVSS

[00229] The amino acid sequence of the VH Domain of anti-human CD3 mAb-1 Fast (**SEQ ID NO:118**) is shown below (CDR_H residues are shown underlined; differences relative to the VH Domain of **CD3 mAb-1 VH(1)** (**SEQ ID NO:113**) are shown in double underline):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS **TYAMN**WVRQA PGKGLEWVG**R**
IRSKYNNYAT **YYADSVKG**RF TISRDDSKNS LYLQMNSLKT EDTAVYYCVR
H*K*NFGNSYV*T* **WFAY**WGQGTL VTVSS

[00230] Another anti-CD3 antibody, which may be utilized is antibody Muromonab-CD3 "**OKT3**" (Xu *et al.* (2000) *"In Vitro Characterization Of Five Humanized OKT3 Effector Function Variant Antibodies,"* Cell. Immunol. 200:16-26; Norman, D.J. (1995) "*Mechanisms Of Action And Overview Of OKT3*," Ther. Drug Monit. 17(6):615-620;

Canafax, D.M. *et al.* (1987) "*Monoclonal Antilymphocyte Antibody (OKT3)* Treatment *Of Acute Renal Allograft Rejection*," Pharmacotherapy 7(4):121-124; Swinnen, L.J. *et al.* (1993) "*OKT3 Monoclonal Antibodies Induce Interleukin-6 And Interleukin-10: A Possible Cause Of Lymphoproliferative Disorders Associated With Transplantation*," Curr. Opin. Nephrol. Hypertens. 2(4):670-678).

[00231] The amino acid sequence of the VH Domain of **OKT3** (**SEQ ID NO:119**) is shown below (CDR_H residues are shown underlined):

QVQLQQSGAE LARPGASVKM SCKASGYTFT **RYTMH**WVKQR PGQGLEWIG**Y**
INPSRGYTNY **NQKFKD**KATL TTDKSSSTAY MQLSSLTSED SAVYYCAR**YY**
DDHYCLDYWG QGTTLTVSS

[00232] The amino acid sequence of the VL Domain of **OKT3** (**SEQ ID NO:120**) is shown below (CDR_L residues are shown underlined):

QIVLTQSPAI MSASPGEKVT MTC**SASSSVS** **YMN**WYQQKSG TSPKRWIY**DT**
SKLASGVPAH FRGSGSGTSY SLTISGMEAE DAATYYC**QQW** **SSNPFTF**GSG
TKLEINR

[00233] Additional anti-CD3 antibodies that may be utilized include but are not limited to those described in PCT Publication Nos. WO 2008/119566; and WO 2005/118635.

C. CD8 Binding Capabilities

[00234] In one embodiment, the bispecific, trispecific or multispecific ADAM9-binding molecules of the invention are capable of binding to an epitope of ADAM9 and an epitope of CD8. CD8 is a T-cell co-receptor composed of two distinct chains (Leahy, D.J., (1995) "*A Structural View of CD4 and CD8*," FASEB J., 9:17-25) that is expressed on Cytotoxic T-cells. The activation of $CD8^+$ T-cells has been found to be mediated through co-stimulatory interactions between an antigen:major histocompability class I (**MHC I**) molecule complex that is arrayed on the surface of a target cell and a complex of CD8 and the T-cell Receptor, that are arrayed on surface of the $CD8^+$ T-cell (Gao, G., and Jakobsen, B., (2000). "*Molecular interactions of coreceptor CD8 and MHC class I: the molecular basis for functional coordination with the T-Cell Receptor*". Immunol Today 21: 630–636). Unlike MHC II molecules, which are expressed by only certain immune system cells, MHC I molecules are very widely expressed. Thus, cytotoxic T-cells are capable of binding to a wide variety of cell types. Activated cytotoxic T-cells mediate cell killing through their

release of the cytotoxins perforin, granzymes, and granulysin. Antibodies that specifically bind CD8 include the anti-CD8 antibodies "**OKT8**" and "**TRX2**."

[00235] The amino acid sequence of the VH Domain of OKT8 (**SEQ ID NO:121**) is shown below (CDR_H residues are shown underlined):

QVQLLESGPE LLKPGASVKM SCKA**SGYTFT** **DYNMH**WVKQS HGKSLEWIG**Y**
IYPYTGGTGY **NQKFKN**KATL TVDSSSSTAY MELRSLTSED SAVYYCARNF
RYTYWYFDVW GQGTTVTVSS

[00236] The amino acid sequence of the VL Domain of OKT8 (**SEQ ID NO:122**) is shown below (CDR_L residues are shown underlined):

DIVMTQSPAS LAVSLGQRAT ISCRASESVD **SYDNSLMH**WY QQKPGQPPKV
LIY**LASNLES** GVPARFSGSG SRTDFTLTID PVEADDAATY YC**QQNNEDPY**
TFGGGTKLEI KR

[00237] The amino acid sequence of the VH Domain of TRX2 (**SEQ ID NO:123**) is shown below (CDR_H residues are shown underlined):

QVQLVESGGG VVQPGRSLRL SCAASGFTFS **DFGMN**WVRQA PGKGLEWVA**L**
IYYDGSNKFY **ADSVKG**RFTI SRDNSKNTLY LQMNSLRAED TAVYYCAK**PH**
YDGYYHFFDS WGQGTLVTVS S

[00238] The amino acid sequence of the VL Domain of TRX2 (**SEQ ID NO:124**) is shown below (CDR_L residues are shown underlined):

DIQMTQSPSS LSASVGDRVT ITC**KGSQDIN** **NYLA**WYQQKP GKAPKLLIY**N**
TDILHTGVPS RFSGSGSGTD FTFTISSLQP EDIATYYC**YQ** **YNNGYT**FGQG
TKVEIK

D. CD16 Binding Capabilities

[00239] In one embodiment, multispecific ADAM9-binding molecules of the invention are capable of binding to an epitope of ADAM9 and an epitope of CD16. CD16 is the FcγRIIIA receptor. CD16 is expressed by neutrophils, eosinophils, natural killer (NK) cells, and tissue macrophages that bind aggregated but not monomeric human IgG (Peltz, G.A. *et al.* (1989) "*Human Fc Gamma RIII: Cloning, Expression, And Identification Of The Chromosomal Locus Of Two Fc Receptors For IgG*," Proc. Natl. Acad. Sci. (U.S.A.) 86(3):1013-1017; Bachanova, V. *et al.* (2014) "*NK Cells In Therapy Of Cancer*," Crit. Rev. Oncog. 19(1-2):133-141; Miller, J.S. (2013) "*Therapeutic Applications: Natural Killer Cells In The Clinic*," Hematology Am. Soc. Hematol. Educ. Program. 2013:247-253; Youinou, P. *et al.* (2002) "*Pathogenic Effects Of Anti-Fc Gamma Receptor IIIB (CD16) On*

Polymorphonuclear Neutrophils In Non-Organ-Specific Autoimmune Diseases," Autoimmun Rev. 1(1-2):13-19; Peipp, M. *et al.* (2002) "*Bispecific Antibodies Targeting Cancer Cells*," Biochem. Soc. Trans. 30(4):507-511). Molecules that specifically bind CD16 include the anti-CD16 antibodies "**3G8**" and "**A9**." Humanized A9 antibodies are described in PCT Publication No. WO 03/101485.

[00240] The amino acid sequence of the VH Domain of 3G8 (**SEQ ID NO:125**) is shown below (CDR_H residues are shown underlined):

QVTLKESGPG ILQPSQTLSL TCSFSGFSLR **TSGMGVG**WIR QPSGKGLEWL
A**HIWWDDDKR** **YNPALKS**RLT ISKDTSSNQV FLKIASVDTA DTATYYCAQ**I**
NPAWFAYWGQ GTLVTVSA

[00241] The amino acid sequence of the VL Domain of 3G8 (**SEQ ID NO:126**) is shown below (CDR_L residues are shown underlined):

DTVLTQSPAS LAVSLGQRAT ISC**KASQSVD** **FDGDSFMN**WY QQKPGQPPKL
LIY**TTSNLES** GIPARFSASG SGTDFTLNIH PVEEEDTATY YC**QQSNEDPY**
TFGGGTKLEI K

[00242] The amino acid sequence of the VH Domain of A9 (**SEQ ID NO:127**) is shown below (CDR_H residues are shown underlined):

QVQLQQSGAE LVRPGTSVKI SCKASGYTFT **NYWLG**WVKQR PGHGLEWIG**D**
IYPGGGYTNY **NEKFKG**KATV TADTSSRTAY VQVRSLTSED SAVYFCAR**SA**
SWYFDVWGAR TTVTVSS

[00243] The amino acid sequence of the VL Domain of A9 (**SEQ ID NO:128**) is shown below (CDR_L residues are shown underlined):

DIQAVVTQES ALTTSPGETV TLTC**RSNTGT** **VTTSNYAN**WV QEKPDHLFTG
LIG**HTNNRAP** GVPARFSGSL IGDKAALTIT GAQTEDEAIY FC**ALWYNNHW**
VFGGGTKLTVL

[00244] Additional anti-CD16 antibodies that may be utilized include but are not limited to those described in PCT Publication Nos. WO 03/101485; and WO 2006/125668.

E. TCR Binding Capabilities

[00245] In one embodiment, the bispecific, trispecific or multispecific ADAM9-binding molecules of the invention are capable of binding to an epitope of ADAM9 and an epitope of the T Cell Receptor (TCR). The T Cell Receptor is natively expressed by $CD4^+$ or $CD8^+$ T cells, and permits such cells to recognize antigenic peptides that are bound and presented by class I or class II MHC proteins of antigen-presenting cells. Recognition of a

pMHC (peptide–MHC) complex by a TCR initiates the propagation of a cellular immune response that leads to the production of cytokines and the lysis of the antigen-presenting cell (see, *e.g.*, Armstrong, K.M. *et al.* (2008) "*Conformational Changes And Flexibility In T-Cell Receptor Recognition Of Peptide–MHC Complexes*," Biochem. J. 415(Pt 2):183–196; Willemsen, R. (2008) "*Selection Of Human Antibody Fragments Directed Against Tumor T-Cell Epitopes For Adoptive T-Cell Therapy*," Cytometry A. 73(11):1093-1099; Beier, K.C. *et al.* (2007) "*Master Switches Of T-Cell Activation And Differentiation*," Eur. Respir. J. 29:804-812; Mallone, R. *et al.* (2005) "*Targeting T Lymphocytes For Immune Monitoring And Intervention In Autoimmune Diabetes*," Am. J. Ther. 12(6):534–550). CD3 is the receptor that binds to the TCR (Thomas, S. *et al.* (2010) "*Molecular Immunology Lessons From Therapeutic T-Cell Receptor Gene Transfer*," Immunology 129(2):170-177; Guy, C.S. *et al.* (2009) "*Organization Of Proximal Signal Initiation At The TCR:CD3 Complex*," Immunol. Rev. 232(1):7-21; St. Clair, E.W. (Epub 2009 Oct 12) "*Novel Targeted Therapies For Autoimmunity*," Curr. Opin. Immunol. 21(6):648-657; Baeuerle, P.A. *et al.* (Epub 2009 Jun 9) "*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*," Cancer Res. 69(12):4941-4944; Smith-Garvin, J.E. *et al.* (2009) "*T Cell Activation*," Annu. Rev. Immunol. 27:591-619; Renders, L. *et al.* (2003) "*Engineered CD3 Antibodies For Immunosuppression*," Clin. Exp. Immunol. 133(3):307-309).

[00246] Molecules that specifically bind to the T Cell Receptor include the anti-TCR antibody "**BMA 031**" (EP 0403156; Kurrle, R. *et al.* (1989) "*BMA 031 – A TCR-Specific Monoclonal Antibody For Clinical Application*," Transplant Proc. 21(1 Pt 1):1017-1019; Nashan, B. *et al.* (1987) "Fine Specificity Of A Panel Of Antibodies Against The TCR/CD3 Complex," Transplant Proc. 19(5):4270-4272; Shearman, C.W. *et al.* (1991) "Construction, Expression, And Biologic Activity Of Murine/Human Chimeric Antibodies With Specificity For The Human α/β T Cell," J. Immunol. 146(3):928-935; Shearman, C.W. *et al.* (1991) "*Construction, Expression And Characterization of Humanized Antibodies Directed Against The Human α/β T Cell Receptor*," J. Immunol. 147(12):4366-4373; and PCT Publication No. WO 2010/027797).

[00247] The amino acid sequence of a VH Domain of BMA 031 (**SEQ ID NO:129**) is shown below (CDR_H residues are shown underlined):

QVQLVQSGAE VKKPGASVKV SCKASGYKFT **SYVMH**WVRQA PGQGLEWIG**Y**
INPYNDVTKY **NEKFKG**RVTI TADKSTSTAY LQMNSLRSED TAVHYCAR**GS**
YYDYDGFVYW GQGTLVTVSS

[00248] The amino acid sequence of the VL Domain of BMA 031 (**SEQ ID NO:130**) is shown below (CDR_L residues are shown underlined):

EIVLTQSPAT LSLSPGERAT LSC**SATSSVS** **YMH**WYQQKPG KAPKRWIY**DT** **SKLAS**GVPSR FSGSGSGTEF TLTISSLQPE DFATYYC**QQW** **SSNPLT**FGQG TKLEIK

F. NKG2D Binding Capabilities

[00249] In one embodiment, multispecific ADAM9-binding molecules of the invention are capable of binding to an epitope of ADAM9 and an epitope of the NKG2D receptor. The NKG2D receptor is expressed on all human (and other mammalian) Natural Killer cells (Bauer, S. *et al.* (1999) "*Activation Of NK Cells And T Cells By NKG2D, A Receptor For Stress-Inducible MICA*," Science 285(5428):727-729; Jamieson, A.M. *et al.* (2002) "*The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing*," Immunity 17(1):19-29) as well as on all $CD8^+$ T cells (Groh, V. *et al.* (2001) "*Costimulation Of CD8αβ T Cells By NKG2D Via Engagement By MIC Induced On Virus-Infected Cells*," Nat. Immunol. 2(3):255-260; Jamieson, A.M. *et al.* (2002) "*The Role Of The NKG2D Immunoreceptor In Immune Cell Activation And Natural Killing*," Immunity 17(1):19-29). Such binding ligands, and particularly those which are not expressed on normal cells, include the histocompatibility 60 (H60) molecule, the product of the retinoic acid early inducible gene-1 (RAE-1), and the murine UL16-binding proteinlike transcript 1 (MULT1) (Raulet D.H. (2003) "*Roles Of The NKG2D Immunoreceptor And Its Ligands*," Nature Rev. Immunol. 3:781-790; Coudert, J.D. *et al.* (2005) "*Altered NKG2D Function In NK Cells Induced By Chronic Exposure To Altered NKG2D Ligand-Expressing Tumor Cells*," Blood 106:1711-1717). Molecules that specifically bind to the NKG2D Receptor include the anti-NKG2D antibodies "**KYK-1.0**" and "**KYK-2.0**" (Kwong, KY *et al.* (2008) "*Generation, Affinity Maturation, And Characterization Of A Human Anti-Human NKG2D Monoclonal Antibody With Dual Antagonistic And Agonistic Activity*," J. Mol. Biol. 384:1143-1156).

[00250] The amino acid sequence of the VH Domain of KYK-1.0 (**SEQ ID NO:131**) is shown below (CDR_H residues are shown underlined):

EVQLVESGGG VVQPGGSLRL SCAASGFTFS **SYGMH**WVRQA PGKGLEWVA**F** **IRYDGSNKYY** **ADSVKG**RFTI SRDNSKNTKY LQMNSLRAED TAVYYCAK**DR** **FGYYLDY**WGQ GTLVTVSS

[00251] The amino acid sequence of the VL Domain of KYK-1.0 (**SEQ ID NO:132**) is shown below (CDR_L residues are shown underlined):

QPVLTQPSSV SVAPGETARI PC**GGDDIETK** **SVH**WYQQKPG QAPVLVIY**DD**
DDRPSGIPER FFGSNSGNTA TLSISRVEAG DEADYYC**QVW** **DDNNDEWV**FG
GGTQLTVL

[00252] The amino acid sequence of a VH Domain of KYK-2.0 (**SEQ ID NO:133**) is shown below (CDR_H residues are shown underlined):

QVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYGMH**WVRQA PGKGLEWVA**F**
IRYDGSNKYY **ADSVKG**RFTI SRDNSKNTLY LQMNSLRAED TAVYYCAK**DR**
GLGDGTYFDY WGQGTTVTVS S

[00253] The amino acid sequence of a VL Domain of KYK-2.0 (**SEQ ID NO:134**) is shown below (CDR_L residues are shown underlined):

QSALTQPASV SGSPGQSITI SC**SGSSSNIG** **NNAVN**WYQQL PGKAPKLLIY
YDDLLPSGVS DRFSGSKSGT SAFLAISGLQ SEDEADYYC**A** **AWDDSLNGPV**
FGGGTKLTVL

XI. Multispecific ADAM9-Binding Molecules

A. ADAM9 x CD3 Bispecific Two Chain Diabodies

[00254] The VL and VH Domains of the above-described optimized humanized anti-ADAM9 **MAB-A** antibody is used to construct ADAM9 x CD3 bispecific diabodies composed of two covalently linked polypeptide chains and comprising the above-discussed optimized humanized VL and VH Domains of **MAB-A**. The general structure and amino acid sequences of such ADAM9 x CD3 bispecific diabodies is provided below.

[00255] The first polypeptide chain of one exemplary ADAM9 x CD3 bispecific two chain diabody comprises, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of an anti-ADAM9 antibody (*e.g.*, **hMAB-A VL (2)** (**SEQ ID NO:55**); an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:69**)); the VH Domain of an anti-CD3 antibody (*e.g.*, CD3 mAb 1 (D65G) (**SEQ ID NO:116**)); a cysteine-containing intervening spacer peptide (**Linker 2:** GGCGGG (**SEQ ID NO:70**)); a Heterodimer-Promoting (*e.g.*, an E-coil) Domain (EVAALEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:82**)); and a C-terminus.

[00256] The second polypeptide chain of such an exemplary ADAM9 x CD3 bispecific two chain diabody comprises, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of a corresponding anti-CD3 antibody (*e.g.*, a VL domain that in association with the VH Domain of the first polypeptide chain forms a CD3-binding site, *e.g.*, the VL Domain of **CD3 mAb-1** (**SEQ ID NO:115**); an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:69**)); the VH Domain of a corresponding anti-ADAM9 antibody (*e.g.*, a VH domain that in association with the VL Domain of the first polypeptide chain forms an ADAM9-binding site, *e.g.*, **hMAB-A VH (2)** (**SEQ ID NO:17**); a cysteine-containing intervening spacer peptide (**Linker 2:** GGCGGG (**SEQ ID NO:70**)); a Heterodimer-Promoting (*e.g.*, K-coil) Domain (KVAALKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:83**)); and a C-terminus.

[00257] As provided herein, alternative linkers and/or alternative Heterodimer-Promoting Domains may be utilized in the generation of such diabodies. For example, the first polypeptide chain of an alternative exemplary ADAM9 x CD3 bispecific two chain diabody may comprise, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of an anti-ADAM9 antibody; the intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:69**)); the VH Domain of the anti-CD3 antibody or of a corresponding anti-CD3 antibody; an intervening spacer peptide (**Linker 2:** ASTKG (**SEQ ID NO:74**)); a cysteine-containing Heterodimer-Promoting (*e.g.*, K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:85**)); and a C-terminus. The second polypeptide chain of such alternative exemplary diabody may comprise, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of a corresponding anti-CD3 antibody; an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:69**)); the VH Domain of a corresponding anti-ADAM9 antibody; an intervening spacer peptide (**Linker 2:** ASTKG (**SEQ ID NO:74**)); a cysteine-containing Heterodimer-Promoting (*e.g.*, E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:84**)); and a C-terminus.

[00258] A representative ADAM9 x CD3 bispecific two chain diabody ("**DART-1**") comprising the VH and VL Domains of **hMAB-A (2.2)** and the VH and VL Domains of a **CD3 mAb-1** is constructed.

[00259] The amino acid sequence of the first polypeptide chain of **DART-1** (**SEQ ID NO:135**) is shown below (the sequence of the **hMAb-A VL(2)** Domain (**SEQ ID NO:55**) is underlined; the sequence of the **CD3 mAb-1 (D65G) VH** Domain (**SEQ ID NO:116**) is italicised):

DIVMTQSPDS LAVSLGERAT ISCKASQSVD YSGDSYMNWY QQKPGQPPKL
LIYAASDLES GIPARFSGSG SGTDFTLTIS SLEPEDFATY YCQQSHEDPF
TFGQGTKLEI KGGGSGGGG*E VQLVESGGGL VQPGGSLRLS CAASGFTFST*
YAMNWVRQAP GKGLEWVGRI RSKYNNYATY YADSVKGRFT ISRDDSKNSL
*YLQMNSLKTE DTAVYYCVRH GNFGNSYVSW FAYWGQGTLV TVSS*GGCGGG
EVAALEKEVA ALEKEVAALE KEVAALEK

[00260] The amino acid sequence of the second polypeptide chain of **DART-1** (**SEQ ID NO:136**) is shown below (the sequence of the **hMAB-A VH (2)** Domain (**SEQ ID NO:17**) is underlined; the sequence of the **CD3 mAb-1 VL** Domain (**SEQ ID NO:115**) is italicised):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
*GGGTKLTVL*G GGGSGGGGEV QLVESGGGLV KPGGSLRLSC AASGFTFSSY
WMHWVRQAPG KGLEWVGEII PIFGHTNYNE KFKSRFTISL DNSKNTLYLQ
MGSLRAEDTA VYYCARGGYY YYGSRDYFDY WGQGTTVTVS SGGCGGGKVA
ALKEKVAALK EKVAALKEKV AALKE

B. ADAM9 x CD3 Bispecific Three Chain Diabodies

[00261] An ADAM9 x CD3 diabody having three chains and possessing an Fc Region is generated having one binding site specific for ADAM9 (comprising humanized/optimized VH and VL Domains of **hMAB-A**) and one binding site specific for CD3 (comprising the VL and VH Domains of CD3 mAb 1 (D65G)). The diabody is designated "**DART-2**."

[00262] The first polypeptide chain of the exemplary ADAM9 x CD3 bispecific three chain diabodies comprises, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of an anti-ADAM9 antibody (*e.g.*,**hMAB-A VL (2)** (**SEQ ID NO:55**)); an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:69**)); the VH Domain of CD3 mAb 1 (D65G) (**SEQ ID NO:116**); an intervening spacer peptide (**Linker 2:** ASTKG (**SEQ ID NO:74**)); a cysteine-containing Heterodimer-Promoting (E-coil) Domain (EVAACEK-EVAALEK-EVAALEK-EVAALEK (**SEQ ID NO:82**)); an intervening spacer peptide (**Linker 3:** GGGDKTHTCPPCP (**SEQ ID NO:94**)); a knob-bearing IgG1 CH2-CH3 Domain (**SEQ ID NO:109**); and a C-terminus. Polynucleotides encoding this polypeptide

chain may encode the C-terminal lysine residue of **SEQ ID NO:109** (*i.e.*, X of **SEQ ID NO:109**), however, as discussed above, this lysine residue may be post-translationally removed in some expression systems. Accordingly, the invention encompasses such a first polypeptide chain that contains such lysine residue (*i.e.*, **SEQ ID NO:109**, wherein X is lysine), as well as a first polypeptide chain that lacks such lysine residue (*i.e.*, **SEQ ID NO:109**, wherein X is absent). The amino acid sequences of such first polypeptide chain of **DART-2** (**SEQ ID NO:137**) is provided below (the sequence of the **hMAB-A VL (2)** Domain (**SEQ ID NO:55**) is underlined; the sequence of the **CD3 mAb-1 (D65G) VH** Domain (**SEQ ID NO:116**) is italicised):

DIVMTQSPDS LAVSLGERAT ISCKASQSVD YSGDSYMNWY QQKPGQPPKL
LIYAASDLES GIPARFSGSG SGTDFTLTIS SLEPEDFATY YCQQSHEDPF
TFGQGTKLEI KGGGSGGGG*E VQLVESGGGL VQPGGSLRLS CAASGFTFST*
YAMNWVRQAP GKGLEWVGRI RSKYNNYATY YADSVKGRFT ISRDDSKNSL
*YLQMNSLKTE DTAVYYCVRH GNFGNSYVSW FAYWGQGTLV TVSS*ASTKGE
VAACEKEVAA LEKEVAALEK EVAALEKGGG DKTHTCPPCP APEAAGGPSV
FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD GVEVHNAKTK
PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA PIEKTISKAK
GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE WESNGQPENN
YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE ALHNHYTQKS
LSLSPGX

wherein X is Lysine (K) or is absent.

[00263] The second polypeptide chain of the exemplary ADAM9 x CD3 bispecific three chain diabodies comprises, in the N-terminal to C-terminal direction: an N-terminus; the VL Domain of CD3 mAb 1 (**SEQ ID NO:115**); an intervening spacer peptide (**Linker 1:** GGGSGGGG (**SEQ ID NO:69**)); the VH Domain of an anti-ADAM9 antibody (*e.g.*, **hMAB-A VH (2)** (**SEQ ID NO:17**)); an intervening spacer peptide (**Linker 2:** ASTKG (**SEQ ID NO:74**)); a cysteine-containing Heterodimer-Promoting (K-coil) Domain (KVAACKE-KVAALKE-KVAALKE-KVAALKE (**SEQ ID NO:85**)); and a C-terminus. The amino acid sequence of such second polypeptide chain of **DART-2** (**SEQ ID NO:138**) is provided below (the sequence of the **hMAB-A VH (2)** Domain (**SEQ ID NO:17**) is underlined; the sequence of the **CD3 mAb-1 VL** Domain (**SEQ ID NO:115**) is italicised):

QAVVTQEPSL TVSPGGTVTL TCRSSTGAVT TSNYANWVQQ KPGQAPRGLI
GGTNKRAPWT PARFSGSLLG GKAALTITGA QAEDEADYYC ALWYSNLWVF
*GGGTKLTVL*G GGGSGGGGEV QLVESGGGLV KPGGSLRLSC AASGFTFSSY
WMHWVRQAPG KGLEWVGEII PIFGHTNYNE KFKSRFTISL DNSKNTLYLQ
MGSLRAEDTA VYYCARGGYY YYGSRDYFDY WGQGTTVTVS SASTKGKVAA
CKEKVAALKE KVAALKEKVA ALKE

[00264] The third polypeptide chain of the exemplary ADAM9 x CD3 bispecific three chain diabodies comprises, in the N-terminal to C-terminal direction: an N-terminus; a spacer peptide (DKTHTCPPCP (**SEQ ID NO:93**)); a hole-bearing IgG1 CH2-CH3 Domain (**SEQ ID NO:110**); and a C-terminus. Polynucleotides encoding this polypeptide chain may encode the C-terminal lysine residue of **SEQ ID NO:110** (*i.e.*, X of **SEQ ID NO:110**), however, as discussed above, this lysine residue may be post-translationally removed in some expression systems. Accordingly, the invention encompasses such a third polypeptide chain that contains such lysine residue (*i.e.*, **SEQ ID NO:110**, wherein X is lysine), as well as a third polypeptide chain that lacks such lysine residue (*i.e.*, **SEQ ID NO:110**, wherein X is absent). The amino acid sequence of such third polypeptide chain (**SEQ ID NO:139**) is provided below:

DKTHTCPPCP APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLVSKL TVDKSRWQQG
NVFSCSVMHE ALHNRYTQKS LSLSPGX

wherein X is Lysine (K) or is absent.

[00265] It will be appreciated in view of the teachings provided herein that different domain orientations, VH Domains, VL Domains, linkers, and/or heterodimer promoting domains, could be utilized to generate alternative ADAM9 x CD3 bispecific three chain diabodies. In particular, the VH Domain and VL Domain of different **hMAB-A** variants may be utilized.

C. ADAM9 x CD3 x CD8 Trivalent Binding Molecules

[00266] Exemplary trivalent "**ADAM9 x CD3 x CD8**" binding molecules having one binding site specific for ADAM9 (comprising a parental and/or humanized anti-ADAM9-VL Domain and a corresponding anti-ADAM9-VH Domain, as described above), one binding site specific for CD3 (comprising, for example, the VL Domain of **CD3 mAb-1** (**SEQ ID NO:115**) and the VH Domain of anti-CD3 antibody (*e.g.*, **CD3 mAb 1 (D65G)** (**SEQ ID NO:116**)), and one binding site specific for CD8 (comprising, for example, the VH and VL Domains of **TRX2** (**SEQ ID NOs:123** and **124**, respectively). Such trivalent binding molecules may have two polypeptide chains (see, *e.g.*, **Figure 6E**, and **Figure 6F**), three polypeptide chains (see, *e.g.*, **Figure 6C** and **Figure 6D**), four polypeptide chains (see, *e.g.*, **Figure 6A** and **Figure 6B**), or five polypeptide chains (see, *e.g.*, **Figure 5**).

XII. Methods of Production

[00267] The ADAM9-binding molecules of the present invention are most preferably produced through the recombinant expression of nucleic acid molecules that encode such polypeptides, as is well-known in the art.

[00268] Polypeptides of the invention may be conveniently prepared using solid phase peptide synthesis (Merrifield, B. (1986) "S*olid Phase Synthesis*," Science 232(4748):341-347; Houghten, R.A. (1985) "*General Method For The Rapid Solid-Phase Synthesis Of Large Numbers Of Peptides: Specificity Of Antigen-Antibody Interaction At The Level Of Individual Amino Acids*," Proc. Natl. Acad. Sci. (U.S.A.) 82(15):5131-5135; Ganesan, A. (2006) "*Solid-Phase Synthesis In The Twenty-First Century*," Mini Rev. Med. Chem. 6(1):3-10).

[00269] In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. Antibodies may be made recombinantly by first isolating the antibodies made from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (*e.g.*, CHO cells). Another method that may be employed is to express the antibody sequence in plants {*e.g.*, tobacco) or transgenic milk. Suitable methods for expressing antibodies 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; Lonberg, N. *et al.* (1995) "*Human Antibodies From Transgenic Mice*," Int. Rev. Immunol 13:65-93; and Pollock *et al.* (1999) "*Transgenic Milk As A Method For The Production Of Recombinant Antibodies*," J. Immunol Methods 231:147-157). Suitable methods for making derivatives of antibodies, *e.g.*, humanized, single-chain, *etc.* are known in the art, and have been described above. In another alternative, antibodies may be made recombinantly by phage display technology (see, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; 6,265,150; and Winter, G. *et al.* (1994) "*Making Antibodies By Phage Display Technology*," Annu. Rev. Immunol. 12.433-455).

[00270] Vectors containing polynucleotides of interest (*e.g.*, polynucleotides encoding the polypeptide chains of the ADAM9-binding molecules of the present invention) can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE- dextran, or other substances; microprojectile bombardment; lipofection;

and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

[00271] Any host cell capable of overexpressing heterologous DNAs can be used for the purpose of expressing a polypeptide or protein of interest. Non-limiting examples of suitable mammalian host cells include but are not limited to COS, HeLa, and CHO cells.

[00272] The invention includes polypeptides comprising an amino acid sequence of an ADAM9-binding molecule of this invention. The polypeptides of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (*i.e.*, single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available.

[00273] The invention includes variants of ADAM9-binding molecules, including functionally equivalent polypeptides that do not significantly affect the properties of such molecules as well as variants that have enhanced or decreased activity. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly or deleteriously change the functional activity, or use of chemical analogs. Amino acid residues that can be conservatively substituted for one another include but are not limited to: glycine/alanine; serine/threonine; valine/isoleucine/leucine; asparagine/glutamine; aspartic acid/glutamic acid; lysine/arginine; and phenylalanine/tyrosine. These polypeptides also include glycosylated and non-glycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Preferably, the amino acid substitutions would be conservative, *i.e.*, the substituted amino acid would possess similar chemical properties as that of the original amino acid. Such conservative substitutions are known in the art, and examples have been provided above. Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the Variable Domain. Changes in the Variable

Domain can alter binding affinity and/or specificity. Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay, such as the attachment of radioactive moieties for radioimmunoassay. Modified polypeptides are made using established procedures in the art and can be screened using standard assays known in the art.

[00274] The invention encompasses fusion proteins comprising one or more of the anti-ADAM9-VL and/or VH of this invention. In one embodiment, a fusion polypeptide is provided that comprises a light chain, a heavy chain or both a light and heavy chain. In another embodiment, the fusion polypeptide contains a heterologous immunoglobulin constant region. In another embodiment, the fusion polypeptide contains a Light Chain Variable Domain and a Heavy Chain Variable Domain of an antibody produced from a publicly-deposited hybridoma. For purposes of this invention, an antibody fusion protein contains one or more polypeptide domains that specifically bind to ADAM9 and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region.

[00275] The present invention particularly encompasses ADAM9-binding molecules (*e.g.*, antibodies, diabodies, trivalent binding molecules, *etc.*) conjugated to a diagnostic or therapeutic moiety. For diagnostic purposes, ADAM9-binding molecules of the invention may be coupled to a detectable substance. Such ADAM9-binding molecules are useful for monitoring and/or prognosing the development or progression of a disease as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Examples of detectable substances include various enzymes (*e.g.,* horseradish peroxidase, beta-galactosidase, *etc.*), prosthetic groups (*e.g.,* avidin/biotin), fluorescent materials (e.g., umbelliferone, fluorescein, or phycoerythrin), luminescent materials (*e.g.*, luminol), bioluminescent materials (*e.g.,* luciferase or aequorin), radioactive materials (*e.g.*, carbon-14, manganese-54, strontium-85 or zinc-65), positron emitting metals, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the ADAM9-binding molecule or indirectly, through an intermediate (*e.g.*, a linker) using techniques known in the art.

[00276] For therapeutic purposes ADAM9-binding molecules of the invention may be conjugated to a therapeutic moiety such as a cytotoxin, (*e.g.*, a cytostatic or cytocidal agent),

a therapeutic agent or a radioactive metal ion, *e.g.*, alpha-emitters. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells such as, for example, Pseudomonas exotoxin, Diptheria toxin, a botulinum toxin A through F, ricin abrin, saporin, and cytotoxic fragments of such agents. A therapeutic agent includes any agent having a therapeutic effect to prophylactically or therapeutically treat a disorder. Such therapeutic agents may be may be chemical therapeutic agents, protein or polypeptide therapeutic agents, and include therapeutic agents that possess a desired biological activity and/or modify a given biological response. Examples of therapeutic agents include alkylating agents, angiogenesis inhibitors, anti-mitotic agents, hormone therapy agents, and antibodies useful for the treatment of cell proliferative disorders. The therapeutic moiety may be coupled or conjugated either directly to the ADAM9-binding molecule or indirectly, through an intermediate (*e.g.*, a linker) using techniques known in the art.

XIII. Uses of the ADAM9-Binding Molecules of the Present Invention

[00277] The present invention encompasses compositions, including pharmaceutical compositions, comprising the ADAM9-binding molecules of the present invention (*e.g.*, antibodies, bispecific antibodies, bispecific diabodies, trivalent binding molecules, *etc.*), polypeptides derived from such molecules, polynucleotides comprising sequences encoding such molecules or polypeptides, and other agents as described herein.

[00278] As provided herein, the ADAM9-binding molecules of the present invention, comprising the anti-ADAM9-VL and/or VH Domains provided herein, have the ability to bind ADAM9 present on the surface of a cell and induce antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) and/or mediate redirected cell killing (*e.g.*, redirected T-cell cytotoxicity).

[00279] Thus, ADAM9-binding molecules of the present invention, comprising the anti-ADAM9-VL and/or VH Domains provided herein, have the ability to treat any disease or condition associated with or characterized by the expression of ADAM9. As discussed above, ADAM9 is an onco-embryonic antigen expressed in numerous blood and solid malignancies that is associated with high-grade tumors exhibiting a less-differentiated morphology, and is correlated with poor clinical outcomes. Thus, without limitation, the ADAM9-binding molecules of the present invention may be employed in the diagnosis or treatment of cancer, particularly a cancer characterized by the expression of ADAM9.

[00280] In particular, ADAM9-binding molecules of the present invention may be used in the treatment of bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, liver cancer, lymphoid cancer, non-small-cell lung cancer, myeloid cancer ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, thyroid cancer, testicular cancer, and uterine cancer.

[00281] In further embodiments, ADAM-9-binding molecules of the present invention may be useful in the treatment of non-small-cell lung cancer (squamous cell, adenocarcinoma, or large-cell undifferentiated carcinoma) and colorectal cancer (adenocarcinoma, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma, or squamous cell carcinoma).

[00282] The bispecific ADAM9-binding molecules of the present invention augment the cancer therapy provided by ADAM9 by promoting the redirected killing of tumor cells that express the second specificity of such molecules (*e.g.*, CD2, CD3, CD8, CD16, the T Cell Receptor (TCR), NKG2D, *etc.*). Such ADAM9-binding molecules are particularly useful for the treatment of cancer.

[00283] In addition to their utility in therapy, the ADAM9-binding molecules of the present invention may be detectably labeled and used in the diagnosis of cancer or in the imaging of tumors and tumor cells.

XIV. Pharmaceutical Compositions

[00284] The compositions of the invention 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-binding molecules of the present invention, or a combination of such agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of the ADAM9-binding molecules of the present invention and a pharmaceutically acceptable carrier. The invention also encompasses 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.

[00285] In a specific embodiment, the term "**pharmaceutically acceptable**" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "**carrier**" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete), excipient, or vehicle with which the therapeutic is administered. Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette 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 or saline can be provided so that the ingredients may be mixed prior to administration.

[00286] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with an ADAM9-binding molecule of the present invention, alone or with such pharmaceutically acceptable carrier. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[00287] The present invention provides kits that can be used in the above methods. A kit can comprise any of the ADAM9-binding molecules of the present invention. 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.

XV. Methods of Administration

[00288] The compositions of the present invention may be provided for the treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of an ADAM9-binding molecule (e.g. an antibody, bispecific antibody, diabody, trivalent binding molecule, fusion

protein, *etc.*) or a conjugated ADAM9-binding molecule of the invention, or a pharmaceutical composition comprising an ADAM9-binding molecule or a conjugated ADAM9-binding molecule of the invention. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgus monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[00289] Various delivery systems are known and can be used to administer the compositions of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (See, *e.g.*, Wu *et al.* (1987) *"Receptor-Mediated In Vitro Gene Transformation By A Soluble DNA Carrier System,"* J. Biol. Chem. 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

[00290] Methods of administering an ADAM9-binding molecule of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the ADAM9-binding molecules of the present invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety.

[00291] The invention also provides that preparations of the ADAM9-binding molecules of the present invention are packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the molecule. In one embodiment, such molecules are supplied as a dry sterilized lyophilized powder or water free concentrate in a

hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the ADAM9-binding molecules of the present invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container.

[00292] The lyophilized preparations of the ADAM9-binding molecules of the present invention should be stored at between 2°C and 8°C in their original container and the molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, such molecules are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, such ADAM9-binding molecules when provided in liquid form are supplied in a hermetically sealed container.

[00293] As used herein, an "**effective amount**" of a pharmaceutical composition is an amount sufficient to effect beneficial or desired results including, without limitation, clinical results such as decreasing symptoms resulting from the disease, attenuating a symptom of infection (*e.g.*, viral load, fever, pain, sepsis, etc.) or a symptom of cancer (e.g., the proliferation, of cancer cells, tumor presence, tumor metastases, *etc.*), thereby increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing the effect of another medication such as via targeting and/or internalization, delaying the progression of the disease, and/ or prolonging survival of individuals.

[00294] An effective amount can be administered in one or more administrations. For purposes of this invention, an effective amount of drug, compound, or pharmaceutical composition is an amount sufficient to: kill and/or reduce the proliferation of cancer cells, and/or to eliminate, reduce and/or delay the development of metastasis from a primary site of cancer. In some embodiments, an effective amount of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective amount" may be considered in the context of administering one or more chemotherapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[00295] For the ADAM9-binding molecules encompassed by the invention, the dosage administered to a patient is preferably determined based upon the body weight (kg) of the recipient subject.

[00296] The dosage and frequency of administration of an ADAM9-binding molecule of the present invention may be reduced or altered by enhancing uptake and tissue penetration of the molecule by modifications such as, for example, lipidation.

[00297] The dosage of an ADAM9-binding molecule of the invention administered to a patient may be calculated for use as a single agent therapy. Alternatively, the molecule may be used in combination with other therapeutic compositions and the dosage administered to a patient are lower than when said molecules are used as a single agent therapy.

[00298] The pharmaceutical compositions of the invention may be administered locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as SILASTIC® membranes, or fibers. Preferably, when administering a molecule of the invention, care must be taken to use materials to which the molecule does not absorb.

[00299] The compositions of the invention can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) *"New Methods Of Drug Delivery,"* Science 249:1527-1533); Treat *et al.*, in LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 3 17-327).

[00300] Where the composition of the invention is a nucleic acid encoding an ADAM9-binding molecule of the present invention, the nucleic acid can be administered *in vivo* to promote expression of its encoded ADAM9-binding molecule by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (*See* U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (See *e.g.*, Joliot *et al.* (1991) *"Antennapedia Homeobox Peptide Regulates Neural Morphogenesis,"* Proc.

Natl. Acad. Sci. (U.S.A.) 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

EXAMPLES

[00301] Having now generally described the invention, 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 invention. The examples are intended to illustrate, but in no way limit, the scope of the invention.

Example 1
Tumor Cell Specificity of the Anti-ADAM9 Antibody MAB-A

[00302] A murine anti-ADAM9 antibody (designated herein as **MAB-A**) was identified that: (1) blocks the target protein processing activity of ADAM9; (2) is internalized; and (3) has anti-tumor activity (see, *e.g.*, US Patent No. 8361475). The tumor cell specificity of **MAB-A** was investigated by IHC. Tumor tissue was contacted with **MAB-A** (0.4 μg/mL) or an isotype control (0.4 μg/mL) and the extent of staining was visualized. **MAB-A** was found to strongly label a variety of large cell carcinoma, squamous cell carcinoma, and adenocarcinoma non-small cell lung cancer cell types (**Figure 7A, Panels 1-8**), breast cancer cells, prostate cancer cells, gastric cancer cells (**Figure 7B, Panels 1-6**), as well as colon cancer samples (**Figure 7C, Panels 1-8**). Normal tissue was contacted with **MAB-A** (1.25 μg/mL) and the extent of staining was visualized. As summarized in **Table 1** above, **MAB-A** exhibited little or no staining of a wide variety of normal tissues. It will be noted that the concentration of **MAB-A** used in these studies was nearly 3-times that used for staining of tumor cells. The results of these IHC studies indicate that **MAB-A** exhibits strong preferential binding to tumor cells over normal cells.

Example 2
Species Cross Reactivity

[00303] The binding of **MAB-A** to human ADAM9 (huADAM9) and cynomolgus monkey ADAM9 (cynoADAM9) was examined. Briefly, 293-FT and CHO-K cells transiently expressing huADAM9, cynoADAM9, an unrelated antigen, or the untransfected parental cells were incubated with **MAB-A** followed by goat anti-murine-PE secondary antibody and analyzed by FACS. As shown in **Figures 8A-8B**, **MAB-A** exhibited strong binding to huADAM9 transiently expressed on both cells types. **MAB-A** exhibited poor

binding to cynoADAM9. **MAB-A** did not bind to the parental cells or cells expressing an irrelevant antigen. Similar poor binding to cynoADAM were seen in ELISA assays.

Example 2
Humanization and Initial Optimization

[00304] Humanization of **MAB-A** yielded a humanized VH Domain, designated herein as **"hMAB-A VH(1)"** and a humanized VL Domain designated herein as "**hMAB-A VL(1)**." The humanized Variable Domains were then optimized to enhance binding activity and/or to remove potentially labile amino acid residues as described in more detail below. This first round of optimization yielded three additional humanized VH Domains, designated herein as **"hMAB-A VH(2)**," "**hMAB-A VH(3)**," and "**hMAB-A VH(4)**," and three additional humanized VL Domains designated herein as "**hMAB-A VL(2)**," "**hMAB-A VL(3)**," and "**hMAB-A VL(4)**." In addition, a chimeric version of **MAB-A** ("**chMAB-A**") having the murine VH and VL Domains and human constant regions was generated. The amino acid sequences of the murine and the humanized/optimized VH and VL Domains are provided above, an alignment is provided in **Figures 9A** and **9B**. The consensus sequence of these humanized/optimized VH and VL Domains is provided above. Where multiple humanized Variable Domains were generated the humanized heavy and light chain Variable Domains of a particular anti-ADAM9 antibody (*e.g.*, **MAB-A**) may be used in any combination and particular combinations of humanized chains are referred to by reference to the specific VH/VL Domains, for example a molecule (*e.g.*, an antibody or diabody) comprising **hMAB-A VH(1)** and **hMAB-A VL(2)** is specifically referred to as **"hMAB-A (1.2)**."

[00305] **hMAB-A VH(1)** was generated having framework regions derived from human germlines VH3-21 and VH3-64, and **hMAB-A VL(1)** was generated having framework regions derived from human germlines B3 and L6. The murine CDRs were retained in these humanized variable domains.

[00306] A potential deamidation site was identified in the CDR_H2 (shown in single underlining in **Figure 9A**) and a potential aspartic acid isomerization site was identified in CDR_L1 (shown in single underlining in **Figure 9B**). Amino acid substitutions at these positions were examined to identify substitutions to remove these sites while maintaining binding affinity. A substitution of phenylalanine at position 54 (N54F) of CDR_H2 (present in **hMAB-A VH(2)**) and at serine at position 28 (D28S) of CDR_L1 (present in **hMAB-A**

VL(2)) were selected, wherein the numbering is accordingly to Kabat. The identified substitutions may be used separately or in combination. Surprisingly, antibodies comprising the N54F substitution were found to exhibit about a 2-fold increase in affinity for human ADAM9, and to exhibit slightly improved binding to cynomolgus ADAM9.

[00307] Additional, optimized variants were generated to minimize the number of lysine residues present in the CDRs. Two lysine residues are present in CDR_H2 (indicated with a double underline in **Figure 9A**), and one lysine is present in CDR_L1 (indicated with a double underline in **Figure 9B**). Amino acid substitutions at these positions were examined to identify substitutions that maintained binding affinity. Substitutions of arginine at position 62 (K62R), of glutamine at position 64 (K64Q), and serine at position 65 (S65G) were selected for CDR_H2 (present in **hMAB-A VH(3))**, wherein the numbering is accordingly to Kabat. A substitution of an arginine at position 24 (K24R) was selected for CDR_L1 (present in **hMAB-A VL(3))**. The identified substitutions may be used separately or in combination.

[00308] Other potentially labile resides present in the CDRs were identified (indicated with a dotted underline in **Figures 9A-9B**), one methionine residue within CDR_H1 at position 34 (M34), one methionine residue within CDR_L1 at position 33 (M33), and histidine, glutamic acid, and aspartic acid residues at positions 92 (H93), 93 (E93), and 94 (D94), within CDR_L3, wherein the numbering is accordingly to Kabat. Amino acid substitutions at these positions were examined to identify substitutions that maintained binding affinity. Substitution of isoleucine at position 34 (M34I) was selected for CDR_H1 and substitutions of leucine, tyrosine, serine and threonine were selected for positions 33 (M33L), 92 (H93Y), 93 (E93S), and 94 (D94T) of CDR_L3, wherein the numbering is according to Kabat. Each of these positions could readily be substituted in combination with all of the substitutions detailed above to yield **hMAB-A VH(4)** and **hMAB-A VL(4)**, which when paired together generate an antibody that retained a small improvement in affinity as compared to the parental murine antibody, and that has a greatly reduced potential for deamidation or oxidation and no lysine residues in the CDRs.

[00309] The relative binding affinity of the humanized/optimized antibodies **hMAB-A** (1.1), **hMAB-A** (2.2), **hMAB-A** (2.3), **hMAB-A** (3.3), **hMAB-A** (4.4) and the chimeric **chMAB-A** (having murine VH/VL Domains) to huADAM was investigated using BIACORE® analysis, in which His-tagged soluble human ADAM9 ("**shADAM9-His**,"

containing an extracellular portion of human ADAM9 fused to a histidine-containing peptide) was passed over a surface coated with immobilized antibody. Briefly, each antibody was captured onto an Fab_2 goat-anti-human Fc-coated surface and then incubated in the presence of different concentrations (6.25-100 nM) of the shADAM9-His peptide. The kinetics of binding were determined via BIACORE® analysis binding (normalized 1:1 Langmuir binding model). The calculated k_a, k_d and K_D from these studies are presented in **Table 7**. Binding to cynoADAM9 was examined by FACS as described above and by ELISA.

Table 7

Antibody	pI	huADAM9		
		k_a ($x10^6$)	k_d ($x10^{-3}$)	KD (nM)
ch**MAB-A**	6.61	1.3	4.7	3.6
h**MAB-A** (1.1)	6.44	1.5	5.2	3.5
h**MAB-A** (2.2)	6.58	1.1	1.5	1.4
h**MAB-A** (2.3)	6.58	1.3	1.7	1.3
h**MAB-A** (3.3)	6.44	1.1	1.5	1.4
h**MAB-A** (4.4)	6.73	1.0	2.0	2.0

[00310] The results of these studies demonstrate that the humanized/optimized antibodies have the same or higher binding affinity to human ADAM9 than the parental murine antibody. In particular, it was observed that the introduction of the N54F mutation in the humanized antibodies resulted in improved binding to huADAM9 (*i.e.*, h**MAB-A** (2.2), h**MAB-A** (2.3) and h**MAB-A** (3.3)). This mutation also provided a slight improvement in binding to cynoADAM9 as determined by FACS and ELISA, however these antibodies continued to exhibit poor binding to cynoADAM9. These studies also identified additional substitutions that could be introduced to remove lysine residues from the CDRs without reducing affinity. Additional substitutions were identified to remove other potentially labile residues with a minimal impact on affinity.

Example 4
Optimization of Binding to Non-Human Primate ADAM9

[00311] Random mutagenesis was used to introduce substitutions within the Heavy Chain CDR_H2 (Kabat positions 53-58) and CDR_H3 (Kabat positions 95-100 and 100a-100f) domains of **hMAB-A** (2.2). The mutants were screened to identify clones having enhanced binding to non-human primate ADAM9 (*e.g.*, cynoADAM9) and that retained high affinity binding to huADAM9. 48 clones were selected from two independent screens of mutations within CDR_H3 (Kabat positions 100a-100f). **Table 8** provides an alignment of the amino

acid sequence of CDR_H3 Kabat residues 100a-f from **hMAB-A (2.2)** clones selected for enhanced binding to cynoADAM9 from two independent screens. Additional clone alignments are provided in **Table 9**. As indicated in such Tables, similar clones emerged in each experiment, which fell into discrete substitution patterns.

Table 8
Substitutions within Sub-Domain of the Heavy Chain CDRH3 of MAB-A (Kabat Positions 100a-100f)

Screen 1			Screen 2		
Clone ID	**SEQ ID NO**	**CDR_H3 Sub-Domain Sequence**	**Clone ID**	**SEQ ID NO**	**CDR_H3 Sub-Domain Sequence**
MAB-A	140	GSRDYF	**MAB-A**	140	GSRDYF
1	141	DGEGVM	1	171	DGKAVL
2	141	DGEGVM	2	172	FNKAVL
3	142	FHSGLL	3	143	FNSATL
4	143	FNSATL	4	173	FNSGTW
5	144	FNSGTL	5	174	FNTGVF
6	145	FNSSTL	6	175	GKSRFH
7	146	GKSKWL	7	150	IGKGVF
8	147	GMGGTL	8	151	IGKGVL
9	148	HAKGGM	9	176	IGKNVY
10	149	IGEAVL	10	177	MGKGVM
11	150	IGKGVF	11	178	NGESVF
12	150	IGKGVF	12	179	PDFGWM
13	151	IGKGVL	13	180	PGSGVM
14	152	KHDSVL	14	181	PKDAWL
15	153	LNTAVM	15	158	PKFGWK
16	154	NGEGTL	16	158	PKFGWK
17	155	NGKNTL	17	182	PKFGWL
18	156	NSAGIL	18	183	PKIGWH
19	157	PKEGWM	19	183	PKIGWH
20	158	PKFGWK	20	183	PKIGWH
21	159	PKMGWV	21	184	PKMGWA
22	160	PRLGHL	22	185	PKMGWM
23	161	PSFGWA	23	185	PKMGWM
24	162	QAKGTM	24	185	PKMGWM
25	163	RGMGVM	25	185	PKMGWM
26	164	RKEGWM	26	186	PQMGWL
27	165	TGKGVL	27	187	PRFGWL
28	166	TGMGTL	28	187	PRFGWL
29	167	TGNGVM	29	187	PRFGWL
30	167	TGNGVM	30	188	PRMGFL
31	168	WNAGTF	31	189	PRMGFM
32	169	YHHTPL	32	190	PSFGWM
33	169	YHHTPL	33	191	RREGWM
34	170	YQSATL	34	192	SGEGVL
			35	193	SGNGVM
			36	194	VGKAVL

Table 9 Substitutions within Sub-Domain of the Heavy Chain CDRH3 of MAB-A (Kabat Positions 100a-100f)		
Clone ID	**SEQ ID NO**	**CDR_H3 Sub-Domain Sequence**
MAB-A VH (2A)	144	FNSGTL
MAB-A VH (2B)	151	IGKGVL
MAB-A VH (2C)	187	PRFGWL
MAB-A VH (2D)	165	TGKGVL
MAB-A VH (2E)	195	DSNAVL
MAB-A VH (2F)	196	FHSGTL
MAB-A VH (2G)	172	FNKAVL
MAB-A VH (2H)	197	GGSGVL
MAB-A VH (2I)	198	PRQGFL
MAB-A VH (2J)	199	YNSGTL

[00312] For all the clones examined, Gly and Ala are the preferred amino acid residues at positon 4 (P4) and Leu, Met, and Phe are the preferred amino acid residues at position 6 (P6). The preferred amino acid residues at other positions (*e.g.*, position 2 (P2), position 3 (P3) and position 5 (P5)) depend on the amino acid residue found at P1. For clones having a Pro residue at position 1 (P1), Lys and Arg were preferred at P2, Phe and Met at P3, Gly at P4, and Trp or Phe at P5. For clones having a Phe, Tyr or Trp at P1, Asn and His were preferred at P2, Ser and His at P3, and Leu at P6. For clones having Ile, Leu or Val at P1, Gly was preferred at P2, Lys at P3, Val at P5 and hydrophobic at P6. In addition, as can be seen in **Table 8**, for clones having a Thr residue at P1, Gly was preferred at P2, Lys, Met, and Asn were preferred at P3, Gly was preferred at P4, Val or Thr were preferred at P5 and Leu and Met at P6. Additional clones having an Asp, Gly, Arg, His, or Ser residue at P1 were also identified at lower frequencies (see **Table 8** and **Table 9**).

[00313] The VH Domain of the ten clones shown in **Table 9** were used to generate further optimized variants of **hMAB-A (2.2)** designated **hMAB-A (2A.2)**. The binding of the selected clones was examined by ELISA assay. Briefly, antibodies that bind to histidine-containing peptides, and that had been coated onto microtiter plates, were used to capture His peptide-tagged soluble cynoADAM9 ("**cynoADAM9-His**") (1 μg/mL) or His peptide-tagged soluble huADAM9 (1 μg/mL), and the binding of serial dilutions of the parental **hMAB-A (2.2)** and the ten CDR_H3 **hMAB-A (2A.2)** variants was examined. The binding curves for cynoADAM9 and huADAM9 are presented in **Figure 10A** and **Figure 10B**, respectively. **hMAB-A (2A.2)** variants comprising each of the selected VH Domains

exhibited improved binding to cynoADAM9 with **MAB-A** VH(2B), **MAB-A** VH(2C), **MAB-A** VH(2D), and **MAB-A** VH(2I), showing the greatest enhancement in cynoADAM9 binding while maintaining similar binding to huADAM9 as the parental h**MAB-A** (2.2) antibody.

[00314] The relative binding affinity of the humanized/further optimized antibodies **MAB-A** VH(2B.2), **MAB-A** VH(2C.2), **MAB-A** VH(2D.2), and **MAB-A** VH(2I.2), and the parental h**MAB-A** (2.2), to huADAM9-His and cynoADAM9-His was investigated using BIACORE® analysis essentially as described above. The calculated k_a, k_d and K_D from these studies are presented in **Table 10**.

Table 10

Antibody	huADAM9			cynoADAM9		
	k_a ($x10^5$) ($M^{-1}s^{-1}$)	k_d ($x10^{-4}$) (s^{-1})	KD (nM)	k_a ($x10^5$) ($M^{-1}s^{-1}$)	k_d ($x10^{-4}$) (s^{-1})	KD (nM)
hMAB-A (2.2)	9.0	5.5	0.6	2.0	220	110
hMAB-A (2B.2)	6.1	3.9	0.6	3.4	0.66	0.2
hMAB-A (2C.2)	5.9	8.1	1.4	3.5	<0.1	<0.3
hMAB-A (2D.2)	6.9	5.8	0.8	4.2	3.0	0.7
hMAB-A (2I.2)	6.6	2.3	0.4	4.0	0.85	0.2

[00315] The binding studies demonstrate that the four top clones exhibited between 150-550-fold enhancement in binding affinity to cynoADAM9 while maintaining the same high affinity binding to huADAM9 as the parental antibody. **hMAB-A (2C.2)** and **hMAB-A (2I.2)** was selected for further studies.

Example 5
Immunohistochemistry Study of Antibody hMAB-A (2I.2)

[00316] The cell specificity of **hMAB-A (2I.2)** was investigated by IHC. Positive and negative control cells, and normal human and cynomolgus monkey tissues were contacted with **hMAB-A (2I.2)** (2.5 μg/mL) or an isotype control (2.5 μg/mL) and the extent of staining was visualized. The results of the study are summarized in **Table 11**.

Table 11		
Cell/Tissue	**hMAB-A (2I.2) (2.5 µg/ml)**	**IgG1 Negative Control (2.5 µg/ml)**
Cho-K parental cells	-	-
Cho-K/huADAM9 medium expression P:1	2-4+ (gr c > m) rare to occasional and 1+ (gr c > m) occasional	-
Cho-K/huADAM9 high expression	2-4+ (gr c > m) frequent	-
Cho-K/ cynoADAM9 clone 2	2-4+ (gr c > m) frequent	-
Cho-K/cynoADAM9 clone #16	2-4+ (gr c > m) frequent	-
A498 cells	2-4+ (gr c > m) rare to occasional and 1+ (gr c > m) occasional to frequent	-
Colon MG06-CHTN-96 B	-	numerous 2-4+ (gr c) cells consistent with macrophages
Lung MG06-CHtN-162B1 A	-	occasional 2-4+ (gr c) cells consistent with macrophages
Liver ILS11103 B	-	hepatocytes 1+ (gr c) rare to occasional
Pancreas ILS10266	-	-
Heart Life Legacy 0910035D	-	cardiac muscle cells with numerous 1-3+ small foci of (gr c) consistent with lipofuscin pigment
Kidney ILS10241 B	-	tubule epi 1+ (gr c) rare
Bladder ILSD8011 J	-	occasional 2-4+ (gr c) cells consistent with macrophages
Cyno Colon #1	-	mucosal epi (luminal m) 2-4+ rare to occasional and 1+ rare to occasional; numerous 2-3+ (gr c) cells consistent with macrophages predominantly within LP
Cyno Lung #1	-	very rare 2-4+ (gr c) cells consistent with macrophages
Cyno Liver #1	-	-
Cyno Pancreas #1	-	-
Cyno Heart #1	-	-
Cyno Kidney #070368M	-	tubule epi 2+ (gr c) rare and 1+ (gr c) rare to occasional
Cyno Bladder #1	transitional cell epi ± (gr c) rare	rare 1-4+ (gr c) cells consistent with macrophages
Lung CA ILS10108	H score 150	tu -
Lung CA ILS7223	H score 180	tu -
Lung CA ILS2156 A	H score 80	tu -
Lung CA ILS7295 A	H score 60	tu -

[00317] IHC studies were also conducted to assess binding of humanized/optimized **hMAB-A (2I.2)** at a concentration of 12.5 µg/mL (5x optimal staining concentration). Positive and negative control cells, normal human tissues, and cynomolgus monkey tissues were employed in this study. The results of the study are summarized in **Table 12**.

Table 12		
Cell/Tissue	**hMAB-A (2I.2) (12.5 µg/ml)**	**IgG1 Negative Control (12.5 µg/ml)**
Cho-K parental cells	-	-
Cho-K/huADAM9 medium expression P:1	2-4+ (gr c > m) occasional to frequent	-
Cho-K/huADAM9 high expression	3-4+ (gr c > m) occasional to frequent	-
Cho-K/cynoADAM9 clone 2	3-4+ (gr c > m) frequent	-
Cho-K/cynoADAM9 clone #16	3-4+ (gr c > m) frequent	-
A498 cells	2-4+ (gr c > m) occasional to frequent	-
Colon MG06-CHTN-96 B	epi ± - 1+ rare to occasional	numerous 2-4+ (gr c) cells consistent with macrophages predominantly within LP in test article and negative control
Lung MG06-CHtN-162B1 A	alveolar cells (favor pneumocytes) 2-3+ (gr c > m) rare, 1+ (gr c > m) rare to occasional; EC 2-4+ (c,m) rare, 1+ (c,m) rare	occasional scattered 2-4+ (gr c) cells consistent with macrophages in test article and negative control
Liver ILS11103 B	-	occasional scattered 2-4+ (gr c) cells consistent with macrophages in test article and negative control
Pancreas ILS10266	ductal epi 1+ (gr c > m) very rare	cells (favor acinar cells) 1+ (gr c) very rare; occasional scattered 2-4+ (gr c) cells consistent with macrophages in test article and negative
Heart Life Legacy 0910035D	-	numerous small foci 1-3+ granular staining with cardiac muscle cells consistent with lipofuscin pigment consistent with artifact in test article and negative control
Kidney ILS10241 B	tubule epi 1+ (gr c) rare to occasional	tubule epi ± (gr c) rare
Bladder ILSD8011 J	transitional cell epi 1+ (gr c) rare	rare 2-4+ (gr c) cells consistent with macrophages in test article and negative control
Cyno Colon #1	-	mucosal epi (luminal m) 2-4+ occasional and 1+ rare to occasional
Cyno Lung #1	bronchial epi 1+ (gr c > m) rare to occasional and ± (gr c > m) occasional to frequent	-
Cyno Liver #1	-	-
Cyno Pancreas #1	-	-
Cyno Heart #1	-	-
Cyno Kidney #070368M	-	tubule epi 1+ (gr c) rare and ± (gr c) rare
Cyno Bladder #1	transitional cell epi 2+ (gr c > m) rare and 1+ (gr c > m) rare to occasional	-
Lung CA ILS10108	H score 180	tu -
Lung CA ILS7223	H score 180	tu -
Lung CA ILS2156 A	H score 115	tu -
Lung CA ILS7295 A	H score 115	tu -

[00318] A comparative IHC study was conducted in order to assess differences in binding by **hMAB-A (2.2)**, **hMAB-A (2.3)**, **hMAB-A (2C.2)**, and **hMAB-A (2I.2)** at 2.5 µg/mL or 5 µg/mL. Positive and negative control cells, normal human tissues, and cynomolgus monkey tissues were employed in this study. The results of the study are summarized in **Table 13**.

Table 13

Tissue	hMAB-A (2.3) 5 ug/mL	hMAB-A (2.2) 2.5 µg/mL	hMAB-A (2C.2) 2.5 µg/mL	hMAB-A (2I.2) 2.5 µg/mL	Isotype control 5 µg/mL
Cho-K parental P:3	-	-	-	-	-
Cho-K/hu ADAM9.2 medium expression P:1	1+ (c) occasional	2-4+ (gr c > m) rare and 1+ (gr c > m) rare to occasional	2-4+ (gr c > m) rare to occasional and 1+ (gr c > m) rare to occasional	2-4+ (gr c > m) rare to occasional and 1+ (gr c > m) occasional	-
Cho-K/hu ADAM9.18 high expression P:1	3+ (m,c) frequent	2-4+ (gr c > m) occasional to frequent and 1+ (gr c > m) occasional	2-4+ (gr c > m) occasional to frequent and 1+ (gr c > m) occasional	2-4+ (gr c > m) frequent	-
Cho-K Cyno #2	1+ (c) occasional	-	3-4+ (gr c > m) frequent	2-4+ (gr c > m) frequent	-
Cho-K Cyno #16	2+ (c,m) occasional to frequent	2-4+ (gr c > m) rare and 1+ (gr c > m) rare to occasional	3-4+ (gr c > m) frequent	2-4+ (gr c > m) frequent	-
A498 072210	3-4+ (c,m) frequent	2-4+ (gr c > m) rare and 1+ (gr c > m) occasional to frequent	2-4+ (gr c > m) rare and 1+ (gr c > m) occasional	2-4+ (gr c > m) rare to occasional and 1+ (gr c > m) occasional to frequent	-
Lung CA ILS10108	IHC score 3	H Score 55	H Score 17	H score 150	-
Lung CA ILS7223	IHC score 3	H Score 205	H Score 160	H score 180	-
Lung CA ILS2156 A	IHC score 1	H Score 5	H Score 0	H score 80	-
Lung CA ILS7295 A	IHC score 1	H Score 1	H Score 0	H score 60	-

[00319] A further comparative IHC study was conducted in order to assess differences in binding by **hMAB-A (2.2)**, **hMAB-A (2.3)**, **hMAB-A (2C.2)**, and **hMAB-A (2I.2)** and murine **MAB-A** at 2.5 µg/mL 5 µg/mL or 12.5 µg/mL. Positive and negative control cells, normal human tissues, and cynomolgus monkey tissues were employed in this study. The results of the study are summarized in **Table 14**.

Table 14

Tissue	hMAB-A (2.3) 5 ug/mL	hMAB-A (2.2) 2.5 µg/mL	hMAB-A (2C.2) 2.5 µg/mL	hMAB-A (2I.2) 12.5 µg/ml	MAB-A 5 µg/mL
Colon MG06-CHTN-96 B	epi 1+ (c,m) rare; sm negative	-	-	epi ± - 1+ rare to occasional	Epithelium 1-3+ [m, c] (occas to freq); Others (Neg)
Lung MG06-CHtN-162B1 A	pneumocytes/macrophages 2+ (c,m) occasional	-	-	alveolar cells (favor pneumocytes) 2-3+ (gr c > m) rare, 1+ (gr c > m) rare to occasional; EC 2-4+ (c,m) rare, 1+ (c,m) rare	Monoctyes 1+ [c] (rare to occas); Others (Neg)
Liver ILS11103 B	hepatocytes 1+ (c) rare to occasional	hepatocytes 1+ (gr c) frequent	hepatocytes 2+ (gr c) rare and 1+ (gr c) frequent	-	Kupffer cells 3+ [c] (occas); Others (Neg)
Pancreas ILS10266	epi 1+ (c) rare; Islet Cells 1+ (c) very rare	-	-	ductal epi 1+ (gr c > m) very rare	Ductal epithelium 1-2+ [c, m] (rare to occas); Fibril 2+ (rare); Others (Neg)
Heart Life Legacy 0910035D	±	-	-	-	Neg
Kidney ILS10241 B	epi 2-3+ (c,m) frequent	tubule epi 2+ (gr c) rare to occasional and 1+ (gr c) occasional to frequent	tubule epi 2+ (gr c) rare to occasional and 1+ (gr c) occasional to frequent	tubule epi 1+ (gr c) rare to occasional	Epithelium 1+ [c] (rare); Others (Neg)
Bladder ILSD8011 J	transitional epi 1+ (c) rare to occasional	-	-	transitional cell epi 1+ (gr c) rare	Transitional epithelium 2+ [c, m] (occas to freq); Stromal cells 3+ [c] (rare); Others (Neg)
Cyno Colon #1	epi 1+ (c,m) rare	-	-	-	
Cyno Lung #1	Macrophage and pneumocytes 1+ (c) very rare	-	bronchial epi 3-4+ (gr c) rare, 2+ (gr c) occasional, and 1+ (gr c) occasional	bronchial epi 1+ (gr c > m) rare to occasional and ± (gr c > m) occasional to frequent	
Cyno Liver #1	hepatocytes 1+ (c) frequent	hepatocytes 2+ (gr c) rare to occasional and 1+ (gr c) rare to occasional	hepatocytes 2+ (gr c) rare to occasional and 1+ (gr c) occasional; ductal epi 1+ (gr c) occasional	-	
Cyno Pancreas #1	epi and Islet Cells 1+ (c) very rare	-	islet cells ± (gr c) frequent; ductal epi 1+ (gr c) rare to occasional	-	positive

Table 14					
Tissue	**hMAB-A (2.3) 5 ug/mL**	**hMAB-A (2.2) 2.5 µg/mL**	**hMAB-A (2C.2) 2.5 µg/mL**	**hMAB-A (2I.2) 12.5 µg/ml**	**MAB-A 5 µg/mL**
Cyno Heart #1	myocardium 1+ (c) frequent	-	-	-	
Cyno Kidney #070368M	epi 2+ (c) frequent	tubule epi 2+ (gr c) rare to occasional and 1+ (gr c) rare to occasional	tubule epi 2+ (gr c) rare to occasional and 1+ (gr c) occasional to frequent	-	positive
Cyno Bladder #1	transitional epi ± (c); macrophages very rare	-	transitional cell epi 2-3+ (gr c > m) rare and 1+ (gr c > m) occasional	transitional cell epi 2+ (gr c > m) rare and 1+ (gr c > m) rare to occasional	

[00320] The results thus demonstrate that **hMAB-A (2.2)** exhibited an overall low-level staining of human hepatocytes and kidney tubules at optimal concentration, with a lower staining intensity/frequency of reactivity in hepatocytes and kidney tubules observed in the negative control. **hMAB-A (2.2)** exhibited similar low-level staining of cyno hepatocytes and kidney tubules at optimal concentration, with lower staining intensity/frequency of reactivity in kidney tubules observed in the negative control.

[00321] The results also demonstrate that **hMAB-A (2C.2)** exhibited an overall low-level staining of human hepatocytes and kidney tubules at optimal concentration, with lower staining intensity/frequency of reactivity in hepatocytes and kidney tubules observed in the negative control. **hMAB-A (2C.2)** exhibited similar low-level staining in cyno hepatocytes and kidney tubules at optimal concentration. Additional minimal findings in cyno lung epithelium, pancreas islets/ epithelium and bladder epithelium for **hMAB-A (2C.2)** was not observed in the corresponding human tissue; lower staining intensity/frequency of reactivity was observed in lung epithelium, kidney tubules, bladder epithelium in negative control.

[00322] The results also demonstrate that **hMAB-A (2I.2)** exhibited no staining of human or cyno tissues at optimal concentration, with rare +/- bladder transitional cell epithelium staining. **hMAB-A (2I.2)** also exhibited overall low level and frequency staining of human lung alveolar cells, pancreas ductal epithelium, kidney tubule, bladder transitional cell epithelium at 5x optimal concentration, and overall low-level staining of cyno bronchial epithelium and bladder transitional cell epithelium at 5x optimal concentration. **hMAB-A (2I.2)** exhibited an overall favorable IHC profile on the human normal tissues tested and a similar profile on corresponding cynomolgus monkey tissues.

[00323] 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 the invention has been described in connection with specific embodiments thereof, 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 invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

WHAT IS CLAIMED IS:

Claim 1. An ADAM9-binding molecule that comprises an ADAM9-binding domain, wherein said ADAM9-binding domain comprises a Light Chain Variable (VL) Domain and a Heavy Chain Variable (VH) Domain, wherein said Heavy Chain Variable Domain comprises a CDR_H1 Domain, a CDR_H2 Domain and a CDR_H3 Domain, and said Light Chain Variable Domain comprises a CDR_L1 Domain, a CDR_L2 Domain, and a CDR_L3 Domain, wherein:

(A) said CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain have the amino acid sequence of the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of a Heavy Chain Variable (VH) Domain of an optimized variant of **MAB-A**; and said CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain have the amino acid sequence of the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of the Light Chain Variable (VL) Domain of **MAB-A**; or

(B) said CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain have the amino acid sequence of the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of the Heavy Chain Variable (VH) Domain of **MAB-A**; and said CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain have the amino acid sequence of the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of a Light Chain Variable (VL) Domain of an optimized variant of **MAB-A**; or

(C) said CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain have the amino acid sequence of the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of a Heavy Chain Variable (VH) Domain of an optimized variant of **MAB-A**; and said CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain have the amino acid sequence of the CDR_L1 Domain, CDR_L2 Domain, and CDR_L3 Domain of a Light Chain Variable (VL) Domain of an optimized variant of **MAB-A**.

Claim 2. The ADAM9-binding molecule of claim 1, wherein said ADAM9-binding domain possesses:

(A) (1) the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of the Heavy Chain Variable (VH) Domain of **MAB-A**; and

(2) the FR1, FR2, FR3 and FR4 of a VH Domain of a humanized variant of **MAB-A**; or

(B) (1) the CDR_L1 Domain, CDR_L2 Domain and CDR_L3 Domain of the Light Chain Variable (VL) Domain **MAB-A**; and

(2) the FR1, FR2, FR3 and FR4 of a VL Domain of a humanized variant of **MAB-A**; or

(C) (1) the CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of a Heavy Chain Variable (VH) Domain of an optimized variant of **MAB-A**; and

(2) the FR1, FR2, FR3 and FR4 of the VH Domain of a humanized variant of **MAB-A**; or

(D) (1) the CDR_L1 Domain, CDR_L2 Domain and CDR_L3 Domain of a Light Chain Variable (VL) Domain of an optimized variant of **MAB-A**; and

(2) the FR1, FR2, FR3 and FR4 of the VL Domain of a humanized variant of **MAB-A**; or

(E) (1) the Heavy Chain Variable (VH) Domain of a humanized/optimized variant of **MAB-A**; and

(2) the VL Light Chain Variable (VL) Domain of a humanized/optimized variant of **MAB-A**.

Claim 3. The ADAM9-binding molecule of any one of claims 1 or 2, wherein said Heavy Chain Variable (VH) Domain of said optimized variant of **MAB-A** comprises the amino acid sequence of **SEQ ID NO:15**:

EVQLVESGGG LVKPGGSLRL SCAASGFTFS **SYWX_1H**WVRQA
PGKGLEWVG**E** **IIPIX_2GHTNY** **NEX_3F$X_4$$X_5$**RFTI SLDNSKNTLY
LQMGSLRAED TAVYYCAR**GG** **YYYY$X_6$$X_7$$X_8$$X_9$$X_{10}$$X_{11}$**
DYWGQGTTVT VSS

wherein: X_1, X_2, X_3, X_4, X_5, and X_6 are independently selected,

wherein: X_1 is M or I; X_2 is N or F;

X_3 is K or R; X_4 is K or Q;

X_5 is S or G, and X_6 is P, F, Y, W, I, L, V, T, G or D;

wherein: X_7, X_8, X_9, X_{10}, and X_{11} are selected such that:

when X_6 is P; X_7 is K or R; X_8 is F or M; X_9 is G; X_{10} is W or F; and X_{11} is M, L or K;

when X_6 is F, Y or W; X_7 is N or H; X_8 is S or K; X_9 is G or A; X_{10} is T or V; and X_{11} is M, L or K;

when X_6 is I, L or V; X_7 is G; X_8 is K; X_9 is G or A; X_{10} is V; and X_{11} is M, L or K;

when X_6 is T; X_7 is G; X_8 is K, M or N; X_9 is G; X_{10} is V or T; and X_{11} is L or M;

when X_6 is G; X_7 is G; X_8 is S; X_9 is G; X_{10} is V; and X_{11} is L;

when X_6 is D; X_7 is S; X_8 is N; X_9 is A; X_{10} is V; and X_{11} is L.

Claim 4. The ADAM9-binding molecule of any one of claims 1-3, wherein said CDR_H1 Domain, CDR_H2 Domain and CDR_H3 Domain of said Heavy Chain Variable (VH) Domain of said optimized variant of **MAB-A** respectively have the amino acid sequences of:

(1) **SEQ ID NO:47** ($SYWX_1H$)

wherein: X_1 is M or I;

(2) **SEQ ID NO:48** ($EIIPIX_2GHTNYNEX_3FX_4X_5$)

wherein: X_2, X_3, X_4, and X_5 are independently selected, and

wherein: X_2 is N or F; X_3 is K or R;

X_4 is K or Q; and

X_5 is S or G; and

(3) **SEQ ID NO:49** ($GGYYYYYX_6X_7X_8X_9X_{10}X_{11}DY$)

wherein: X_6, is P, F, Y, W, I, L, V, T, G or D, and X_7, X_8, X_9, X_{10}, and X_{11} are selected such that:

(A) when X_6 is P:

X_7 is K or R; X_8 is F or M; X_9 is G;

X_{10} is W or F; and X_{11} is M, L or K;

(B) when X_6 is F, Y or W:

X_7 is N or H; X_8 is S or K; X_9 is G or A;

X_{10} is T or V; and X_{11} is M, L or K;

(C) when X_6 is I, L or V:

X_7 is G; X_8 is K; X_9 is G or A;

X_{10} is V; and X_{11} is M, L or K;

(D) when X_6 is T:

X_7 is G; X_8 is K, M or N; X_9 is G;

X_{10} is V or T; and X_{11} is L or M;

(E) when X_6 is G:

X_7 is G; X_8 is S; X_9 is G;

X_{10} is V; and X_{11} is L; and

(F) when X_6 is D:

X_7 is S; X_8 is N; X_9 is A;

X_{10} is V; and X_{11} is L.

Claim 5. The ADAM9-binding molecule of claim 4, wherein said Heavy Chain Variable (VH) Domain of said optimized variant of **MAB-A** is selected from the group consisting of:

(1) **hMAB-A VH(1) (SEQ ID NO:16)**;

(2) **hMAB-A VH(2) (SEQ ID NO:17)**;

(3) **hMAB-A VH(3) (SEQ ID NO:18)**;

(4) **hMAB-A VH(4) (SEQ ID NO:19)**;

(5) **hMAB-A VH(2A) (SEQ ID NO:20)**;

(6) **hMAB-A VH(2B) (SEQ ID NO:21)**;

(7) **hMAB-A VH(2C) (SEQ ID NO:22)**;

(8) **hMAB-A VH(2D) (SEQ ID NO:23)**;

(9) **hMAB-A VH(2E) (SEQ ID NO:24)**;

(10) **hMAB-A VH(2F) (SEQ ID NO:25)**;

(11) **hMAB-A VH(2G) (SEQ ID NO:26)**;

(12) **hMAB-A VH(2H) (SEQ ID NO:27)**;

(13) **hMAB-A VH(2I) (SEQ ID NO:28)**; and

(14) **hMAB-A VH(2J) (SEQ ID NO:29)**.

Claim 6. The ADAM9-binding molecule of any one of claims 1-5, wherein said Light Chain Variable (VL) Domain comprises the amino acid sequence of **SEQ ID NO:53**:

DIVMTQSPDS LAVSLGERAT ISC**X_{12}ASQSVD**
$YX_{13}GDSYX_{14}N$WY QQKPGQPPKL LIY**AASDLES**
GIPARFSGSG SGTDFTLTIS SLEPEDFATY
YC**$QQSX_{15}X_{16}X_{17}PF$** **T**FGQGTKLEI K

wherein: $\mathbf{X_{12}}$, $\mathbf{X_{13}}$, $\mathbf{X_{14}}$, $\mathbf{X_{15}}$, $\mathbf{X_{16}}$, and $\mathbf{X_{17}}$, are independently selected, and

wherein: $\mathbf{X_{12}}$ is K or R; $\mathbf{X_{13}}$ is D or S;

$\mathbf{X_{14}}$ is M or L; $\mathbf{X_{15}}$ is H or Y;

$\mathbf{X_{16}}$ is E or S; and $\mathbf{X_{17}}$ is D or T.

Claim 7. The ADAM9-binding molecule of any one of claims 1-6, wherein said CDR_L1 Domain, CDR_L2 Domain and CDR_L3 Domain of said Light Chain Variable (VL) Domain of said optimized variant of **MAB-A** respectively have the amino acid sequences of:

(1) **SEQ ID NO:66** (X_{12}ASQSVDYX_{13}GDSYX_{14}N)

wherein: $\mathbf{X_{12}}$, $\mathbf{X_{13}}$, $\mathbf{X_{14}}$, are independently selected, and

wherein: $\mathbf{X_{12}}$ is K or R; $\mathbf{X_{13}}$ is D or S; and $\mathbf{X_{14}}$ is M or L;

(2) **SEQ ID NO:13** (AASDLES); and

(3) **SEQ ID NO:67** (QQS$X_{15}X_{16}X_{17}$PFT)

wherein: $\mathbf{X_{15}}$, $\mathbf{X_{16}}$, and $\mathbf{X_{17}}$, are independently selected, and

wherein: $\mathbf{X_{15}}$ is H or Y; $\mathbf{X_{16}}$ is E or S; and $\mathbf{X_{17}}$ is D or T.

Claim 8. The ADAM9-binding molecule of claim 7, wherein said Light Chain Variable (VL) Domain of said optimized variant of **MAB-A** is selected from the group consisting of:

(1) **hMAB-A VL(1) (SEQ ID NO:54)**;

(2) **hMAB-A VL(2) (SEQ ID NO:55)**;

(3) **hMAB-A VL(3) (SEQ ID NO:56)**;

(4) **hMAB-A VL(4) (SEQ ID NO:57)**;

(5) **hMAB-A VL(2A) (SEQ ID NO:20)**.

Claim 9. The ADAM9-binding molecule of claim 1, wherein said ADAM9-binding domain comprises:

(A) (1) a CDR_H1 Domain that comprises the amino acid sequence SYWMH (**SEQ ID NO:8**);

(2) a CDR_H2 Domain that comprises the amino acid sequence EIIPIFGHTNYNEKFKS (**SEQ ID NO:35**); or

(3) a CDR_H3 Domain that comprises the amino acid sequence GGYYYYPRQGFLDY (**SEQ ID NO:45**);

or

(B) (1) a CDR_L1 Domain that comprises the amino acid sequence KASQSVDYSGDSYMN (**SEQ ID NO:62**);

(2) a CDR_L2 Domain that comprises the amino acid sequence AASDLES (**SEQ ID NO:13**); or

(3) a CDR_L3 Domain that comprises the amino acid sequence QQSHEDPFT (**SEQ ID NO:14**);

Claim 10. The ADAM9-binding molecule of claim 9, wherein said ADAM9-binding domain comprises said CDR_H1 Domain that comprises the amino acid sequence SYWMH (**SEQ ID NO:8**), said CDR_H2 Domain that comprises the amino acid sequence EIIPIFGHTNYNEKFKS (**SEQ ID NO:35**), and said CDR_H3 Domain that comprises the amino acid sequence GGYYYYPRQGFLDY (**SEQ ID NO:45**).

Claim 11. The ADAM9-binding molecule of any one of claims 9 or 10, wherein said ADAM9-binding domain comprises said CDR_L1 Domain that comprises the amino acid sequence KASQSVDYSGDSYMN (**SEQ ID NO:62**), said CDR_L2 Domain that comprises the amino acid sequence AASDLES (**SEQ ID NO:13**), and said CDR_L3 Domain that cpomprises the amino acid sequence QQSHEDPFT (**SEQ ID NO:14**).

Claim 12. The ADAM9-binding molecule of any one of claims 9-11, wherein said ADAM9-binding domain comprises:

(A) the Heavy Chain Variable (VH) Domain of **hMAB-A (2I.2)** (**SEQ ID NO:28**); or

(B) the Light Chain Variable (VL) Domain of **hMAB-A (2I.2)** (**SEQ ID NO:55**); or

(C) the Heavy Chain Variable (VH) Domain of **hMAB-A (2I.2)** (**SEQ ID NO:28**) and the Light Chain Variable (VL) Domain of **hMAB-A (2I.2)** (**SEQ ID NO:55**).

Claim 13. The ADAM9-binding molecule of claim 1, wherein said ADAM9-binding domain comprises a CDR_H1 domain, a CDR_H2 domain, and a CDR_H3 domain and a CDR_L1 domain, a CDR_L2 domain, and a CDR_L3 domain having the sequences selected from the group consisting of:

(a) **SEQ ID NOs:8**, **35** and **10** and **SEQ ID NOs:62**, **13** and **14**, respectively

(b) **SEQ ID NOs:8**, **35** and **10** and **SEQ ID NOs:63**, **13** and **14**, respectively;

(c) **SEQ ID NOs:8**, **36** and **10** and **SEQ ID NOs:63**, **13** and **14**, respectively; and

(d) **SEQ ID NOs:34**, **36** and **10** and **SEQ ID NO:64**, **13** and **65**, respectively.

Claim 14. The ADAM9-binding molecule of claim 13, wherein said ADAM9-binding domain comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) having sequences that are at least 90%, at least 95%, or at least 99% identical to sequences selected from the group consisting of:

(a) **SEQ ID NO:17** and **SEQ ID NO:55**, respectively;

(b) **SEQ ID NO:17** and **SEQ ID NO:56**, respectively;

(c) **SEQ ID NO:18** and **SEQ ID NO:56**, respectively; and

(d) **SEQ ID NO:19** and **SEQ ID NO:57**, respectively.

Claim 15. The ADAM9-binding molecule of claim 14, wherein said ADAM9-binding domain comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) having the sequences selected from the group consisting of:

(a) **SEQ ID NO:17** and **SEQ ID NO:55**, respectively;

(b) **SEQ ID NO:17** and **SEQ ID NO:56**, respectively;

(c) **SEQ ID NO:18** and **SEQ ID NO:56**, respectively; and

(d) **SEQ ID NO:19** and **SEQ ID NO:57**, respectively.

Claim 16. The ADAM9-binding molecule of claim 1, wherein said ADAM9-binding domain has at least a 150-fold enhancement in binding affinity to cyno ADAM9 and retains high affinity binding to human ADAM9 as compared to **MAB-A**.

Claim 17. The ADAM9-binding molecule of claim 16, wherein said ADAM9-binding domain comprises a CDR_H1 domain, a CDR_H2 domain, and a CDR_H3

domain and a CDR_L1 domain, a CDR_L2 domain, and a CDR_L3 domain having the sequences selected from the group consisting of:

(a) **SEQ ID NOs:8**, **35** and **37** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(b) **SEQ ID NOs:8**, **35** and **38** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(c) **SEQ ID NOs:8**, **35** and **39** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(d) **SEQ ID NOs:8**, **35** and **40** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(e) **SEQ ID NOs:8**, **35** and **41** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(f) **SEQ ID NOs:8**, **35** and **42** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(g) **SEQ ID NOs:8**, **35** and **43** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(h) **SEQ ID NOs:8**, **35** and **44** and **SEQ ID NOs:62**, **13** and **14**, respectively;

(i) **SEQ ID NOs:8**, **35** and **45** and **SEQ ID NOs:62**, **13** and **14**, respectively; and

(j) **SEQ ID NOs:8**, **35** and **46** and **SEQ ID NOs:62**, **13** and **14**, respectively.

Claim 18. The ADAM9-binding molecule of claim 19, wherein said ADAM9-binding domain comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) having sequences that are at least 90%, at least 95%, or at least 99% identical to sequences selected from the group consisting of:

(a) **SEQ ID NO:20** and **SEQ ID NO:55**, respectively;

(b) **SEQ ID NO:21** and **SEQ ID NO:55**, respectively;

(c) **SEQ ID NO:22** and **SEQ ID NO:55**, respectively;

(d) **SEQ ID NO:23** and **SEQ ID NO:55**, respectively;

(e) **SEQ ID NO:24** and **SEQ ID NO:55**, respectively;

(f) **SEQ ID NO:25** and **SEQ ID NO:55**, respectively;

(g) **SEQ ID NO:26** and **SEQ ID NO:55**, respectively;

(h) **SEQ ID NO:27** and **SEQ ID NO:55**, respectively;
(i) **SEQ ID NO:28** and **SEQ ID NO:55**, respectively; and
(j) **SEQ ID NO:29** and **SEQ ID NO:55**, respectively.

Claim 19. The ADAM9-binding molecule of claim 18, wherein said ADAM9-binding domain comprises a heavy chain variable domain (VH) and a light chain variable domain (VL) having the sequences selected from the group consisting of:

(a) **SEQ ID NO:20** and **SEQ ID NO:55**, respectively;
(b) **SEQ ID NO:21** and **SEQ ID NO:55**, respectively;
(c) **SEQ ID NO:22** and **SEQ ID NO:55**, respectively;
(d) **SEQ ID NO:23** and **SEQ ID NO:55**, respectively;
(e) **SEQ ID NO:24** and **SEQ ID NO:55**, respectively;
(f) **SEQ ID NO:25** and **SEQ ID NO:55**, respectively;
(g) **SEQ ID NO:26** and **SEQ ID NO:55**, respectively;
(h) **SEQ ID NO:27** and **SEQ ID NO:55**, respectively;
(i) **SEQ ID NO:28** and **SEQ ID NO:55**, respectively; and
(j) **SEQ ID NO:29** and **SEQ ID NO:55**, respectively.

Claim 20. The ADAM9-binding molecule of any one of claims 1-19, wherein said molecule is a monospecific ADAM9-binding antibody or an ADAM9-binding fragment thereof.

Claim 21. The ADAM9-binding molecule of any one of claims 1-19, wherein said molecule is a bispecific antibody.

Claim 22. The ADAM9-binding molecule of any one of claims 1-19, wherein said molecule is a diabody, said diabody being a covalently bonded complex that comprises two, three, four or five polypeptide chains.

Claim 23. The ADAM9-binding molecule of any one of claims 1-19, wherein said molecule is a trivalent binding molecule, said trivalent binding molecule being a covalently bonded complex that comprises three, four, five, or more polypeptide chains.

Claim 24. The ADAM9-binding molecule of any one of claims 22-23, wherein said molecule comprises an Albumin-Binding Domain (ABD).

Claim 25. The ADAM9-binding molecule of any one of claims 21-24, wherein said ADAM9-binding molecule comprises an Fc Region.

Claim 26. The ADAM9-binding molecule of claim 21, wherein said Fc Region is a variant Fc Region that comprises:

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

(b) one or more amino acid modification(s) that enhance(s) the serum half-life of said ADAM9-binding molecule.

Claim 27. The ADAM9-binding molecule of claim 26, wherein said one or more amino acid modification(s) that reduce(s) the affinity of the variant Fc Region for an FcγR comprise:

(A) L234A;

(B) L235A; or

(C) L234A and L235A;

wherein said numbering is that of the EU index as in Kabat.

Claim 28. The ADAM9-binding molecule of any one of claims 26 or 27, wherein said one or more amino acid modification(s) that that enhance(s) the serum half-life of said ADAM9-binding molecule comprise:

(A) M252Y;

(B) M252Y and S254T;

(C) M252Y and T256E;

(D) M252Y, S254T and T256E; or

(E) K288D and H435K;

wherein said numbering is that of the EU index as in Kabat.

Claim 29. The ADAM9-binding molecule of any one of claims 1-20 or 22-28, wherein said molecule is bispecific and comprises an epitope-binding site capable of immunospecific binding to an epitope of ADAM9 and an epitope-binding site capable of immunospecific binding to an epitope of a molecule present on the surface of an effector cell.

Claim 30. The ADAM9-binding molecule of claim 22, wherein said molecule comprises two epitope-binding sites capable of immunospecific binding to epitope(s) of ADAM9 and two epitope-binding sites capable of immunospecific binding to epitope(s) of a molecule present on the surface of an effector cell.

Claim 31. The ADAM9-binding molecule of any one of claims 1-19 or 23-28, wherein said molecule is trispecific and comprises:

(a) one epitope-binding site capable of immunospecific binding to an epitope of ADAM9;

(b) one epitope-binding site capable of immunospecific binding to an epitope of a first molecule present on the surface of an effector cell; and

(c) one epitope-binding site capable of immunospecific binding to an epitope of a second molecule present on the surface of an effector cell.

Claim 32. The ADAM9-binding molecule of any one of claims 29-31, wherein said molecule is capable of simultaneously binding to ADAM9 and said molecule present on the surface of an effector cell.

Claim 33. The ADAM9-binding molecule of any one of claims 29-32, wherein said molecule present on the surface of an effector cell is CD2, CD3, CD8, TCR, or NKG2D.

Claim 34. The ADAM9-binding molecule of any one of claims 29-33, wherein said effector cell is a cytotoxic T-cell or a Natural Killer (NK) cell.

Claim 35. The ADAM9-binding molecule of any of claims 29-34, wherein said molecule present on the surface of said effector cell is CD3.

Claims 36. The ADAM9-binding molecule of claim 31, wherein said first molecule present on the surface of an effector cell is CD3 and said second molecule present on the surface of an effector cell is CD8.

Claim 37. The ADAM9-binding molecule of any one of claims 29-36, wherein said ADAM9-binding molecule mediates coordinated binding of a cell expressing ADAM9 and a cytotoxic T cell.

Claim 38. A pharmaceutical composition that comprises an effective amount of the ADAM9-binding molecule of any of claims 1-37 and a pharmaceutically acceptable carrier, excipient or diluent.

Claim 39. Use of the ADAM9-binding molecule of any one of claims 1-37 or the pharmaceutical composition of claim 38 in the treatment of a disease or condition associated with, or characterized by, the expression of ADAM9.

Claim 40. The use of claim 39, wherein said disease or condition associated with, or characterized by, the expression of ADAM9 is cancer.

Claim 41. The use of claim 40, wherein said cancer is selected from the group consisting: bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, liver cancer, non-small-cell lung cancer, myeloid cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, thyroid cancer, testicular cancer, and uterine cancer.

Claim 42. The use of claim 41, wherein said non-small-cell lung cancer is squamous cell carcinoma, adenocarcinoma, or large-cell undifferentiated carcinoma.

Claim 43. The use of claim 41, wherein said colorectal cancer is adenocarcinoma, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma, or squamous cell carcinoma.

Claim 44. A method for treating a disease or condition associated with, or characterized by, the expression of ADAM9 in a subject comprising administering to said subject an effective amount of the ADAM9-binding molecule of any one of claims 1-37 or the pharmaceutical composition of claim 38.

Claim 45. The method of claim 44, wherein said disease or condition associated with, or characterized by, the expression of ADAM9 is cancer.

Claim 46. The method of claim 45, wherein said cancer is selected from the group consisting of bladder cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, gastric cancer, head and neck cancer, liver cancer, non-small-cell lung cancer, myeloid cancer, ovarian cancer, pancreatic cancer, prostate cancer, renal cell carcinoma, thyroid cancer, testicular cancer, and uterine cancer.

Claim 47. The method of claim 46, wherein said non-small-cell lung cancer is squamous cell carcinoma, adenocarcinoma, or large-cell undifferentiated carcinoma.

Claim 48. The method of claim 46, wherein said colorectal cancer is adenocarcinoma, gastrointestinal carcinoid tumors, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma, or squamous cell carcinoma.

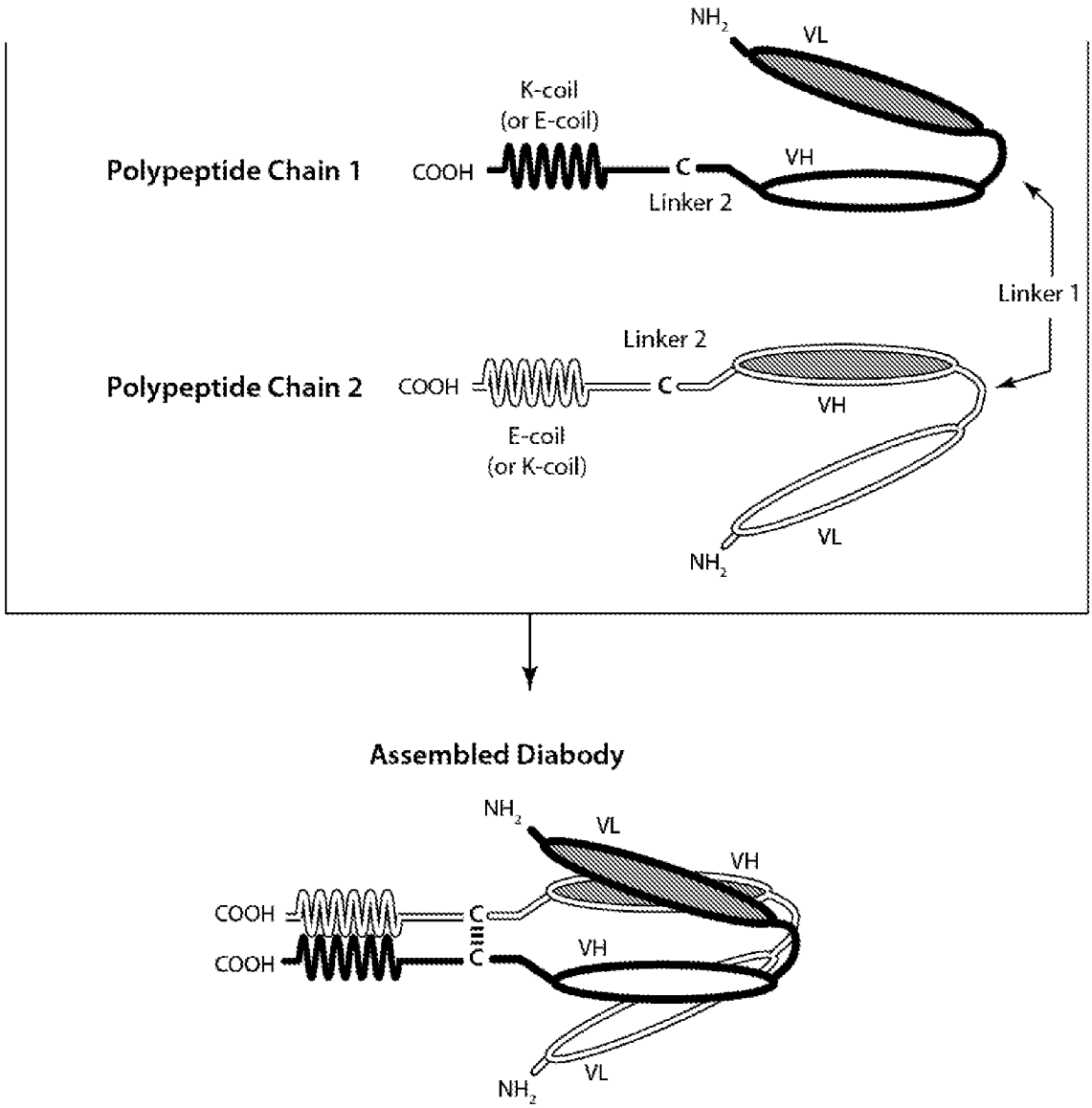
NH2
VL
K-coil
(or E-coil)
Polypeptide Chain 1
COOH
C
VH
Linker 2
Linker 1
Linker 2
Polypeptide Chain 2
COOH
C
VH
E-coil
(or K-coil)
VL
NH2
Assembled Diabody
NH2
VL
VH
COOH
C
COOH
C
VH
VL
NH2

Figure 1

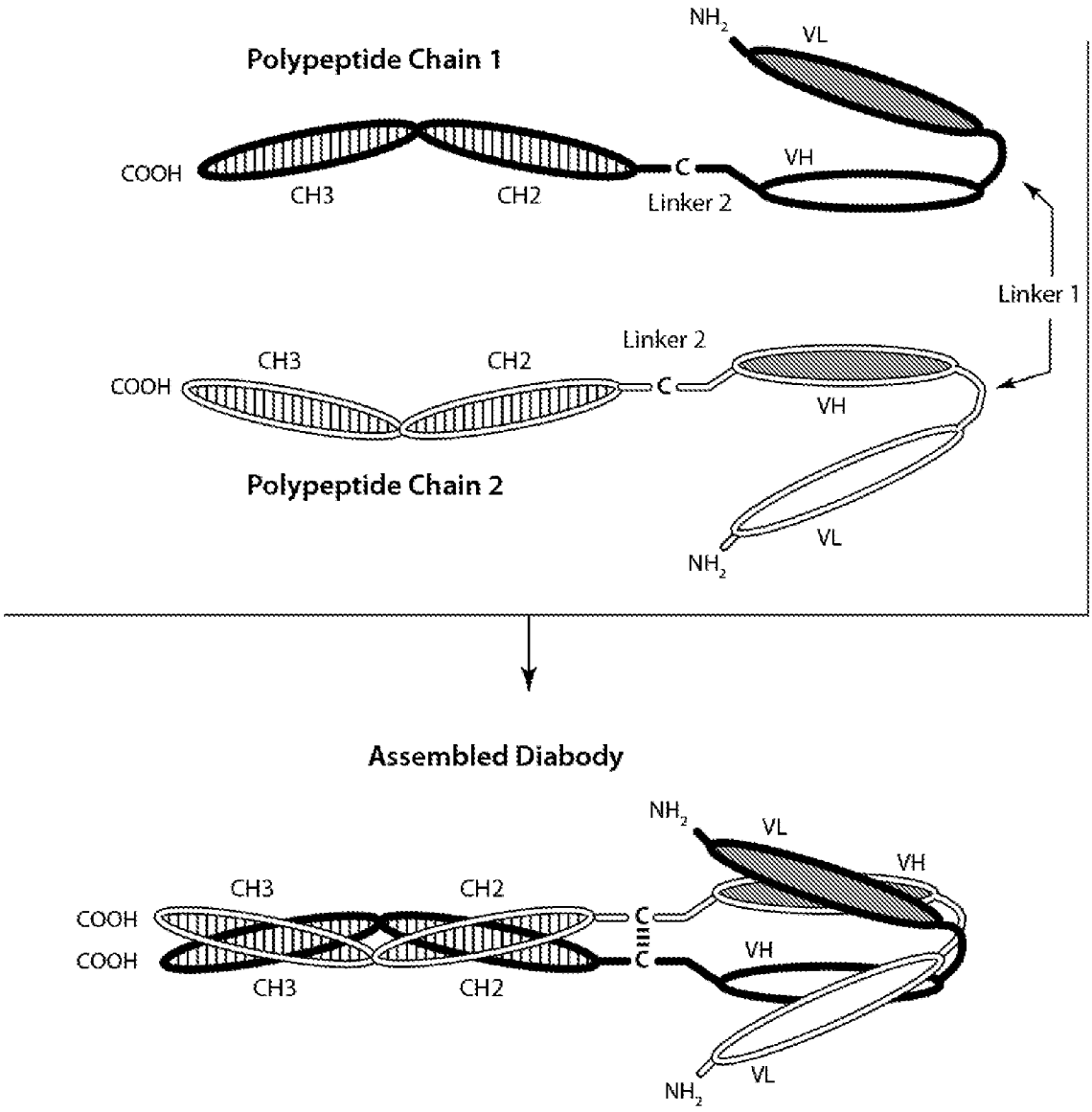
Polypeptide Chain 1
NH2
VL
COOH
CH3
CH2
C
Linker 2
VH
Linker 1
Linker 2
CH3
CH2
COOH
C
VH
Polypeptide Chain 2
VL
NH2
Assembled Diabody
NH2
VL
VH
CH3
CH2
COOH
COOH
C
C
VH
CH3
CH2
VL
NH2

Figure 2

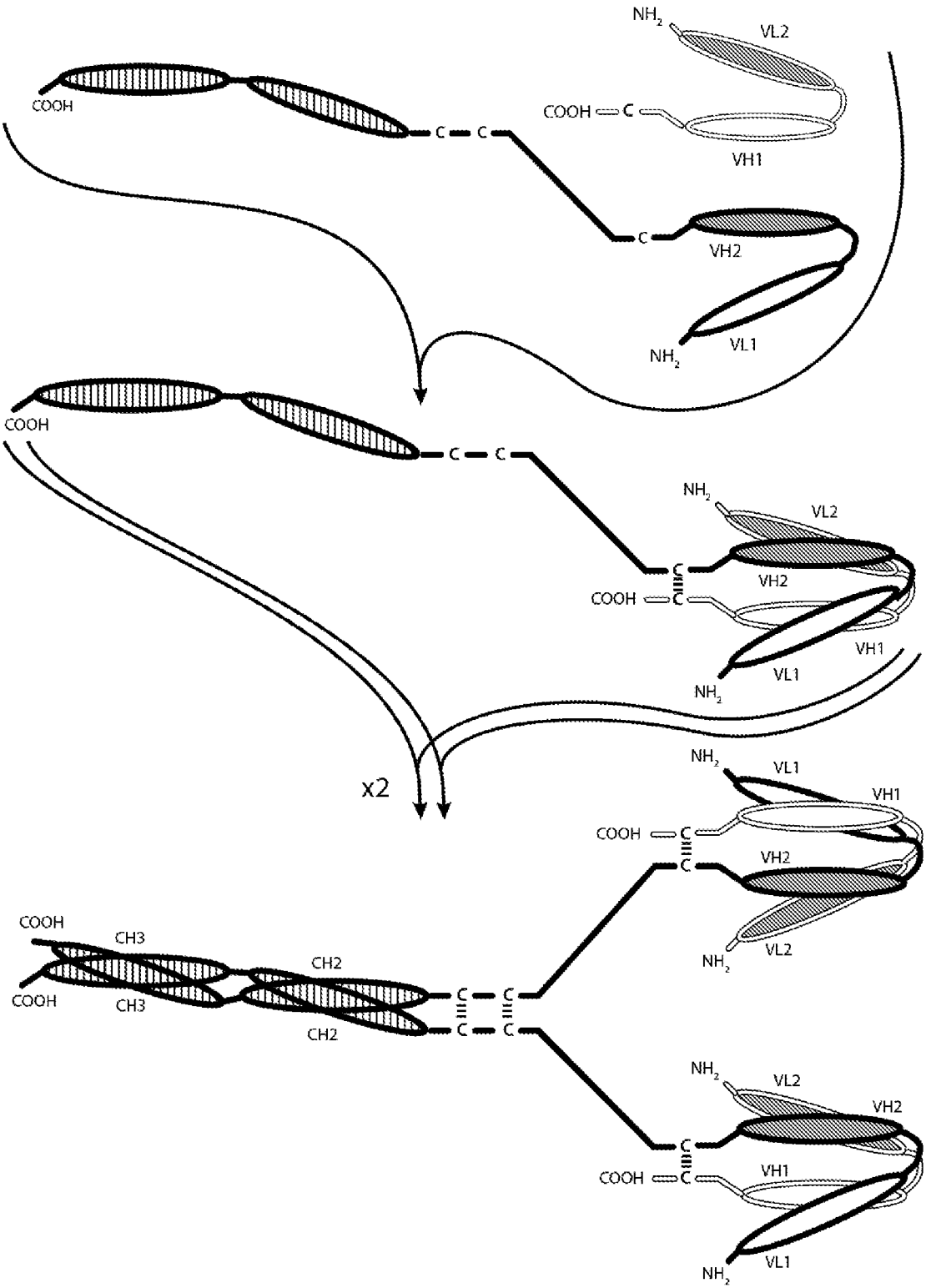
NH2
VL2
COOH
C
C
COOH
C
VH1
C
VH2
NH2
VL1
COOH
C
C
NH2
VL2
VH2
COOH
C
C
VH1
NH2
VL1
x2
NH2
VL1
VH1
COOH
C
C
VH2
NH2
VL2
COOH
CH3
CH2
COOH
CH3
C
C
C
C
CH2
NH2
VL2
VH2
C
C
COOH
VH1
NH2
VL1

Figure 3A

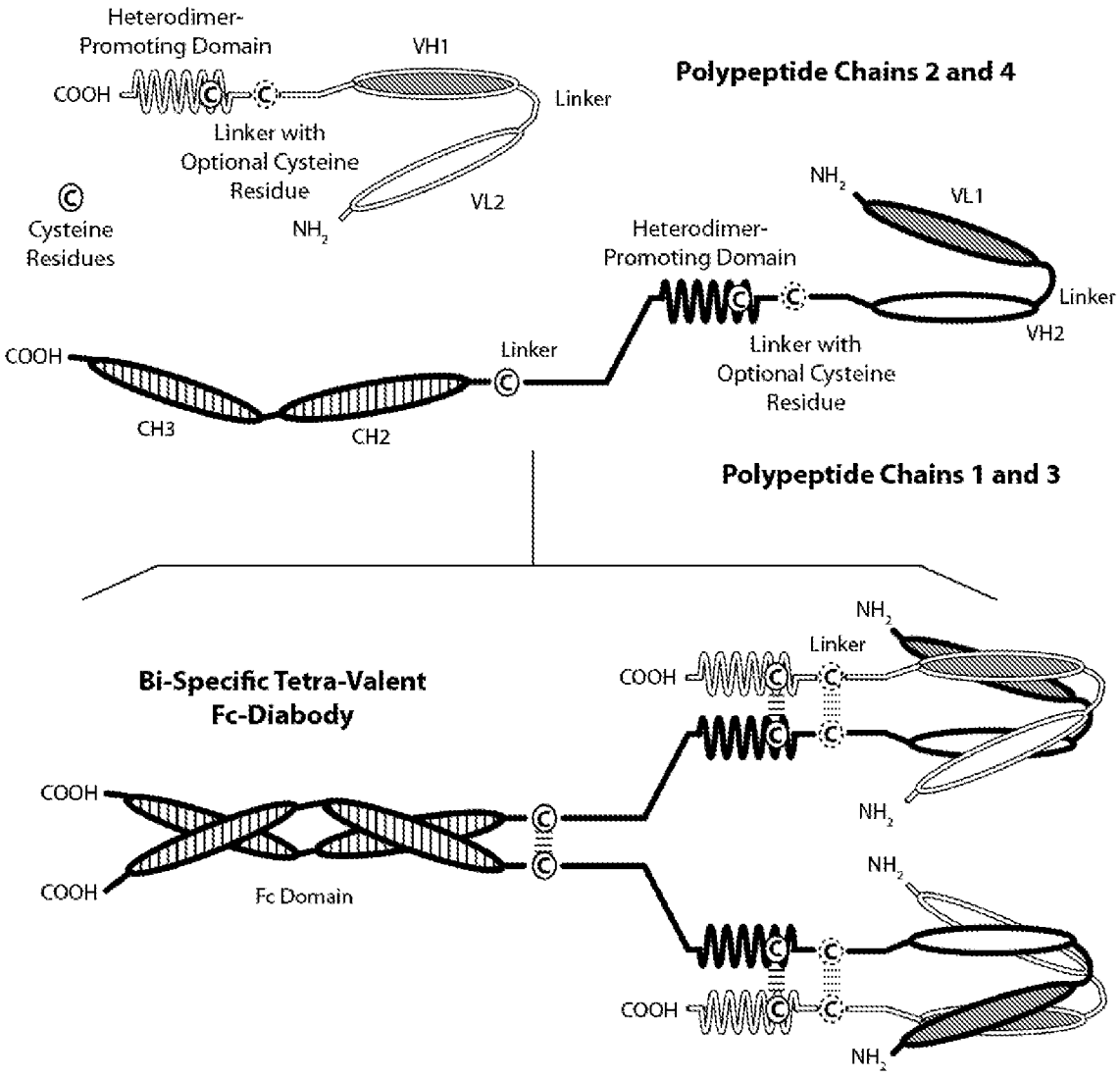
Heterodimer-
Promoting Domain
VH1
COOH
Linker
Linker with
Optional Cysteine
Residue
VL2
NH2
Cysteine
Residues
Polypeptide Chains 2 and 4
NH2
VL1
Heterodimer-
Promoting Domain
Linker
VH2
Linker with
Optional Cysteine
Residue
COOH
Linker
CH3
CH2
Polypeptide Chains 1 and 3
Bi-Specific Tetra-Valent
Fc-Diabody
NH2
Linker
COOH
COOH
COOH
Fc Domain
NH2
NH2
COOH
NH2

Figure 3B

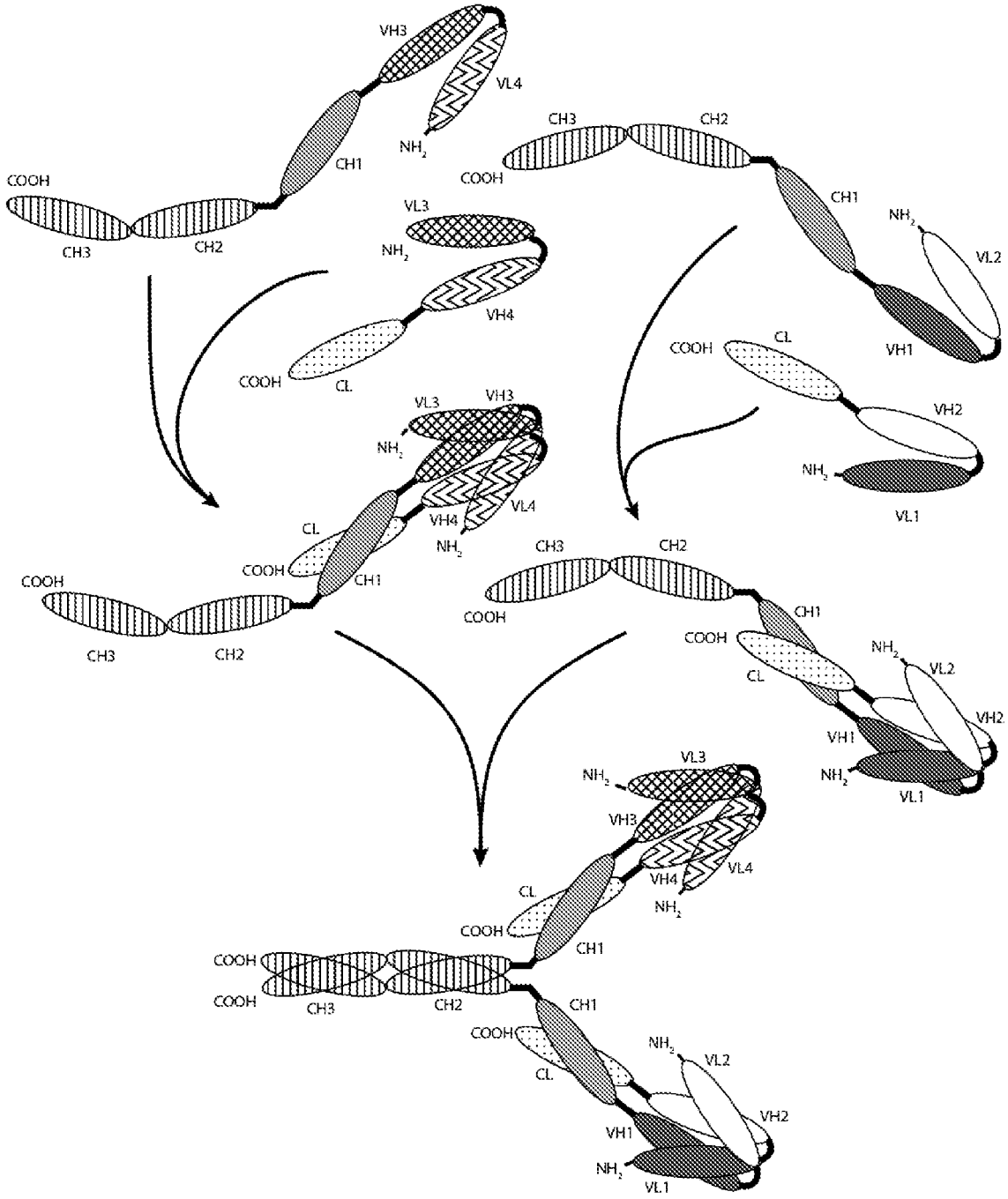
VH3
VL4
NH2
CH1
COOH
CH3
CH2
VL3
NH2
VH4
COOH
CL
CH3
CH2
COOH
CH1
NH2
VL2
VH1
CL
COOH
VH2
NH2
VL1
VL3
VH3
NH2
VH4
VL4
CL
COOH
CH1
NH2
COOH
CH3
CH2
CH3
CH2
COOH
CH1
COOH
CL
NH2
VL2
VH1
VH2
NH2
VL1
VL3
NH2
VH3
VH4
VL4
NH2
CL
COOH
CH1
COOH
COOH
CH3
CH2
CH1
COOH
CL
NH2
VL2
VH1
VH2
NH2
VL1

Figure 3C

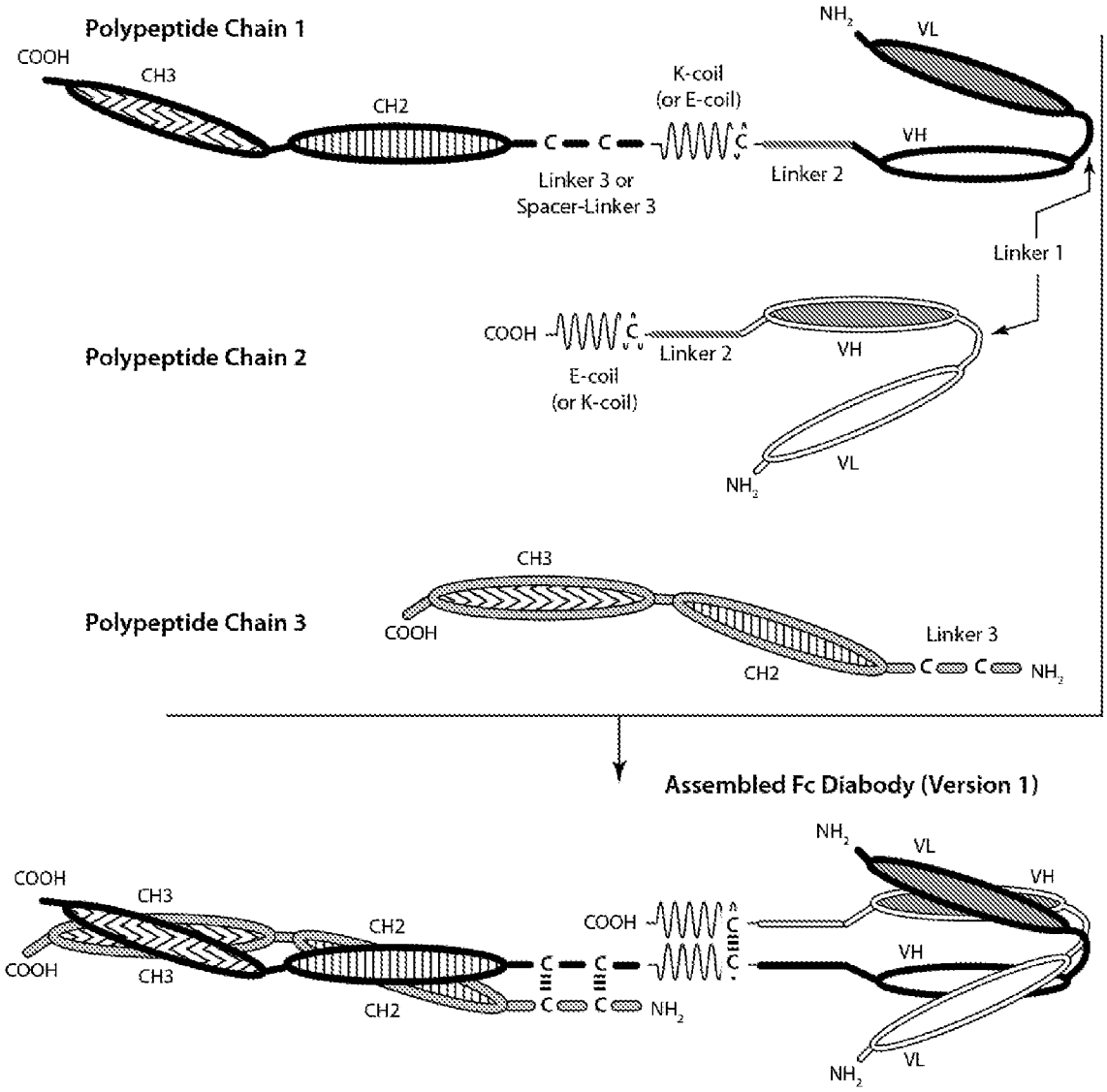
Polypeptide Chain 1
COOH
CH3
CH2
K-coil
(or E-coil)
Linker 3 or
Spacer-Linker 3
Linker 2
NH2
VL
VH
Linker 1
Polypeptide Chain 2
COOH
E-coil
(or K-coil)
Linker 2
VH
VL
NH2
Polypeptide Chain 3
CH3
COOH
CH2
Linker 3
NH2
Assembled Fc Diabody (Version 1)
COOH
CH3
CH3
COOH
CH2
CH2
NH2
VL
VH
VH
VL
NH2

Figure 4A

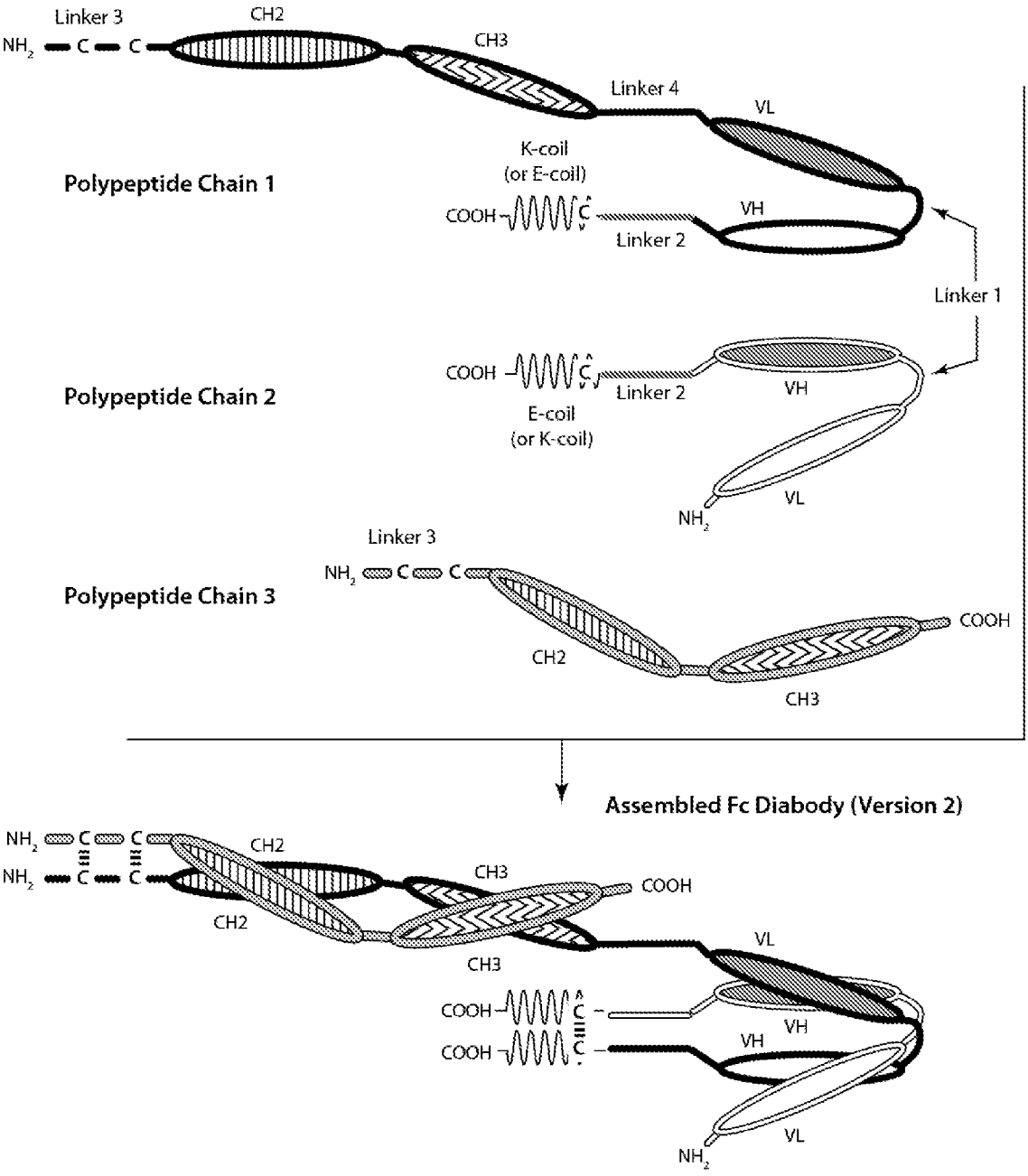
Linker 3
CH2
CH3
Linker 4
VL
K-coil
(or E-coil)
Polypeptide Chain 1
VH
Linker 2
Linker 1
Polypeptide Chain 2
E-coil
(or K-coil)
Linker 3
Polypeptide Chain 3
COOH
Assembled Fc Diabody (Version 2)

Figure 4B

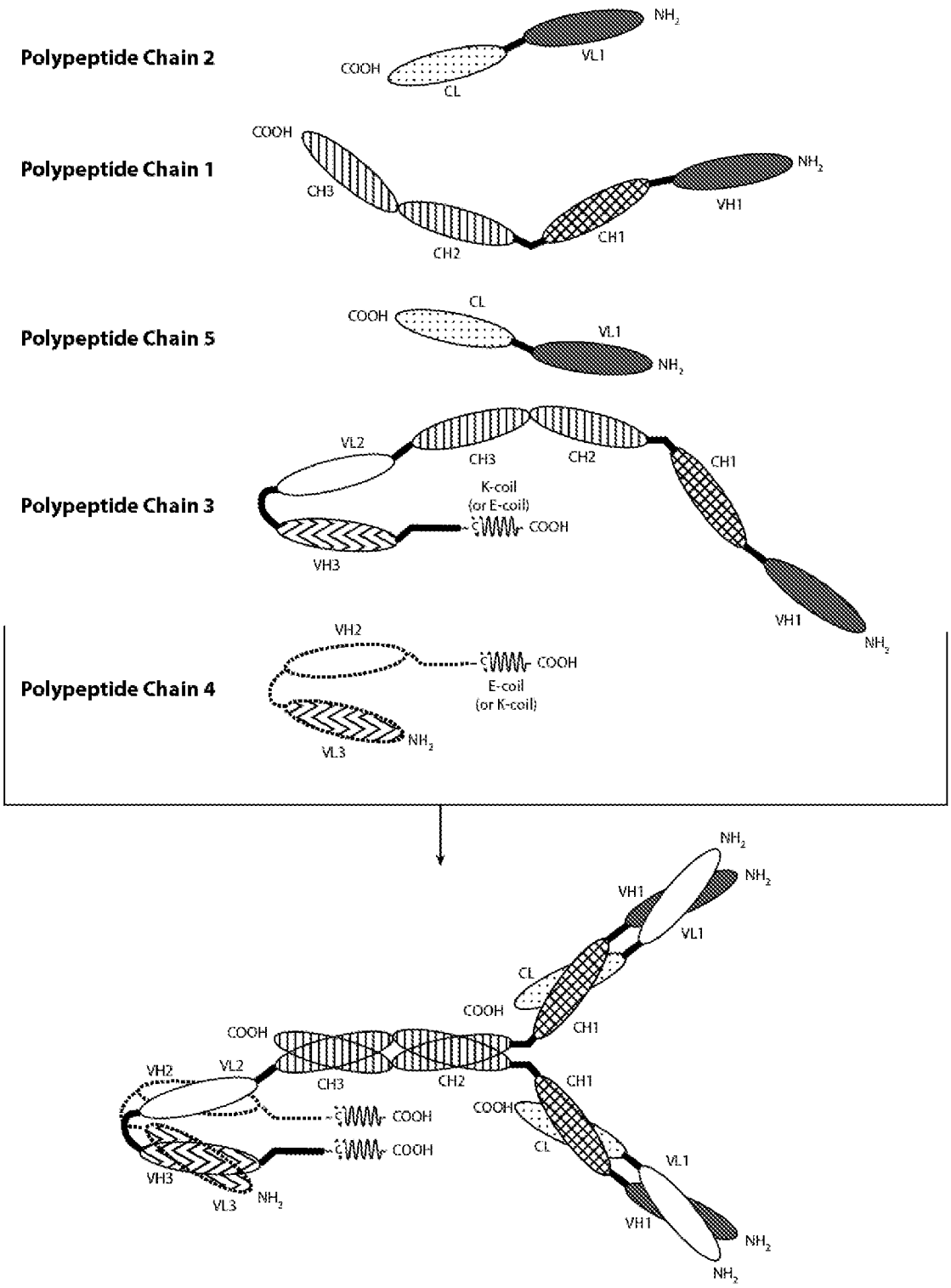
Polypeptide Chain 2
NH2
VL1
COOH
CL
Polypeptide Chain 1
COOH
CH3
CH2
CH1
VH1
NH2
Polypeptide Chain 5
CL
COOH
VL1
NH2
Polypeptide Chain 3
VL2
CH3
CH2
CH1
K-coil
(or E-coil)
COOH
VH3
VH1
NH2
Polypeptide Chain 4
VH2
COOH
E-coil
(or K-coil)
VL3
NH2
NH2
NH2
VH1
VL1
CL
COOH
CH1
COOH
VH2
VL2
CH3
CH2
CH1
COOH
COOH
CL
VL1
VH3
VL3
NH2
VH1
NH2
NH2

Figure 5

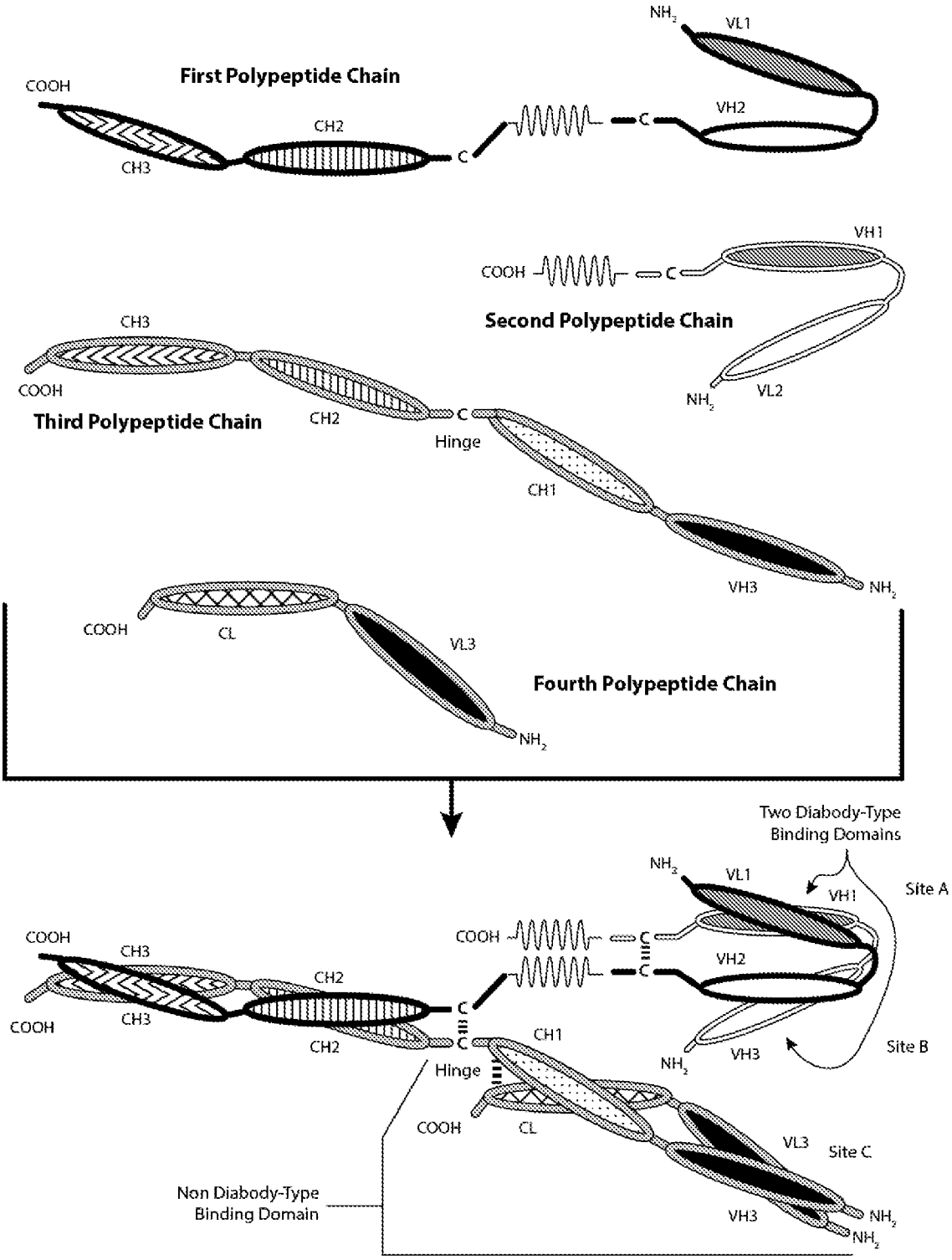
First Polypeptide Chain
COOH
CH3
CH2
C
NH2
VL1
VH2
Second Polypeptide Chain
VH1
COOH
C
NH2
VL2
Third Polypeptide Chain
CH3
COOH
CH2
C
Hinge
CH1
VH3
NH2
Fourth Polypeptide Chain
COOH
CL
VL3
NH2
Two Diabody-Type
Binding Domains
Site A
Site B
Site C
Non Diabody-Type
Binding Domain

Figure 6A

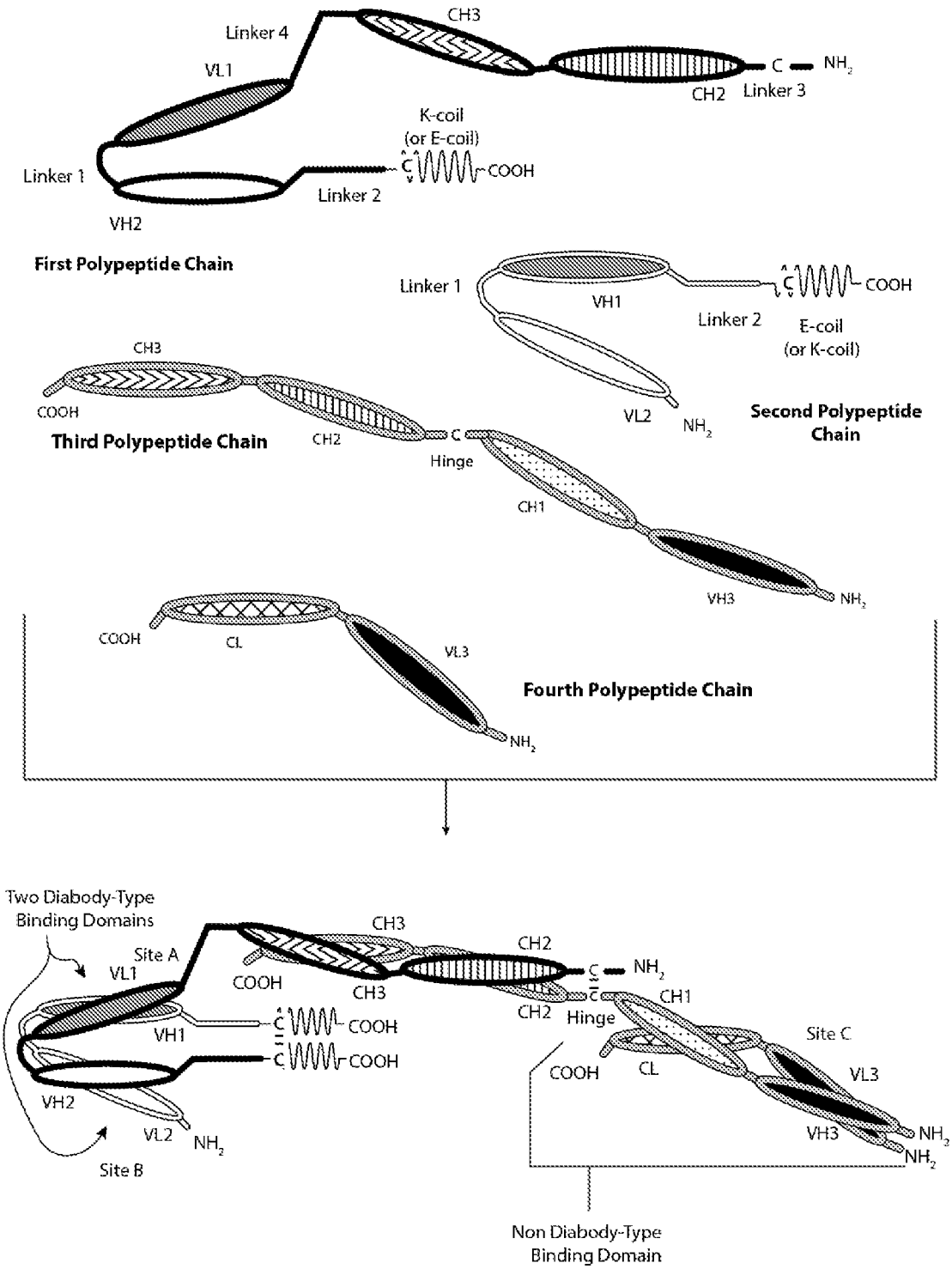
CH3
Linker 4
VL1
C
NH2
CH2
Linker 3
K-coil
(or E-coil)
Linker 1
COOH
Linker 2
VH2
First Polypeptide Chain
Linker 1
VH1
COOH
Linker 2
E-coil
(or K-coil)
CH3
VL2
NH2
Second Polypeptide Chain
COOH
Third Polypeptide Chain
CH2
C
Hinge
CH1
VH3
NH2
COOH
CL
VL3
Fourth Polypeptide Chain
NH2
Two Diabody-Type
Binding Domains
Site A
VL1
CH3
CH2
COOH
C
NH2
CH3
CH1
CH2
Hinge
VH1
COOH
COOH
Site C
COOH
CL
VL3
VH2
VH3
NH2
VL2
NH2
NH2
Site B
Non Diabody-Type
Binding Domain

Figure 6B

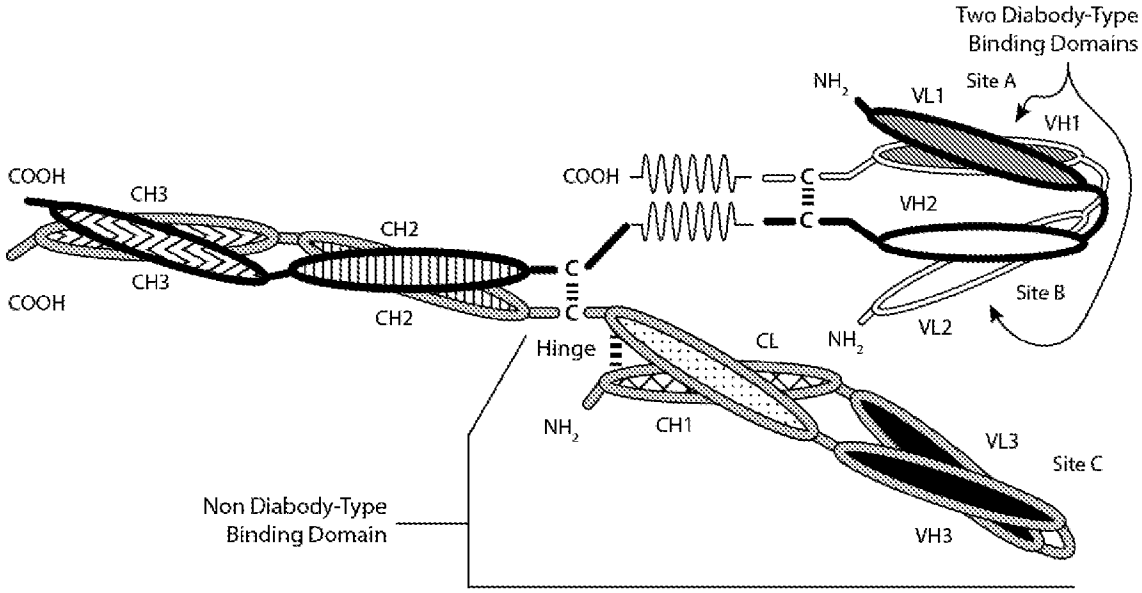

Figure 6C

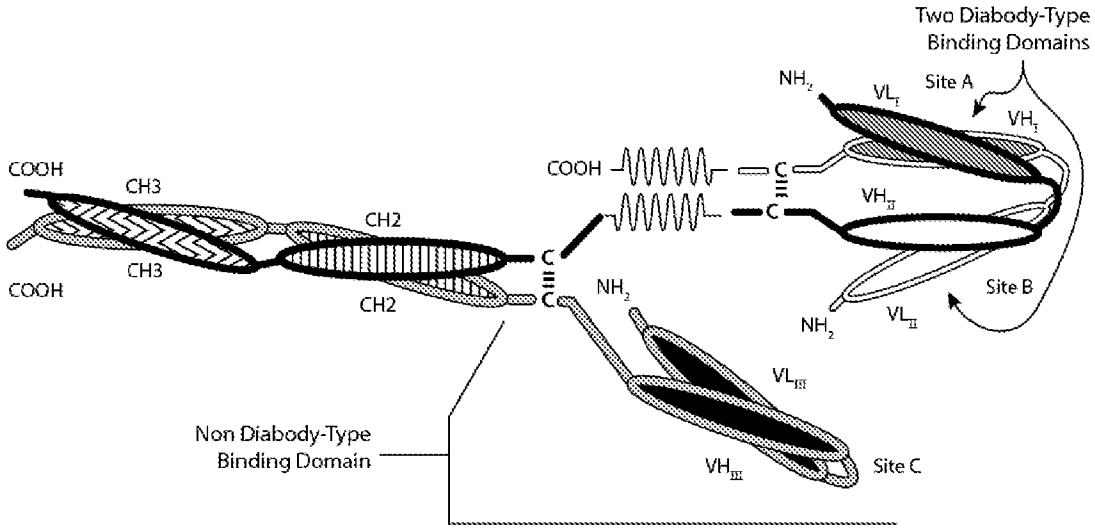

Figure 6D

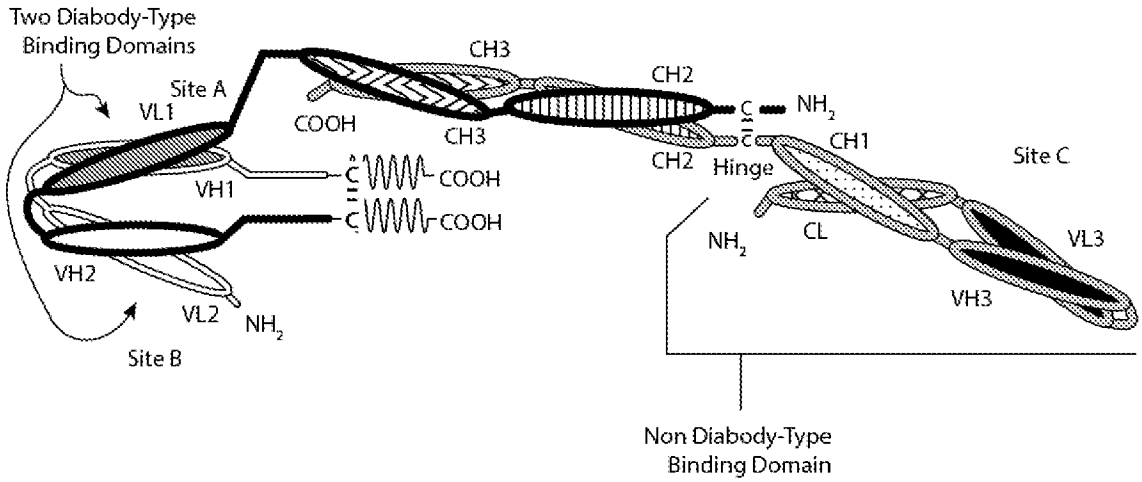

Figure 6E

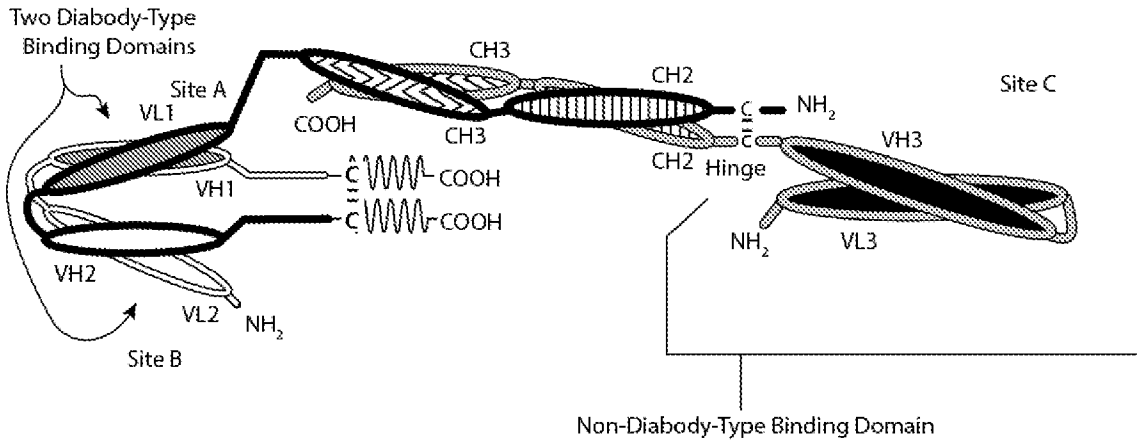

Figure 6F

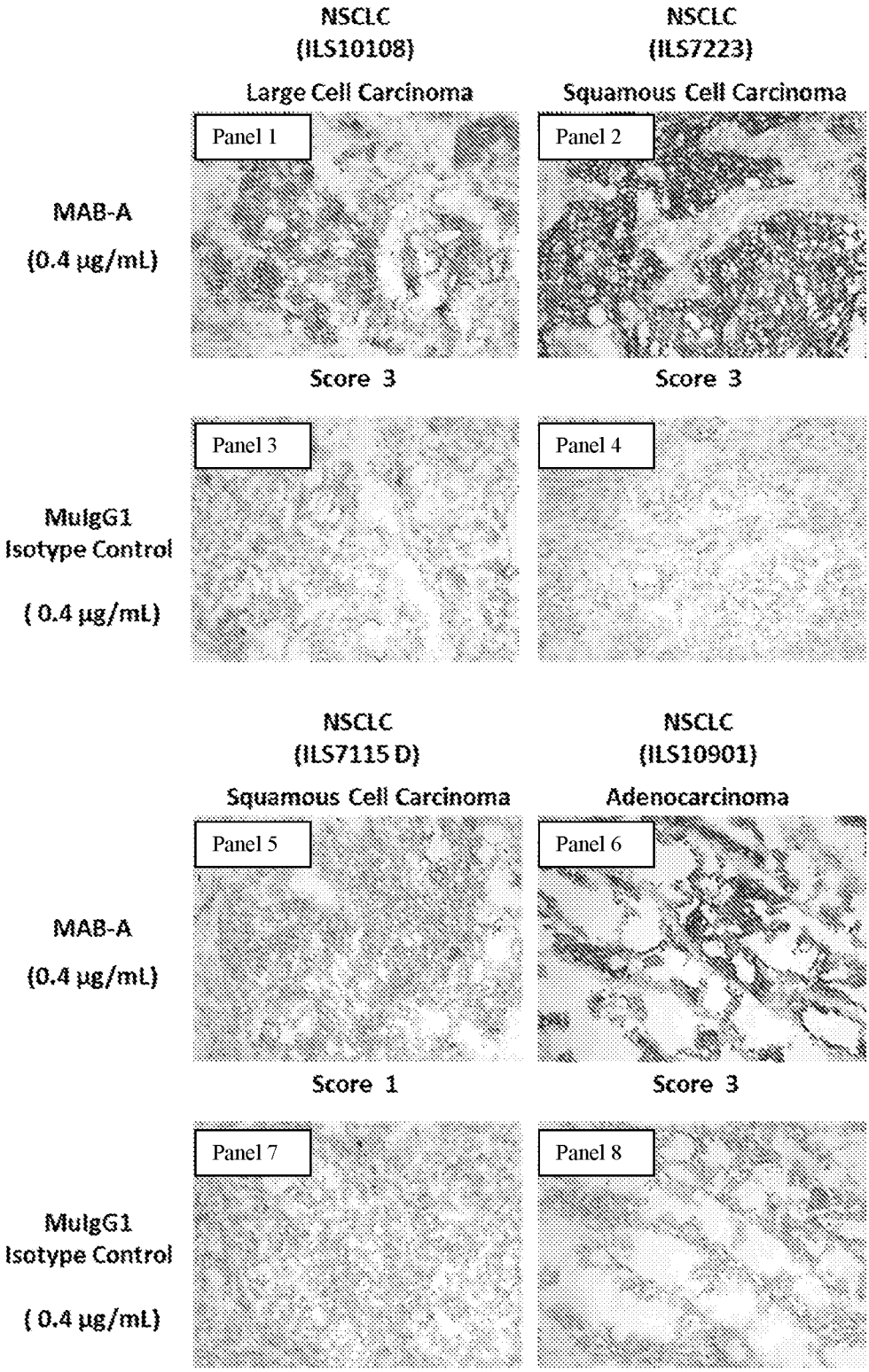
NSCLC
(ILS10108)
Large Cell Carcinoma
NSCLC
(ILS7223)
Squamous Cell Carcinoma
Panel 1
Panel 2
MAB-A
(0.4 µg/mL)
Score 3
Score 3
Panel 3
Panel 4
MuIgG1
Isotype Control
(0.4 µg/mL)
NSCLC
(ILS7115 D)
Squamous Cell Carcinoma
NSCLC
(ILS10901)
Adenocarcinoma
Panel 5
Panel 6
MAB-A
(0.4 µg/mL)
Score 1
Score 3
Panel 7
Panel 8
MuIgG1
Isotype Control
(0.4 µg/mL)

Figure 7A

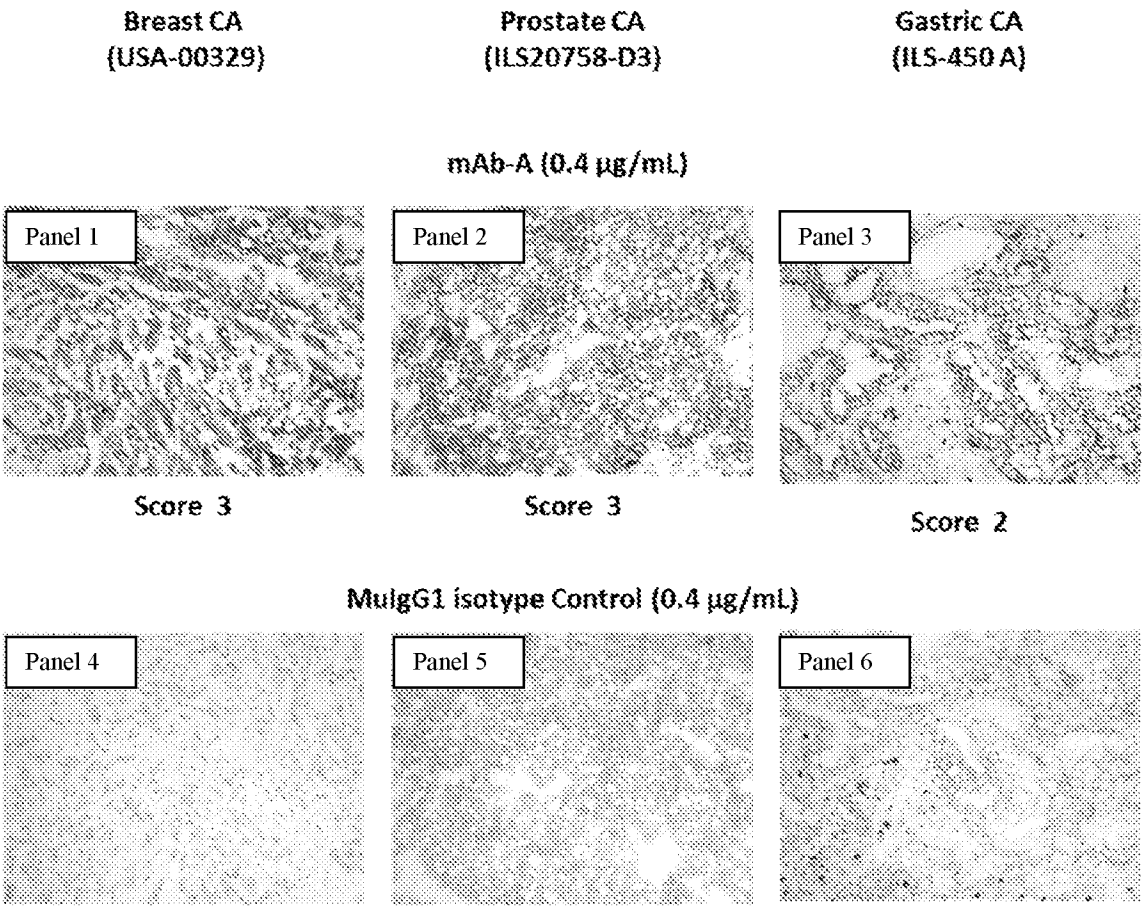
Breast CA
(USA-00329)
Prostate CA
(ILS20758-D3)
Gastric CA
(ILS-450 A)
mAb-A (0.4 µg/mL)
Panel 1
Panel 2
Panel 3
Score 3
Score 3
Score 2
MuIgG1 isotype Control (0.4 µg/mL)
Panel 4
Panel 5
Panel 6

Figure 7B

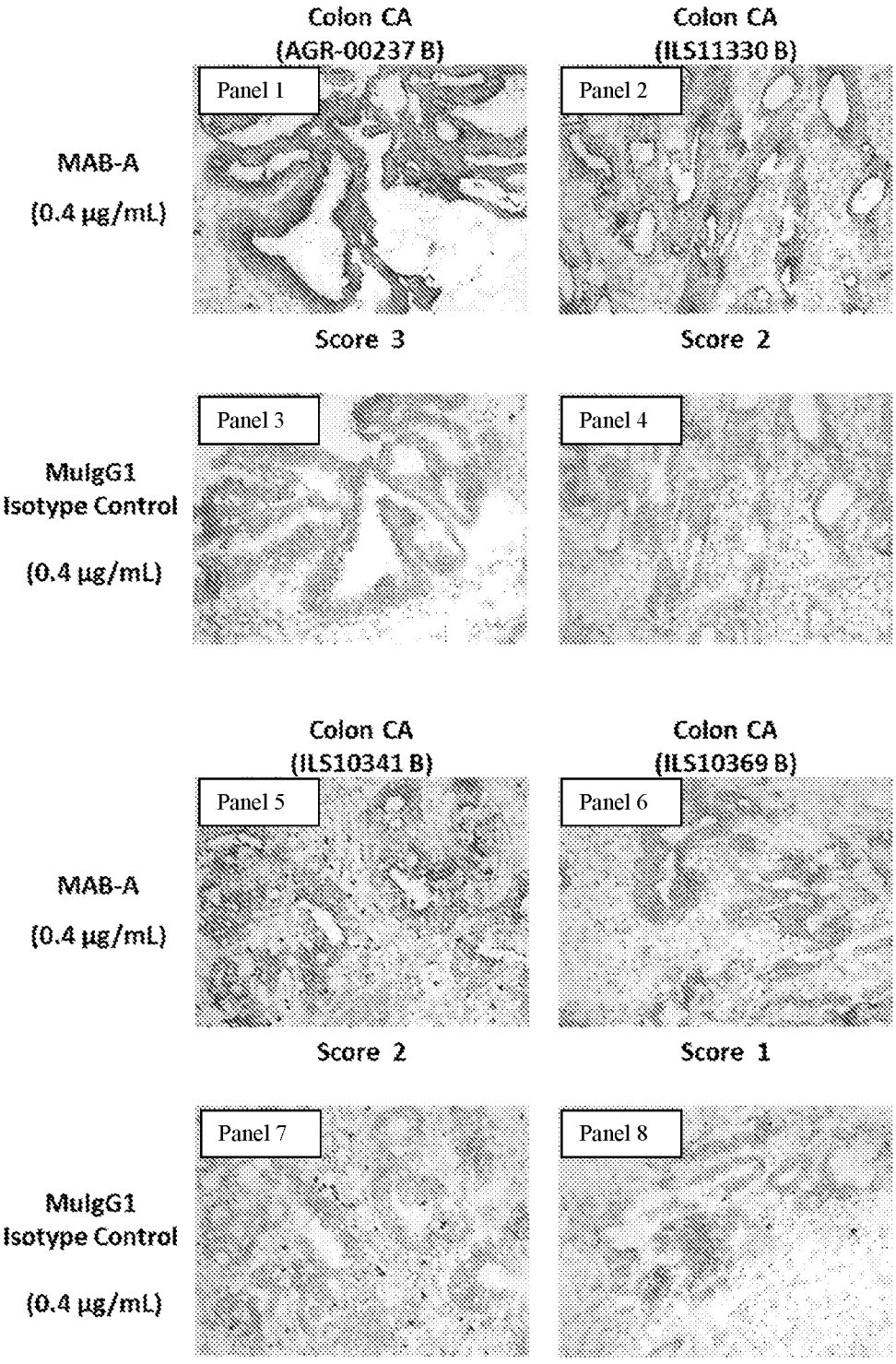
Colon CA
(AGR-00237 B)
Colon CA
(ILS11330 B)
Panel 1
Panel 2
MAB-A
(0.4 µg/mL)
Score 3
Score 2
Panel 3
Panel 4
MuIgG1
Isotype Control
(0.4 µg/mL)
Colon CA
(ILS10341 B)
Colon CA
(ILS10369 B)
Panel 5
Panel 6
MAB-A
(0.4 µg/mL)
Score 2
Score 1
Panel 7
Panel 8
MuIgG1
Isotype Control
(0.4 µg/mL)

Figure 7C

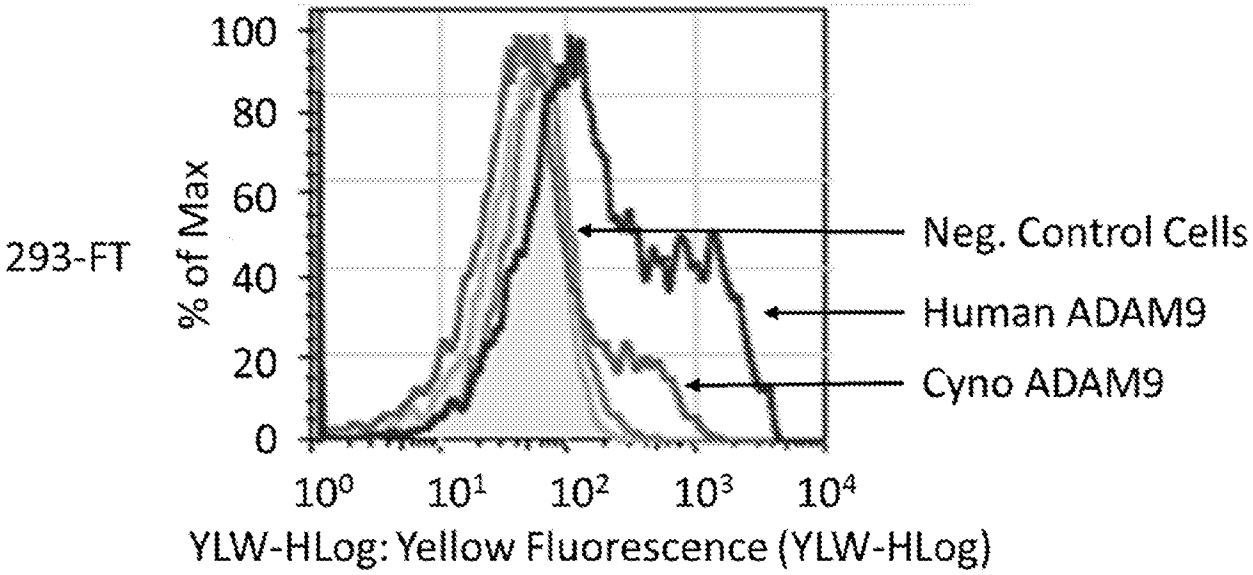

Figure 8A

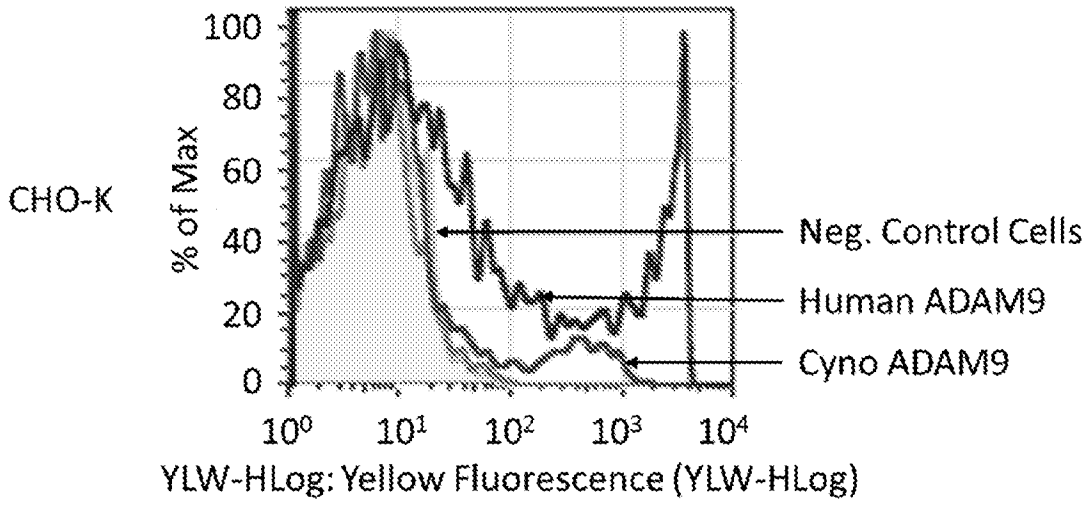

Figure 8B

		FR1	CDR1
Murine MAB-A VH	**SEQ ID NO:7**	QVQLQQPGAELVKPGASVKLSCKASGYTFT	SYWMH
hMAB-A VH(1)	**SEQ ID NO:16**	E...VES.GG.....G.LR...A...F..S	.....
hMAB-A VH(2)	**SEQ ID NO:17**	E...VES.GG.....G.LR...A...F..S	.....
hMAB-A VH(3)	**SEQ ID NO:18**	E...VES.GG.....G.LR...A...F..S	.....
hMAB-A VH(4)	**SEQ ID NO:19**	E...VES.GG.....G.LR...A...F..S	...I.
hMAB-A VH(2B)	**SEQ ID NO:21**	E...VES.GG.....G.LR...A...F..S	.....
hMAB-A VH(2C)	**SEQ ID NO:22**	E...VES.GG.....G.LR...A...F..S	.....
hMAB-A VH(2D)	**SEQ ID NO:23**	E...VES.GG.....G.LR...A...F..S	.....
hMAB-A VH(2I)	**SEQ ID NO:28**	E...VES.GG.....G.LR...A...F..S	.....

FR2	CDR2	FR3
WVKQRPGQGLEWIG	EIIPINGHTNYNEKFKS	KATLTLDKSSSTAYMQLSSLASEDSAVYYCAR
..R.A..K....V.	.....	RF.IS..N.KN.L.L.MG..RA..T.....
..R.A..K....V.	.....F.....	RF.IS..N.KN.L.L.MG..RA..T.....
..R.A..K....V.	.....F.......R.QG	RF.IS..N.KN.L.L.MG..RA..T.....
..R.A..K....V.	.....F.......R.QG	RF.IS..N.KN.L.L.MG..RA..T.....
..R.A..K....V.	.....F.....	RF.IS..N.KN.L.L.MG..RA..T.....
..R.A..K....V.	.....F.....	RF.IS..N.KN.L.L.MG..RA..T.....
..R.A..K....V.	.....F.....	RF.IS..N.KN.L.L.MG..RA..T.....
..R.A..K....V.	.....F.....	RF.IS..N.KN.L.L.MG..RA..T.....

CDR3	FR4
GGYYYYGSRDYFDY	WGQGTTLTVSS
.....	......V....
.....	......V....
.....	......V....
.....	......V....
......IGKGVL..	......V....
......PRFGWL..	......V....
......TGKGVL..	......V....
......PRQGFL..	......V....

Figure 9A

		FR1	CDR1
Murine MAB-A VL	SEQ ID NO:11	DIVLTQSPASLAVSLGQRATISC	KASQSVDYDGDSYMN
hMAB-A VL(1)	SEQ ID NO:54	...M....D.......E.....	....
hMAB-A VL(2)	SEQ ID NO:55	...M....D.......E.....	........S......
hMAB-A VL(3)	SEQ ID NO:56	...M....D.......E.....	R.......S......
hMAB-A VL(4)	SEQ ID NO:57	...M....D.......E.....	R.......S....L.

FR2	CDR2	FR3	CDR3	FR4
WYQQIPGQPPKLLIY	AASDLES	GIPARFSGSGSGTDFTLNIHPVEEEDAATYYC	QQSHEDPFT	FGGGTKLEIK
....K..........	....	..................T.SSL.P..F.....	....	....
....K..........	....	..................T.SSL.P..F.....	....	....
....K..........	....	..................T.SSL.P..F.....	....	....
....K..........	....	..................T.SSL.P..F.....	...YST...	....

Figure 9B

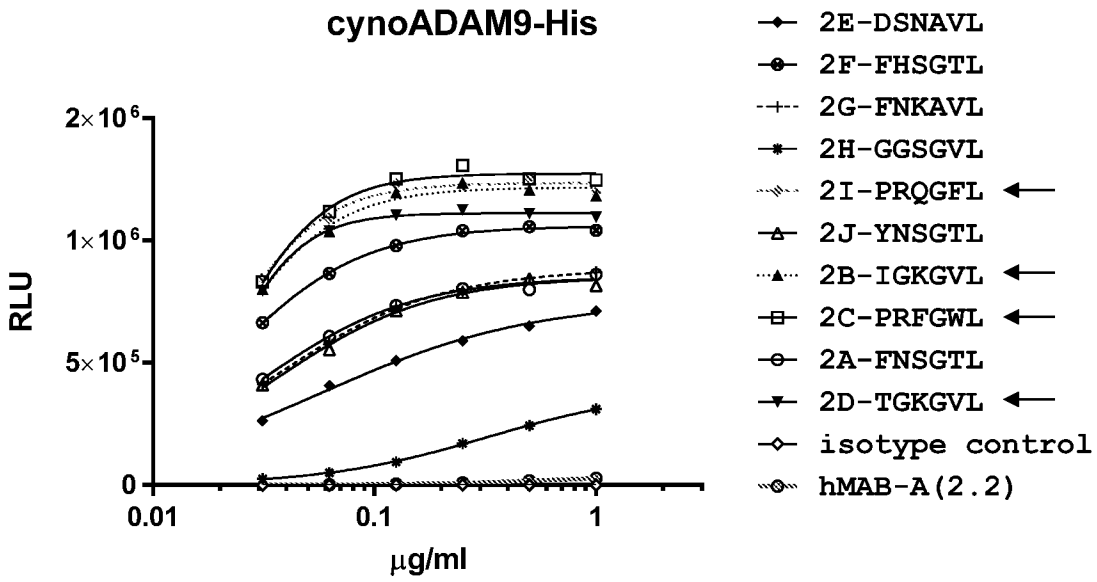

Figure 10A

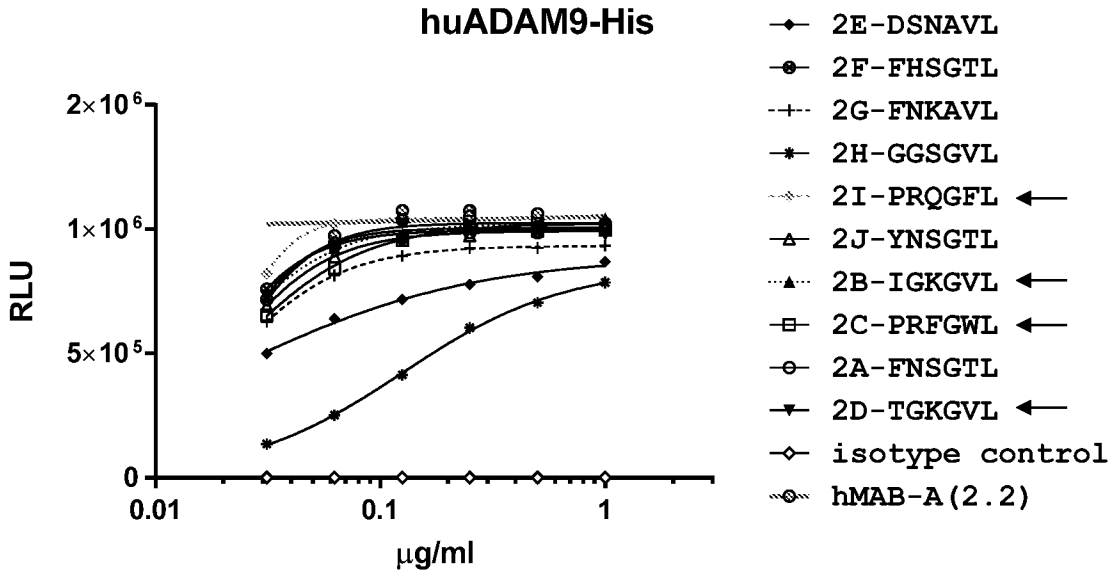

Figure 10B

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 1301.0147PCT	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/US17/67770	International filing date *(day/month/year)* 21 December 2017 (21.12.2017)	(Earliest) Priority Date *(day/month/year)* 23 December 2016 (23.12.2016)
Applicant MacroGenics, Inc.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 9 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☒ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. 1

   ☐ as suggested by the applicant.

   ☒ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☐ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/67770

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/67770

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

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

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

3. ☒ Claims Nos.: 4-8, 12, 20-48
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US17/67770

A. CLASSIFICATION OF SUBJECT MATTER
IPC - A61K 39/395; A61P 35/04; C07K 16/18, 16/40 (2018.01)
CPC - A61K 39/395, 39/39558; A61P 35/04; C07K 16/18, 16/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0111951 A1 (MATHER, J. et al.) 6 May 2010; paragraphs [0014], [0023], [0053], [0133].	1-2, 3/1-2, 9-10, 11/9-10, 13-19
A	US 2010/0297664 A1 (WADHWA, R. et al.) 25 November 2010; paragraphs [0031], [0043]-[0045], [0144], [0156]; claim 57.	1-2, 3/1-2, 9-10, 11/9-10, 13-19
A	US 2013/0243795 A1 (JANSSEN BIOTECH, INC.) 19 September 2013; paragraphs [0011], [0014], [0048], [0129].	1-2, 3/1-2, 9-10, 11/9-10, 13-19
A	WO 2012/018687 A1 (MACROGENICS, INC.) 9 February 2012; paragraphs [0015], [0042], [00151].	1-2, 3/1-2, 9-10, 11/9-10, 13-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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05 March 2018 (05.03.2018)	13 MAR 2018
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