UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
63/684,204	**08/16/2024 06:14:27 PM Z ET**	**080015-1445918-041000US**

Title of Invention

ENGINEERED CD9 ANTIBODIES AND THEIR USE

Application Information

APPLICATION TYPE	Utility - Provisional Application under 35 USC 111(b)	PATENT #	-
CONFIRMATION #	7481	FILED BY	Jeffrey Luo
PATENT CENTER #	66821877	FILING DATE	-
CUSTOMER #	20350	FIRST NAMED INVENTOR	Kam Tong LEUNG
CORRESPONDENCE ADDRESS	-	AUTHORIZED BY	Chuan Gao

Documents

TOTAL DOCUMENTS: 5

DOCUMENT		PAGES	DESCRIPTION	SIZE (KB)
ADS_080015_1445918_041000US.pdf		10	Application Data Sheet	1227 KB
PROV_APP_080015_1445918_041000US.pdf		54	-	2810 KB
PROV_APP_080015_1445918_041000US-SPEC.pdf	(1-39)	39	Specification	424 KB
PROV_APP_080015_1445918_041000US-CLM.pdf	(40-42)	3	Claims	90 KB
PROV_APP_080015_1445918_041000US-ABST.pdf	(43-43)	1	Abstract	86 KB
PROV_APP_080015_1445918_041000US-DRW.NONBW.pdf	(44-54)	11	Drawings-other than black and white line drawings	2598 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
ADS_080015_1445918_041000US.pdf	6A1D8577FE52BBC2B5F7AF660CB0E479451E09E237C45C1537AD91EF54E9D5A719BD9CFE116B9CF27C8ECD7F100D295CD9129982332B09B2F37859F89E4F1D8B
PROV_APP_080015_1445918_041000US.pdf	B3D7EE03EB9B3C4029E4F0B114013316B6C89320FF72AB134D5E475503B8AB1E0FA836F099CE39D56BDB56095333621E9740D0DF9E4C9850EB5E00697A14972B
PROV_APP_080015_1445918_041000US-SPEC.pdf	BA1B2D3CC9BD594E26D0FBDA098C0E58D1F60CCEB551D63CA66BA94908E8526070C33515F91F235E07F4E88A7A29DA4D792F1C818450D485905AE165397D1B03
PROV_APP_080015_1445918_041000US-CLM.pdf	B4EA30692A7A051F980C31064444AE415F0BA10238D9F8C4E952581D24DF6DFBC96891B9BE36B0DD01C81B7CE4A508B6A9BA0C8C0C662997F561F1D5F92E5376
PROV_APP_080015_1445918_041000US-ABST.pdf	6236BDADDB981DE4D0FA9F9315673B0106D231546B76094A2CCB40B2E8C3B0C963BCD5974E33461540E70E48CC81E69D2DFE35761AF53F5AE90DC9D6686C12CA
PROV_APP_080015_1445918_041000US-DRW.NONBW.pdf	B0A3C78B9CBFE93DADD18C6BA9FCC811168D1C99C25DFAA6BAC6719DD59558C75482F993218227963A27BA631C30994300DB6ECD6C35B7CFBAE5A32324F3FBAE

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

UNITED STATES
PATENT AND TRADEMARK OFFICE

P.O. Box 1450
Alexandria, VA 22313 - 1450
www.uspto.gov

ELECTRONIC PAYMENT RECEIPT

APPLICATION #	RECEIPT DATE / TIME	ATTORNEY DOCKET #
63/684,204	**08/16/2024 06:14:27 PM Z ET**	**080015-1445918-041000US**

Title of Invention

ENGINEERED CD9 ANTIBODIES AND THEIR USE

Application Information

APPLICATION TYPE	Utility - Provisional Application under 35 USC 111(b)	PATENT #	-
CONFIRMATION #	7481	FILED BY	Jeffrey Luo
PATENT CENTER #	66821877	AUTHORIZED BY	Chuan Gao
CUSTOMER #	20350	FILING DATE	-
CORRESPONDENCE ADDRESS	-	FIRST NAMED INVENTOR	Kam Tong LEUNG

Payment Information

PAYMENT METHOD	PAYMENT TRANSACTION ID	PAYMENT AUTHORIZED BY
CARD / 8428	**E20248FI16065011**	**Jeffrey Luo**

FEE CODE	DESCRIPTION	ITEM PRICE($)	QUANTITY	ITEM TOTAL($)
2005	PROVISIONAL APPLICATION FILING FEE	120.00	1	120.00
			TOTAL AMOUNT:	**$120.00**

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage

submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

Attorney Docket No. 80015-1445918-041000US
Client Ref. No. 23/MED/1262

PROVISIONAL PATENT APPLICATION

ENGINEERED CD9 ANTIBODIES AND THEIR USE

Inventor(s): Kam Tong LEUNG, a citizen of Hong Kong SAR
Research Office, 8/F, Tower A, Hong Kong Children's Hospital
Kowloon Bay, Kowloon
Hong Kong SAR, CHINA

Qi ZHAO, a citizen of Hong Kong SAR
E12-3022, Faculty of Health Sciences, The University of Macau
Taipa, Macau, CHINA

Chi Kong LI, a citizen of Hong Kong SAR
Doctor's Office, 9/F, Tower B, Hong Kong Children's Hospital
Kowloon Bay, Kowloon
Hong Kong SAR, CHINA

Yuen Yee CHAN, a citizen of Hong Kong SAR
Room 84041, 6/F, Lui Che Woo Clinical Sciences Building
Prince Wales Hospital, Sha Tin, New Territories
Hong Kong SAR, CHINA

Wing Hei NG, a citizen of Hong Kong SAR
Lab D, 8/F, Tower A, Hong Kong Children's Hospital
Kowloon Bay, Kowloon
Hong Kong SAR, CHINA

Shengyu FU, a citizen of China
E12-2069, Faculty of Health Sciences, The University of Macau
Taipa, Macau, CHINA

Assignees: The Chinese University of Hong Kong
The University of Macau

Entity: Small

KILPATRICK TOWNSEND & STOCKTON LLP
Two Embarcadero Center, Suite 1900
San Francisco, California 94111-3834
Tel: 415-576-0200

ENGINEERED CD9 ANTIBODIES AND THEIR USE

BACKGROUND OF THE INVENTION

[0001] The CD9 antigen is a 24 kDa tetraspanin membrane protein that contains four putative transmembrane domains, short N- and C-terminal cytoplasmic domains, a small intracellular loop, and two extracellular loops. CD9 is expressed on the surface of a large number of mammalian cell types, such as epithelial cells, endothelial cells, smooth muscle cells, hematopoietic cells, in addition to malignant cells of different origins. Due to its participation in a wide variety of important cellular functions including cell adhesion and migration, cancer progression and metastasis, immune and allergic responses, and viral infection, CD9 is attracting increased attention in the context of biomedical research and development of new therapeutic strategies for CD9-dependent diseases, especially for CD9+ cancers such as leukemia.

[0002] Cancer-related causes are among the top reasons of death in developed nations. In the US alone, the number of new cases of cancer of any type averages about 450 per 100,000 people per year, and the number of deaths averages about 170 per 100,000 people per year. Cancer is a disease with a high mortality rate: while about 1,700,000 newly diagnosed cancer cases are expected each year, over 600,000 deaths annually are attributable to various types of cancer. Based on data from recent years, it is estimated that over 38% of the population will be diagnosed with cancer at some point during their lifetime.

[0003] Because of the prevalence of cancer, its social and economical impact, and the significant role CD9 plays in the pathology of cancer, there exists an urgent need for new and more effective methods for treating cancer by targeting CD9. This invention fulfills this and other related needs.

BRIEF SUMMARY OF THE INVENTION

[0004] This disclosure provides compositions of various modified antibody constructs including single-chain variable fragments (scFvs) and humanized monoclonal antibodies derived from two newly characterized anti-CD9 monoclonal antibodies that specifically bind to human CD9 protein with high affinity. Also provided are methods for treating CD9-related diseases using the CD9 antibody construct of this invention to target CD9-expressing

cells, including those of CD9-positive cancers, such as blood cancers including leukemia, especially B-cell acute lymphoblastic leukemia (B-ALL), as well as solid tumors.

[0005] As such, in a first aspect, this invention provides an anti-CD9 antibody construct that specifically binds CD9 and comprises antibody heavy chain and light chain complementarity determining regions (CDRs) having the amino acid sequences of (1) SEQ ID NOs:5-7 and 8-10; or (2) SEQ ID NOs:11-13 and 14-16, respectively. The anti-CD9 antibody construct of this invention may be a polypeptide in the entirety, or it may be a polypeptide with at least one other polypeptide or a non-polypeptide component conjugated to it.

[0006] In some embodiments, the CD9 antibody construct of this invention comprises an antibody heavy chain variable region (V_H) and an antibody light chain variable region (V_L), each comprising the amino acid sequence set forth in (1) SEQ ID NOs:1 and 2; or (2) SEQ ID NOs:3 and 4, respectively. In some embodiments, the CD9 antibody construct of this invention comprises a V_H and a V_L, each comprising the amino acid sequence set forth in (1) SEQ ID NOs:20 and 21; or (2) SEQ ID NOs:22 and 23, respectively. In some embodiments, the CD9 antibody construct is an anti-CD9 single chain antibody or single chain variable fragment (scFv), for example, it specifically binds CD9 and comprises an antibody heavy chain and light chain variable regions comprising the CDRs having the amino acid sequences set forth in SEQ ID NOs:5-7 and 8-10 or SEQ ID NOs:11-13 and 14-16, respectively. In some embodiments, the antibody construct of this invention is a chimeric antigen receptor (CAR) construct generated from the anti-CD9 monoclonal antibodies or scFv disclosed above and herein, comprises the amino acid sequences set forth in SEQ ID NOs:1 and 2 or SEQ ID NOs:3 and 4 (humanized heavy chain and light chain variable region amino acid sequences) or SEQ ID NOs:20 and 21 or SEQ ID NOs:22 and 23 (murine antibody V_H and V_L amino acid sequences). In some embodiments, the CD9 antibody construct of this invention is a full-length IgG, a bispecific T cell engager (BiTE), or an antibody drug conjugate (ADC). In some embodiments, the CD9 antibody construct is a full-length IgG antibody comprising a modified (N297A substitution) antibody heavy chain constant region. For example, the anti-CD9 IgG antibody comprises the amino acid sequences set forth in SEQ ID NOs:5-7 and 8-10 or SEQ ID NOs:11-13 and 14-16 as the antibody heavy chain and light chain CDRs, respectively, preferably the amino acid sequences set forth in SEQ ID NOs:1 and 2 or SEQ ID NOs:3 and 4 as the antibody heavy chain and light chain variable regions, respectively, along with the amino acid sequence set forth in SEQ ID NO:17 as the antibody heavy chain

constant region and the amino acid sequence set forth in SEQ ID NO:18 as the antibody light chain constant region.

[0007] In a second aspect, the present invention provides a nucleic acid comprising a polynucleotide sequence encoding the anti-CD9 antibody construct, a fusion polypeptide comprising the anti-CD9 antibody construct described above and herein, for example, an anti-CD9 scFv or anti-CD9 CAR construct. The nucleic acid may comprise an expression cassette comprising a promoter operably linked to the polynucleotide sequence encoding the anti-CD9 antibody construct or its fusion polypeptide. In some cases, the nucleic acid is in the form of a vector, such as an expression vector including a plasmid or a viral vector.

[0008] In some embodiments, the nucleic acid is contained and present within a host cell, either in the form of a free vector or in the form of DNA sequence permanently integrated into the host cell genome. The presence of such nucleic acid ensures the expression of the anti-CD9 antibody construct of this invention, *e.g.*, an anti-CD9 scFv or anti-CD9 CAR construct. In some embodiments, the host cell is a T cell or natural killer (NK) cell. In some embodiments, the host cell is a T cell expressing a CAR construct comprising the amino acid sequence set forth in SEQ ID NOs:1 and 2 or SEQ ID NOs:3 and 4.

[0009] In a third aspect, the present invention provides a conjugate comprising the CD9 antibody construct of the present invention. The conjugate includes a polypeptide component, *i.e.*, the anti-CD9 antibody fusion polypeptide of this invention, and one or more conjugation partners, which may or may not be a protein in nature. For example, the conjugation partner may be a solid support, a detectable moiety, or a therapeutic agent. In some embodiments, the conjugate includes an anti-CD9 antibody construct, such as an anti-CD9 scFv, and a therapeutic agent, such as an anti-cancer agent. In this connection, compositions are also provided, which comprise the CD9 antibody construct of this invention, its encoding nucleic acid, including in the form of an expression cassette or vector, a host cell containing the nucleic acid and/or expressing the anti-CD9 antibody construct, or the conjugate comprising the anti-CD9 antibody fusion polypeptide of this invention joined with a conjugation partner, which may be another polypeptide or of a non-protein chemical nature.

[0010] In a fourth aspect, the present invention provides a method for killing CD9-expressing cells. The method includes a step of contacting CD9-expressing cells, *e.g.*, CD9-positive cancer cells, with a composition comprising an effective amount of an anti-CD9

antibody construct, which may or may not be conjugated with a therapeutic agent, *e.g.*, anti-cancer agent, or an adequate number of T cells expressing the CD9 antibody CAR construct or other type of host cells expressing the anti-CD9 humanized antibodies described above and herein, *e.g.*, comprising the amino acid sequence of SEQ ID NOs:1 and 2 or SEQ ID NOs:3 and 4.

[0011] In some embodiments, the CD9+ cells being targeted by the claimed methods are cancer cells characterized by their CD9 expression, for example, leukemia (*e.g.*, B-cell acute lymphoblastic leukemia or B-ALL) cells. In some embodiments, the cancer cells are located in a patient's body. In some embodiments, the method further include these steps, prior to the step of contacting CD9-expressing cells (*e.g.*, CD9-positive cancer cells) with a composition comprising an adequate number of T cells expressing the CD9 antibody CAR construct, (i) isolating T cells from a patient; (ii) transducing the T cells with the nucleic acid encoding the anti-CD9 CAR construct; (iii) cultivating the T cells *ex vivo* to expanding T cells expressing the anti-CD9 CAR construct comprising, from its N-terminus, an anti-CD9 scFv, a CD8 or CD28 transmembrane domain, a CD28 or 4-1BB costimulatory domain, and a CD3ζ T cell activating domain, and (iv) administering the expanded T cells in sufficient number back into the patient's body. Exemplary CAR constructs comprise the amino acid sequence set forth in SEQ ID NOs:1 and 2 or SEQ ID NOs:3 and 4. In some embodiments, for the purpose of treating CD9-positive cancer, a patient in need for the treatment is administered an effective amount of an anti-CD9 antibody construct (*e.g.*, optionally conjugated with an anti-cancer therapeutic agent) or T cells expressing the anti-CD9 CAR construct described above and herein, and the administering step comprises injection, *e.g.*, by subcutaneous, intravenous, intramuscular, intraperitoneal, or intratumoral injection.

[0012] In a fifth aspect, the present invention provides a kit for treating a disease or condition characterized by CD9 expression, for example, CD9+ cancers. The kit often includes a first container containing a first composition comprising an effective amount of an anti-CD9 antibody construct (*e.g.*, optionally conjugated with a therapeutic agent) or an adequate number of host cells expressing an anti-CD9 antibody construct (such as T cells expressing a CAR comprising, for example, from its N-terminus, an anti-CD9 scFv, a CD8 or CD28 transmembrane domain, a CD28 or 4-1BB costimulatory domain, and a CD3ζ T cell activating domain), and a second container containing a second composition comprising an effective amount of another therapeutic agent known for its efficacy for treating the disease or condition, such as a chemotherapeutic or immunotherapeutic agent. In some

embodiments, the CD9+ cancer is leukemia such as B-cell acute lymphoblastic leukemia (B-ALL). In some embodiments, the first or second composition is formulated for injection, *e.g.*, for subcutaneous, intravenous, intramuscular, intraperitoneal, or intratumoral injection. In some cases, the kit may further comprises an instruction manual providing instructions for user to properly use the kit.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] **Figure 1.** Purified CD9 antigens in SDS-PAGE.

[0014] **Figure 2.** Screening of hybridomas from CD9 immunized mice by ELISA. Three clones with the highest CD9 antigen binding were selected.

[0015] **Figure 3.** Purification of anti-CD9 antibodies. Fig. 3A. Purified IgG of mAb-D and mAb-F. Fig. 3B. Purified IgG of hAb-D and hAb-F. Fig. 3C. Purified scFv of hAb-D and hAb-F.

[0016] **Figure 4.** ELISA binding of murine and humanized anti-CD9 antibodies against the different antigens.

[0017] **Figure 5.** Sensorgrams of binding kinetics of murine and humanized anti-CD9 antibodies against CD9.

[0018] **Figure 6.** Flow cytometric analysis of murine and humanized anti-CD9 antibodies against B-ALL cell lines.

[0019] **Figure 7.** Platelet assays of CD9 antibodies.

[0020] **Figure 8.** Binding analysis of Fc-engineered humanized CD9 antibodies by flow cytometry.

[0021] **Figure 9.** Therapy of anti-CD9 antibodies in B-ALL xenografted mice.

[0022] **Figure 10.** Anti-tumor activity of Fc-engineered CD9 antibodies in patient-derived xenografts of resistant B-ALL.

[0023] **Figure 11.** Mechanisms underlying anti-leukemic activities of CD9 antibodies..

DEFINITIONS

[0024] The term "**nucleic acid**" or "**polynucleotide**" refers to deoxyribonucleic acids (DNA) or ribonucleic acids (RNA) and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known

analogues of natural nucleotides that have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions), alleles, orthologs, SNPs, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, *Nucleic Acid Res.* **19**:5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* **260**:2605-2608 (1985); and Rossolini *et al.*, *Mol. Cell. Probes* **8**:91-98 (1994)). The term nucleic acid is used interchangeably with gene, cDNA, and mRNA encoded by a gene.

[0025] The term "**gene**" means the segment of DNA involved in producing a polypeptide chain. It may include regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

[0026] The term "**amino acid**" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, *i.e.*, an α carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. "Amino acid mimetics" refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0027] Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0028] "**Polypeptide**," "**peptide**," and "**protein**" are used interchangeably herein to refer to a polymer of amino acid residues. All three terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. As used herein, the terms encompass amino acid chains of any length, including full-length proteins, wherein the amino acid residues are linked by covalent peptide bonds.

[0029] An "**antibody**" refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, which specifically bind and recognize an analyte (antigen). The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0030] An exemplary immunoglobulin (antibody) structural unit comprises a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to the variable region of the light and heavy chains, respectively.

[0031] Antibodies exist, *e.g.,* as intact immunoglobulins or as a number of well characterized fragments produced by digestion with various peptidases. Thus, for example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)'$_2$, a dimer of Fab which itself is a light chain joined to V_H-C_H1 by a disulfide bond. The F(ab)'$_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)'$_2$ dimer into an Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region (*see*, Paul (Ed.) *Fundamental Immunology*, 3rd Edition, Raven Press, NY (1993)). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized *de novo* either chemically or by utilizing recombinant DNA methodology.

[0032] Further modification of antibodies by recombinant technologies is also well known in the art. For instance, chimeric antibodies combine the antigen binding regions (variable

regions) of an antibody from one animal with the constant regions of an antibody from another animal. Generally, the antigen binding regions are derived from a non-human animal, while the constant regions are drawn from human antibodies. The presence of the human constant regions reduces the likelihood that the antibody will be rejected as foreign by a human recipient. On the other hand, "humanized" antibodies combine an even smaller portion of the non-human antibody with human components. Generally, a humanized antibody comprises the hypervariable regions, or complementarity determining regions (CDR), of a non-human antibody grafted onto the appropriate framework regions of a human antibody. Antigen binding sites may be wild type or modified by one or more amino acid substitutions, *e.g.*, modified to resemble human immunoglobulin more closely. Both chimeric and humanized antibodies are made using recombinant techniques, which are well-known in the art (*see, e.g.*, Jones *et al.* (1986) *Nature* 321:522-525).

[0033] Thus, the term "antibody," as used herein, also includes antibody fragments either produced by the modification of whole antibodies or antibodies synthesized *de novo* using recombinant DNA methodologies (*e.g.*, a Fab', a $F(ab)'_2$, a single chain fragment variable or scFv, a chimeric or humanized antibody, and a chimeric antigen receptor or CAR) that retain the ability to specifically bind the same intended target antigen. For example, an scFv is composed of a V_H and a V_L connected by a peptide linker of a relatively short length (*e.g.*, up to 25 amino acids), whereas a CAR is a recombinant fusion protein composed of an antigen-binding domain (typically an scFv) linked to (typically via a peptide linker) one or more signaling domains for activating immune cells (*e.g.*, T cells or NK cells).

[0034] As used herein, a "**chimeric antigen receptor**" or "**CAR**" describes a single chain polypeptide comprising at least three domains: an extracellular antigen-binding ectodomain, a transmembrane domain, and an intracellular endodomain. The extracellular ectodomain includes a heavy chain variable region (V_H) and a light chain variable region (V_L) of an antibody (*e.g.*, a single chain antibody or scFv). The intracellular endodomain acts to transmit intracellular signals triggered by the binding of an antigen (*e.g.*, CD9) to the extracellular ectodomain. Thus, the CAR endodomain contains at least one signaling domain capable of activating an effector cell, for example, a T cell. As CAR constructs are designed for use in T cells, they are also termed chimeric T cell receptor. The term CAR T is often used to collectively refer to a CAR construct (polypeptide) and T cells expressing the construct. For a review of the CAR T construct design and evolution, see Enblad *et al.*, *Human Gene Therapy* 26(8):498-505, 2015. In the first generation CAR constructs, the

intracellular signaling domain contains only the CD3ζ chain of a T cell receptor (TCR) complex, whereas the second and third generation CAR constructs include one and two (respectively) costimulatory domains, *e.g.*, CD28, 4-1BB, in addition to CD3ζ. Thus, CAR constructs have one effector cell (*e.g.*, T cell) signaling domain, one transmembrane domain (*e.g.*, CD28 transmembrane domain), and at least one signaling domains (*e.g.*, CD3ζ signaling domain). "Chimeric antigen receptor (CAR) therapy" refers to the use of CAR constructs for therapeutic purposes, including for adoptive cell therapy, a therapeutic approach that typically includes first isolation from a patient and *ex vivo* expansion and/or manipulation of immune effector cells (*e.g.*, NK cells or T cells) prior to eventual re-infusion of these cells back to the same patient. One example of such use is for the treatment of cancer. Cells used in CAR therapy are typically autologous (taken from the same individual patient and then put back to the same person) but can be allogeneic (taken from one individual and put into another, different individual of the same species, *e.g.*, both humans, especially with similar genetic background) under some circumstances. Cells may be manipulated to express CAR constructs using any one of the well-known methodologies in the art, for example, transformation by a nucleic acid (in the form or DNA or RNA), transfection by a viral vector, electroporation, *etc.*

[0035] As used herein, the term "**complementarity determining region**" or "**CDR**" refers to three relatively short stretches of amino acid sequences located within each of an antibody variable regions of the heavy chain and the light chain (V_H and V_L). The three CDRs in each of the V_H and V_L are designated CDR1, CDR2 and CDR3, from the N-terminus to the C-terminus, with a full set of CDRs encompassing all six CDRs for both heavy and light chains. The identification of CDRs and numbering of amino acid residues can be based on different numbering systems, among which the commonly used being the Kabat numbering scheme, although alternatives are also know in the art (*e.g.*, the Chothia numbering system).

[0036] In contrast to the CDRs, "**framework**" or "**framework region**" refers to the amino acid sequences within a V_H or V_L other than the CDRs. The presence of three CDRs on each of the heavy and light chains divides the framework regions into four sub-regions (FR1, FR2, FR3, and FR4) on each of V_H and V_L, with CDR1 positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. When used collectively and not specifying any particular sub-regions as FR1, FR2, FR3, or FR4, the term "framework" or "framework region" is used to refer to the entirety of all FRs together within the variable region of each or both of the heavy chain and light chain.

[0037] The term "**immunoassay**" describes an assay that uses an antibody to specifically bind an antigen. The immunoassay is characterized by the use of specific binding properties of a particular antibody to identify, isolate, target, and/or detect the presence or quantity of the antigen.

[0038] The phrase "**specifically binds**," when used to describe the binding relationship between an antibody and its target antigen, refers to a binding reaction that is determinative of the presence of the antigen (*e.g.*, CD9) in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular polypeptide at least two times the background and do not substantially bind in a significant amount to other polypeptides or other antigens present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to a CD9 protein can be selected to obtain only those antibodies that are specifically immunoreactive with that specific protein CD9 and not with other proteins including related proteins, *e.g.*, other variants or homologs of the CD9 protein. This selection may be achieved by subtracting out antibodies that cross-react with molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Antibodies, A Laboratory Manual* (1988) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically, a specific binding reaction (*e.g.*, between the target antigen CD9 and a CD9-specific antibody) will yield at least twice of the background signal or noise (*e.g.*, between a non-target antigen and the CD9-specific antibody) and more typically more than 5, 10, 20, 50, or up to 100 times the background.

[0039] A "**label**," "**detectable label**," or "**detectable moiety**" is a composition detectable by radiological, spectroscopic, photochemical, biochemical, immunochemical, chemical, or other physical means. For example, useful labels include radioisotopes such as ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (*e.g.*, as commonly used in an ELISA), biotin, digoxigenin, or haptens and proteins that can be made detectable, *e.g.*, by incorporating a radioactive component into a polypeptide or used to detect antibodies specifically reactive with the polypeptide. Typically a detectable label is a heterologous moiety attached to a probe or a molecule (*e.g.*, a protein or nucleic acid) with defined binding characteristics (*e.g.*, a polypeptide with a known binding specificity or a polynucleotide), so

as to allow the presence of the probe/molecule (and therefore its binding target) to be readily detectable. The heterologous nature of the label ensures that it has an origin different from that of the probe or molecule that it labels, such that the probe/molecule attached with the detectable label does not constitute a naturally occurring composition (*e.g.*, a naturally occurring polynucleotide or polypeptide sequence).

[0040] The term "**recombinant**" when used with reference, *e.g.*, to a cell, or a nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0041] As used herein, the term "**cancer**" encompasses various malignant neoplasms characterized by the proliferation of anaplastic cells that tend to invade surrounding tissue and metastasize to new body sites. Non-limiting examples of different types of cancer suitable for treatment using the compositions and methods of the present invention include colorectal cancer, colon cancer, anal cancer, liver cancer, ovarian cancer, breast cancer, lung cancer, bladder cancer, thyroid cancer, pleural cancer, pancreatic cancer, cervical cancer, prostate cancer, testicular cancer, bile duct cancer, gastrointestinal carcinoid tumors, esophageal cancer, gall bladder cancer, rectal cancer, appendix cancer, small intestine cancer, stomach (gastric) cancer, renal cancer (*e.g.,* renal cell carcinoma), cancer of the central nervous system, skin cancer, oral squamous cell carcinoma, choriocarcinomas, head and neck cancers, bone cancer, osteogenic sarcomas, fibrosarcoma, neuroblastoma, glioma, melanoma, leukemia (*e.g.,* acute lymphoblastic leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myelogenous leukemia, chronic myelogenous leukemia, or hairy cell leukemia), lymphoma (*e.g.,* non-Hodgkin's lymphoma, Hodgkin's lymphoma, B-cell lymphoma, or Burkitt's lymphoma), and multiple myeloma.

[0042] The term "**immunotherapy**" refers to any treatment that uses certain parts of a patient's immune system to fight diseases such as cancer. The patient's own immune system is stimulated (or suppressed), with administration of one or more agents for that purpose. In some cases, the immunotherapy is "targeted" or cancer cell-specific. In other cases, immunotherapy can be "untargeted," which refers to administration of agents that do not selectively interact with immune system cells, yet modulates immune system function.

Representative examples of untargeted therapies include, without limitation, chemotherapy, gene therapy, and radiation therapy.

[0043] Immunotherapy is one form of targeted therapy that may comprise, for example, the use of cancer vaccines and/or sensitized antigen presenting cells. For example, an oncolytic virus is a virus that is able to infect and lyse cancer cells, while leaving normal cells unharmed, making them potentially useful in cancer therapy. Replication of oncolytic viruses both facilitates tumor cell destruction and also produces dose amplification at the tumor site. They may also act as vectors for anticancer genes, allowing them to be specifically delivered to the tumor site. The immunotherapy can involve passive immunity for short-term protection of a host, achieved by the administration of pre-formed antibody directed against a cancer antigen or disease antigen (*e.g.*, administration of a monoclonal antibody, optionally linked to a chemotherapeutic agent or toxin, to a tumor antigen). For example, anti-VEGF and mTOR inhibitors are known to be effective in treating renal cell carcinoma. Immunotherapy can also focus on using the cytotoxic lymphocyte-recognized epitopes of cancer cell lines. Alternatively, antisense polynucleotides, ribozymes, RNA interference molecules, triple helix polynucleotides and the like, can be used to selectively modulate biomolecules that are linked to the initiation, progression, and/or pathology of a tumor or cancer.

[0044] The term "**immunogenic chemotherapy**" refers to any chemotherapy that has been demonstrated to induce immunogenic cell death, a state that is detectable by the release of one or more damage-associated molecular pattern (DAMP) molecules, including, but not limited to, calreticulin, ATP and HMGB1 (see, *e.g.*, Kroemer *et al.* (2013), *Annu. Rev. Immunol.*, 31:51-72). In addition, the term "immunogenic chemotherapy" further refers to any chemotherapy that results in priming the immune system such that it leads to enhanced immune activity towards cancer. Specific representative examples of consensus immunogenic chemotherapies include 5'-fluorouracil, anthracyclines, such as doxorubicin, and the platinum drug, oxaliplatin, among others.

[0045] The term "**inhibiting**" or "**inhibition**," as used herein, refers to any detectable negative effect on a target biological process, such as RNA/protein expression of a target gene, the biological activity of a target protein, protein-protein specific binding or interaction, cellular signal transduction, cell proliferation, presence/level of an organism especially a micro-organism, any measurable biomarker, bio-parameter, or symptom in a subject, and the like. Typically, an inhibition is reflected in a decrease of at least 10%, 20%, 30%, 40%, 50%,

60%, 70%, 80%, 90% or greater in the target process (*e.g.*, a target cell proliferation rate), or any one of the downstream parameters mentioned above, when compared to a control. "Inhibition" further includes a 100% reduction, *i.e.*, a complete elimination, prevention, or abolition of a target biological process or signal or disease/symptom. The other relative terms such as "suppressing," "suppression," "reducing," and "reduction" are used in a similar fashion in this disclosure to refer to decreases to different levels (*e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater decrease compared to a control level) up to complete elimination of a target biological process or signal or disease/symptom. On the other hand, terms such as "activate," "activating," "activation," "increase," "increasing," "promote," "promoting," "enhance," "enhancing," or "enhancement" are used in this disclosure to encompass positive changes at different levels (*e.g.*, at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, or greater such as 3, 5, 8, 10, 20-fold increase compared to a control level in a target process, signal, or symptom/disease incidence.

[0046] As used in this application, an "**increase**" or a "**decrease**" refers to a detectable positive or negative change in quantity from a comparison control, *e.g.*, an established standard control (such as an average rate of proliferation of a certain cell type), prior to a certain event (*e.g.*, exposure to an agent regulating cellular proliferation). An increase is a positive change that is typically at least 10%, or at least 20%, or 50%, or 100%, and can be as high as at least 2-fold or at least 5-fold or even 10-fold of the control value. Similarly, a decrease is a negative change that is typically at least 10%, or at least 20%, 30%, or 50%, or even as high as at least 80% or 90% of the control value. Other terms indicating quantitative changes or differences from a comparative basis, such as "more," "less," "higher," and "lower," as well as terms indicating an action to cause such changes or differences, such as "increase," "promote," "enhance," "decrease," "inhibit," and "suppress," are used in this application in the same fashion as described above. In contrast, the term "**substantially the same**" or "**substantially lack of change**" indicates little to no change in quantity from the standard control value, typically within ± 10% of the standard control, or within ± 5%, 2%, or even less variation from the standard control.

[0047] As used herein, an "**effective amount**" or a "**therapeutically effective amount**" means the amount of an active agent that, when administered to a subject or patient for treating a disorder, is sufficient to prevent, reduce the frequency of, or alleviate the symptoms of the disorder. The effective amount will vary depending on a variety of the factors, such as

a particular compound or bioactive agent used, the disease and its severity, the age, weight, and other factors of the subject to be treated. Amelioration of a symptom of a particular condition by administration of a pharmaceutical composition described herein refers to any lessening, whether permanent or temporary, that can be associated with the administration of the pharmaceutical composition. For example, the quantity of a therapeutic agent-conjugated anti-CD9 antibody construct administered is considered therapeutically effective for treating a condition involving undesired CD9 expression when administration results in eliminated symptoms, delayed onset of symptoms, or reduced frequency or severity of symptoms including disease progression, for example, in the case of treating CD9-positive cancer, as indicated in tumor mass, metastasis, morbidity and mortality, *etc.* The exact amount "effective" for achieving a desired therapeutic effect will depend on the nature of the therapeutic agent, the manner of administration, and the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (*see*, *e.g.*, Lieberman, *Pharmaceutical Dosage Forms* (vols. 1-3, 1992); Lloyd, *The Art, Science and Technology of Pharmaceutical Compounding* (1999); and Pickar, *Dosage Calculations* (1999)).

[0048] As used herein, the term "**treatment**" or "**treating**" includes both therapeutic and preventative measures taken to address the presence of a disease or condition or the risk of developing such disease or condition at a later time. It encompasses therapeutic or preventive measures for alleviating ongoing symptoms, inhibiting or slowing disease progression, delaying of onset of symptoms, or eliminating or reducing side-effects caused by such disease or condition. A preventive measure in this context and its variations do not require 100% elimination of the occurrence of an event; rather, they refer to a suppression or reduction in the likelihood or severity of such occurrence or a delay in such occurrence.

[0049] A "**subject**," or "**subject in need of treatment**," as used herein, refers to an individual who seeks medical attention due to risk of, or actual sufferance from, a condition involving an undesirable or abnormal, excessive expression of CD9. The term subject can include both animals, especially mammals, and humans. Subjects or individuals in need of treatment include those that demonstrate symptoms caused by or related to CD9 expression, *e.g.*, undesirable or inappropriate cell proliferation, such as tumor and especially malignant tumor/cancer including leukemia or lymphoma or are at risk of later developing these conditions and/or related symptoms.

[0050] A "**pharmaceutically acceptable**" or "**pharmacologically acceptable**" excipient is a substance that is not biologically harmful or otherwise undesirable, *i.e.*, the excipient may

be administered to an individual along with a bioactive agent without causing any undesirable biological effects. Neither would the excipient interact in a deleterious manner with any of the components of the composition in which it is contained.

[0051] The term "**excipient**" refers to any essentially accessory substance that may be present in the finished dosage form of the composition of this invention. For example, the term "excipient" includes vehicles, binders, disintegrants, fillers (diluents), lubricants, glidants (flow enhancers), compression aids, colors, sweeteners, preservatives, suspending/dispersing agents, film formers/coatings, flavors and printing inks.

[0052] The term "**consisting essentially of**," when used in the context of describing a composition containing an active ingredient or multiple active ingredients, refer to the fact that the composition does not contain other ingredients possessing any similar or relevant biological activity of the active ingredient(s) or capable of enhancing or suppressing the activity, whereas one or more inactive ingredients such as physiological or pharmaceutically acceptable excipients may be present in the composition. For example, a composition consisting essentially of active agents effective for treating a CD9-positive cancer by suppressing CD9-positive cell proliferation and/or survival in a subject is a composition that does not contain any other agents that may have any detectable positive or negative effect on the same target process (*e.g.*, efficacy in treating CD9+ cancer or suppression of CD9+ cell proliferation/survival) or that may increase or decrease to any measurable extent of the disease severity or outcome among the receiving subjects.

[0053] A "**promoter**" is defined as an array of polynucleotide control sequences that direct transcription of another polynucleotide sequence. As used herein, a promoter includes necessary polynucleotide sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription. A "constitutive" promoter is a promoter that is active under most environmental and developmental conditions. In contrast, an "inducible" promoter is a promoter that rendered active by environmental or developmental regulation and under certain specific environmental or developmental conditions. The term "operably linked" refers to a functional linkage between a polynucleotide expression control sequence (such as a promoter, or array of transcription factor binding sites) and another polynucleotide sequence (such as a protein-coding sequence), wherein the expression control sequence directs transcription of the second polynucleotide sequence.

[0054] An "**expression cassette**" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified polynucleotide elements that permit transcription of a particular polynucleotide sequence in a host cell or in an *in vitro* transcription system (*e.g.*, partially reconstituted cell lysate). An expression cassette may be the entirety or a part of a plasmid, viral genome, or other replicable nucleic acid construct such as episome. Typically, an expression cassette includes a polynucleotide sequence to be transcribed, operably linked to a promoter.

[0055] As used herein, the term "**heterologous**" is used to describe the relationship of two elements placed adjacent to each other in a construct, referring to these two elements such as two polynucleotide sequences (*e.g.*, a promoter sequence and a polypeptide-encoding sequence) or two polypeptide sequences (*e.g.*, a signal peptide sequence and another peptide sequence) being from two different natural origins, such that these two elements are not found in the same relative positions in nature. Thus, a "heterologous promoter" for a gene refers to a promoter that is not naturally operably linked to that gene. Similarly, a "heterologous polypeptide" or "heterologous polynucleotide" to one particular protein or its encoding sequence is one derived from an origin different from the protein's origin. The fusion of two heterologous polypeptide (or polynucleotide) sequences does not result in a longer polypeptide (or polynucleotide) sequence that can be found in nature as an intact protein (or naturally occurring nucleotide sequence) or a segment thereof.

[0056] The term "**conjugated with**" or "**coupled to**" describes any construction whereby the polypeptide, *e.g.*, a polypeptide of this invention comprising an antigen-bind site capable of specifically binding to CD9, is covalently or non-covalently linked, attached, or joined at its N- or C-terminus or at any side reactive group to a moiety that may serve as a solid support, a detectable element, or a therapeutically active agent for the purpose of detecting, isolating, or imaging of target cells or tissue or for the purpose of treating a relevant medical condition.

[0057] The term "**about**" denotes a range of +/- 10% of a pre-determined value. For example, "about 10" indicates a range of 90% to 110% of 10, *i.e.*, 9 to 11.

DETAILED DESCRIPTION OF THE INVENTION

I. Introduction

[0058] The present invention relates to compositions and methods for treating diseases, disorders, or conditions associated with dysregulated expression of the CD9 antigen. The

invention provides a novel single-chain variable fragment (scFv) that specifically binds to CD9 protein. The invention also provides fully humanized IgG antibodies containing a modification in the heavy chain constant region that specifically target CD9 with significantly reduced side effects such as undesired platelet activation.

II. Recombinant Technology in General

[0059] Basic texts disclosing general methods and techniques in the field of recombinant genetics include Sambrook and Russell, *Molecular Cloning, A Laboratory Manual* (3rd ed. 2001); Kriegler, *Gene Transfer and Expression: A Laboratory Manual* (1990); and Ausubel *et al.*, eds., *Current Protocols in Molecular Biology* (1994).

[0060] For nucleic acids, sizes are given in either kilobases (kb) or base pairs (bp). These are estimates derived from agarose or acrylamide gel electrophoresis, from sequenced nucleic acids, or from published DNA sequences. For proteins, sizes are given in kilodaltons (kDa) or amino acid residue numbers. Proteins sizes are estimated from gel electrophoresis, from sequenced proteins, from derived amino acid sequences, or from published protein sequences.

[0061] Oligonucleotides that are not commercially available can be chemically synthesized, *e.g.*, according to the solid phase phosphoramidite triester method first described by Beaucage & Caruthers, *Tetrahedron Lett.* **22**: 1859-1862 (1981), using an automated synthesizer, as described in Van Devanter *et. al.*, *Nucleic Acids Res.* **12**: 6159-6168 (1984). Purification of oligonucleotides is performed using any art-recognized strategy, *e.g.*, native acrylamide gel electrophoresis or anion-exchange HPLC as described in Pearson & Reanier, *J. Chrom.* **255**: 137-149 (1983). Polynucleotide sequences such as synthetic oligonucleotides can be verified using well-established methodologies, *e.g.*, the chain termination method for sequencing double-stranded templates of Wallace *et al.*, *Gene* **16**: 21-26 (1981).

III. CD9 Antibody Constructs

[0062] The present invention relates to the making and use of antibody constructs that specifically recognize and bind the CD9 protein. In particular, a CD9 antibody construct is derived from an anti-CD9 monoclonal antibody, termed mAb-D or mAb-F, contains at least one set of six CDRs, three in each of the V_H and V_L, and therefore retains the binding specificity and/or affinity of the original monoclonal antibody for the CD9 antigen. The CDRs can be found in SEQ ID NOs:5-10 as the first set, derived from mAb-D, and SEQ ID NOs:11-16 as the second set, derived from mAb-F. The anti-CD9 antibody constructs of this invention in some cases are characterized by the presence of at least one or both of the full

variable region of the heavy chain and light chain, see SEQ ID NOs:1, 2, 3, and 4. Preferably, the CD9 antibody constructs of this invention is humanized. In addition, the CD9 antibody constructs, when including the heavy chain constant region, contains an N297A substitution in the Fc region, as shown in SEQ ID NO:17. Additional modifications in the Fc region that can be made to the anti-CD9 antibody construct of this invention include, but are not limited to, S228P, N297D, N297A, N297Q, N297G, L234A, L234F, L234S, L235E, L235A, L235T, G236A, G236R, G237A, D265A, G236R, D265A, K322A, L328R, P329A, P329G, E233P, L234V, L234A, L235A, L235E, G237A, S267K, P238S, H268A, A330S, P331S, V309L, L234A/L235A, L234F/L235E, L234S/L235T, and S228P/L235E.

A. scFv and CAR

[0063] One embodiment of the CD9 antibody construct of the present invention is a single chain antibody or scFv, where at least one full set of six CDRs, *i.e.*, CDR1-3 from each of the V_H and V_L of the original monoclonal antibody mAb-D or mAb-F, is retained. The framework regions of each of the V_H and V_L are optionally modified, such as for humanization purposes. In some cases, at least one copy of each of the full length V_H and V_L is retained. They may be connected directly or optionally connected through a peptide linker, which has a length of 2-30 amino acids, usually no more than 20 or 25 amino acids. An exemplary scFv of the present invention is described herein has an amino acid sequence comprising SEQ ID NOs:1 and 2 or SEQ ID NOs:3 and 4.

[0064] Another embodiment of the CD9 antibody construct of the present invention is a chimeric antigen receptor or CAR, which includes at least an extracellular antigen-binding ectodomain, a transmembrane domain, and an intracellular endodomain. The extracellular ectodomain contains the antigen-binding site, characterized by the presence of at least one full set of six CDRs, *i.e.*, CDR1-3 from each of the V_H and V_L of the original monoclonal antibody mAb-D or mAb-F. For example, the ectodomain may comprise the anti-CD9 scFv as described above and herein. On the other hand, the intracellular signaling domain includes at least the CD3ζ chain of a T cell receptor (TCR) complex, optionally one and two additional costimulatory domains. In one example, the CAR construct of the present invention includes anti-CD9 scFv, CD8 or CD28 transmembrane domain, CD28 or 4-1BB costimulatory domain, and CD3ζ T cell activating domain. One or more peptide linkers may be used between any two adjacent domains of the CAR construct.

B. Conjugates

[0065] In view of the prevalence of CD9 expression in various conditions and diseases, especially in different types of cancers, and the desirable binding profile of the CD9 antibody constructs of this invention, different forms of conjugates can be devised for use in the detection of CD9 and in targeted delivery of therapeutic agents to CD9+ cells, thus providing new and effective means in the diagnosis and treatment of conditions and diseases involving aberrant CD9 expression.

[0066] More specifically, the CD9 antibody construct of this invention as a polypeptide-based construct can be conjugated with a variety of additional moieties, polypeptide or non-polypeptide in nature, depending on the intended use of the conjugated CD9 antibody construct. Such a conjugate may involve a polypeptide of this invention, comprising an antigen-bind site capable of specifically binding to CD9, being linked, attached, or joined at its N- or C-terminus or at any side reactive group to a moiety to serve as a solid support, a detectable element, or a therapeutically active agent. The linkage may be provided by way of a covalent bond or a non-covalent bond, *e.g.*, through the binding action between a known binding pair, such as biotin-streptavidin or SpyCatcher-SpyTag pair, with one binding partner affixed to the polypeptide of this invention and the other binding partner affixed to the moiety to be conjugated to the polypeptide of this invention. Once made, the conjugate may be used for the purpose of detecting, isolating, imaging, or killing of CD9-expressing cells or tissues or for the purpose of treating a medical condition involving CD9 expression. In this regard, a detectable moiety produces a signal permitting easy detection by radiological, spectroscopic, photochemical, biochemical, immunochemical, chemical, or other means. Therapeutic moieties suitable for use in this invention may include known effective agents for treating conditions or diseases involving CD9 expression, such as anti-cancer therapeutic agents known in the art or described herein, *e.g.*, for radiotherapy, chemotherapy, immunotherapy and the like.

IV. Nucleic Acids and Host Cells

[0067] Upon completion of designing a CD9 antibody construct of this invention (or its conjugate in the fusion polypeptide form), a nucleic acid comprising a polynucleotide sequence encoding the construct (or its conjugate) may be constructed. Such nucleic acid may be in the form of DNA or RNA. In some cases, the nucleic acid takes the form of an expression cassette in which the coding sequence is operably linked to a promoter, typically heterologous promoter, directing the transcription and/or expression of the CD9 antibody

construct. In some embodiments, the nucleic acid is a vector, especially an expression vector, encoding the CD9 antibody construct, such as a plasmid or a viral vector.

A. Expression Cassettes and Vectors

[0068] To obtain high level expression of a nucleic acid construct encoding a desired polypeptide, one typically subclones a polynucleotide sequence encoding the polypeptide into an expression cassette, *e.g.*, an expression vector, that contains a strong promoter to direct transcription, a transcription/translation terminator, and a ribosome binding site for translational initiation. Suitable promoters are well known in the art and described, *e.g.*, in Sambrook and Russell, *supra,* and Ausubel *et al., supra*. Eukaryotic and prokaryotic expression systems for bacterial, mammalian, yeast, insect, or plant cells are well known in the art and are also commercially available. Kits for such expression systems are commercially available. One exemplary eukaryotic expression vector is an adenoviral vector, an adeno-associated vector, a lentiviral vector, or a retroviral vector.

B. Host Cells and Recombinant Protein Expression

[0069] In the context of practicing the present invention, a broad variety of host cells, prokaryotic or eukaryotic, may be used for the expression and/or production of an anti-CD9 antibody construct of this invention. The choice of host cells will depend on the specific purpose of the expression, for example, whether it is for making an antibody construct (*e.g.*, an anti-CD9 IgG or scFv) or for producing T cells expressing an anti-CD9 CAR construct to be used in CAR-T therapy.

[0070] Standard transfection methods can be used to produce bacterial, mammalian, yeast, insect, or plant cell lines that express large quantities of a recombinant polypeptide (*e.g.*, a CD9 antibody construct), which is then purified using standard techniques (*see, e.g.*, Colley *et al.*, *J. Biol. Chem.* **264**: 17619-17622 (1989); *Guide to Protein Purification*, in *Methods in Enzymology*, vol. 182 (Deutscher, ed., 1990)). Transformation of eukaryotic and prokaryotic cells are performed according to standard techniques (*see, e.g.*, Morrison, *J. Bact.* **132**: 349-351 (1977); Clark-Curtiss & Curtiss, *Methods in Enzymology* **101**: 347-362 (Wu *et al.*, eds, 1983).

[0071] Any of the well-known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, liposomes, microinjection, plasma vectors, viral vectors and any of the other well-known methods for introducing a polynucleotide sequence

encoding a protein of interest into a host cell (*see, e.g.*, Sambrook and Russell, *supra*). It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one coding sequence into the host cell capable of expressing the recombinant polypeptide.

[0072] When a recombinant polypeptide, *e.g.*, an anti-CD9 scFv of this invention, is expressed in host cells in satisfying quantity, its isolation/purification can follow the standard protein purification procedure including solubility fractionation, size differential filtration, and column chromatography, as well as various immunoassay-based methods.

[0073] For the purpose of producing recombinant immune effector cells expressing a CD9 CAR construct, such as CAR-T cells of this invention, typically the desired type of immune effector cells, such as natural killer (NK) cells and T cells, including cytotoxic T lymphocytes (CTLs) and regulatory T cells, is first isolated from the peripheral mononuclear cells (PBMCs) taken from a patient being treated for a condition or disease characterized by the inappropriate expression of CD9. Alternatively, the immune effector cells may be obtained from another human subject, preferably with a genetic background similar to that of the intended recipient. The isolated cells are then transformed or transfected with an expression vector, *e.g.*, a viral vector derived from an adenovirus, an adeno-associated virus (AAV), a lentivirus, or a retrovirus, encoding the anti-CD9 CAR construct such that sufficient CD9 expression level can be detected and verified on the surface of these cells. The manipulated immune effector cells, now steadily expressing the anti-CD9 CAR construct on their cell surface, are then cultivated for expansion *ex vivo* to reach an adequate quantity before they are administered back to the patient. Typically, roughly in the range of about $5x10^4$ to about $5x10^7$ of such CAR T cells are re-infused into the patient during each application. For example, about $1x10^5$ to about $3x10^7$, about $3x10^5$ to about $3x10^7$, about $2x10^5$ to about $2x10^7$, about $5x10^6$ to about $1x10^7$ CAR T cells are administered in each infusion.

IV. Pharmaceutical Compositions and Administration

[0074] The present invention also provides pharmaceutical compositions or physiological compositions comprising an effective amount of a CD9 antibody construct, *e.g.*, an anti-CD9 IgG or scFv or a conjugate thereof, that can target CD9-expressing cells and in both prophylactic and therapeutic applications to address conditions and diseases involving aberrant CD9 expression, *e.g.*, different types of cancers. Such pharmaceutical or physiological compositions also include one or more pharmaceutically or physiologically

acceptable excipients or carriers. Pharmaceutical compositions of the invention are suitable for use in a variety of drug delivery systems. Suitable formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, *see*, Langer, *Science* **249**: 1527-1533 (1990).

[0075] The pharmaceutical compositions of the present invention can be administered by various routes, *e.g.*, subcutaneously, transdermally, intramuscularly, intravenously, intraperitoneally, or intratumorally. The preferred routes of administering the pharmaceutical compositions are through injection, especially local delivery by injection (*e.g.*, intratumoral injection) to a relevant organ or tissue in a patient suffering from a solid tumor involving excessive and undesirable cellular proliferation at daily doses of about 10-100,000 μg, about 100-10,000 μg, or about 1,000-5,000 μg of the recombinantly produced an anti-CD9 antibody construct or conjugate for a 70 kg adult human per day. The appropriate dose may be administered in a single daily dose or as divided doses presented at appropriate intervals, for example as two, three, four, or more subdoses per day.

[0076] For preparing pharmaceutical compositions containing a CD9 antibody construct or conjugate thereof, one or more inert and pharmaceutically acceptable carriers are used. The pharmaceutical carrier(s) can be in any form, although liquid form is preferred for formulations intended for injection. Suitable carriers include, for example, magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

[0077] Liquid pharmaceutical compositions include, for example, solutions, suspensions, and emulsions suitable for injection. Sterile buffer solutions of the active component (*e.g.*, a recombinantly anti-CD9 antibody construct or conjugate) or sterile solutions of the active component in solvents comprising water, buffered water, saline, PBS, ethanol, or propylene glycol are examples of liquid compositions suitable for parenteral administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents, detergents, and the like.

[0078] Sterile solutions can be prepared by dissolving the active component in the desired solvent system, and then passing the resulting solution through a membrane filter to sterilize it or, alternatively, by dissolving the sterile active component in a previously sterilized

solvent under sterile conditions. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile aqueous carrier prior to administration. The pH of the preparations typically will be between 3 and 11, more preferably from 5 to 9, and most preferably from 7 to 8.

[0079] The pharmaceutical compositions of a CD9 antibody construct or a conjugate thereof can be administered for prophylactic and/or therapeutic purposes. In therapeutic applications, compositions are administered to a patient already suffering from a condition that involves excessive and undesirable cellular proliferation in an amount sufficient to prevent, cure, reverse, or at least partially slow or arrest the symptoms of the condition and its complications, such as the onset, progression, duration, and severity of the disease. An amount adequate to accomplish this is defined as a "therapeutically effective dose." Amounts effective for this use will depend on the severity of the disease or condition and the weight and general state of the patient, but generally range from about 10-100,000 μg, about 100-10,000 μg, or about 1,000-5,000 μg of the CD9 antibody construct or a conjugate thereof per day for a 70 kg patient, with dosages from 1-1500 μg to about 20-100 μg of the anti-CD9 antibody or conjugate per day per kg for a patient being more commonly used.

[0080] In prophylactic applications, pharmaceutical compositions containing an adequate amount of a CD9 antibody construct or conjugate are administered to a patient susceptible to or otherwise at heightened risk of developing a disease or condition caused or exacerbated by undesirable and excessive cellular proliferation, in an amount sufficient to delay or prevent the onset of the symptoms. Such an amount is defined to be a "prophylactically effective dose." In this use, the precise amounts of the anti-CD9 antibody construct again depend on the patient's state of health and weight, but generally range from about 10-100,000 μg, about 100-10,000 μg, or about 1,000-5,000 μg of the CD9 antibody construct or a conjugate thereof per day for a 70 kg patient, with dosages from 1-1500 μg to about 20-100 μg of the CD9 antibody or conjugate per day per kg for a patient being more commonly used.

[0081] Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event, the pharmaceutical formulations should provide a quantity of the CD9 antibody construct of this invention or its conjugate sufficient to effectively address at least one targeted symptom of a condition or disease involving CD9 expression in the patient, either therapeutically or prophylactically.

V. Anti-Cancer Therapeutic Agents

[0082] One potential use of the anti-CD9 antibody construct of this invention or a conjugate thereof is the treatment of cancers where the relevant tissues or cells exhibit aberrant CD9 expression, which may include various types of blood cancers (such as leukemia or lymphoma) or solid tumors (such as pancreatic cancer or ovarian cancer).

[0083] In such applications, one or more of these previously known effective anti-cancer therapeutic agents, including those named in this application, may be administered concurrently with the CD9 antibody construct or its conjugate to subjects in need of treatment. Optionally, the agent(s) may be used in combination with the CD9 antibody construct of the present invention (*e.g.*, an anti-CD9 IgG, scFv or its conjugate) to suppress cancer growth, inhibit cancer metastasis, and facilitate remission from the disease. In the case of combination therapy, all of the active agents may be administered concurrently each in an effective amount, either together in a single composition or separately in two or more different compositions.

[0084] For example, various chemotherapeutic agents are known to be effective for use to treat various cancers. As used herein, a "chemotherapeutic agent" encompasses any chemical compound exhibiting suppressive effect against cancer cells, thus useful in the treatment of cancer. Classes of chemotherapeutic agents include, but are not limited to: alkylating agents, antimetabolites, kinase inhibitors, spindle poison plant alkaloids, cytoxic/antitumor antibiotics, topoisomerase inhibitors, photosensitizers, anti-estrogens and selective estrogen receptor modulators (SERMs), anti-progesterones, estrogen receptor down-regulators (ERDs), estrogen receptor antagonists, leutinizing hormone-releasing hormone agonists, anti-androgens, aromatase inhibitors, EGFR inhibitors, VEGF inhibitors, and anti-sense oligonucleotides that inhibit expression of genes implicated in abnormal cell proliferation or tumor growth. Chemotherapeutic agents useful in the treatment methods disclosed herein also include cytostatic and/or cytotoxic agents.

[0085] Exemplary anti-cancer therapeutic agents include alkylating agents such as altretamine, bendamustine, busulfan, carboquone, carmustine, chlorambucil, chlormethine, chlorozotocin, cyclophosphamide, dacarbazine, fotemustine, ifosfamide, lomustine, melphalan, melphalan flufenamide, mitobronitol, nimustine, nitrosoureas, pipobroman, ranimustine, semustine, streptozotocin, temozolomide, thiotepa, treosulfan, triaziquone, triethylenemelamine, trofosfamide, and uramustine; anthracyclines such as aclarubicin, daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, pirarubicin, valrubicin, and

zorubicin; cytoskeletal disruptors (taxanes) such as abraxane, cabazitaxel, docetaxel, larotaxel, paclitaxel, taxotere, and tesetaxel; epothilones such as ixabepilone; histone deacetylase inhibitors such as vorinostat, romidepsin, and inhibitors of topoisomerase I such as belotecan, camptothecin, exatecan, gimatecan, irinotecan, and topotecan; inhibitors of topoisomerase II such as etoposide, teniposide, and tafluposide; kinase inhibitors such as bortezomib, erlotinib, gefitinib, imatinib, dasatinib, ponatinib, ruxolitinib, vemurafenib, and vismodegib; BCL-2 inhibitors such as venetoclax and navitoclax; nucleotide analogs and precursor analogs such as azacitidine, azathioprine, capecitabine, cytarabine, doxifluridine, fluorouracil, gemcitabine, hydroxyurea, mercaptopurine, methotrexate, and tioguanine (formerly thioguanine); peptide antibiotics such as actinomycin and bleomycin; platinum-based agents such as carboplatin, cisplatin, dicycloplatin, oxaliplatin, nedaplatin, and satraplatin; retinoids such as alitretinoin, bexarotene, and tretinoin; glucocorticoids such as prednisone and dexamethasone, and vinca alkaloids and derivatives such as vinblastine, vincristine, vindesine, and vinorelbine.

[0086] In addition to targeting CD9, other immunotherapeutic approaches may also be used in combination for cancer treatment. In accordance with the strategy of active immunotherapy, for example, monoclonal antibodies and their conjugates can be used to target other cancer markers. They include adotrastuzumab (HER2), alemtuzumab (CD52), bevaclzumab (VEGF), brentuximab (CD30), capromab (PSMA), cetuximab (EGFR), elotuzumab (SLAMF7), ibritumomab (CD20), necitumumab (EGFR), blinatumomab (CD19), obinutumab (CD20), ofatumumab (CD20), olaratumab (PDGFRA), panitumumab (EGFR), pertuzumab (HER2), ramucirumab (VEGFR2), rituximab (CD20), trastuzumab (HER-2), inotuzumab-ozogamicin (CD22), gemtuzumab-ozogamicin (CD33), and bevacizumab-awwb (VEGF). Moreover, checkpoint inhibitors and cytokines can be used in accordance with the strategy of passive immunotherapy. Currently approved checkpoint inhibitors target molecules CTLA4, PD-1, and PD-L1, including ipilimumab (CTLA4), nivolumab, pembrolizumab, cemiplimab, spartalizumab (PD-1), atezolizumab, avelumab, and durvalumab (PD-L1). Cytokines for use in the treatment of cancer and associated conditions include granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin-2 (IL-2), and interleukin-11 (IL-11).

VI. Kits and Compositions

[0087] The invention provides compositions and kits comprising the anti-CD9 antibody construct of this invention for practicing the methods described herein for detecting/assessing

CD9 expression in cells or tissues, for isolating CD9 protein, and for treating diseases or conditions relating to CD9 expression, including various types of malignancy.

[0088] Kits for carrying out assays for detecting/assessing CD9 expression or for isolating CD9 protein typically include one container containing a CD9 antibody construct (*e.g.*, an anti-CD9 IgG or scFv), optionally conjugated to a solid support or a detectable moiety, such that the specific binding relationship between the CD9 antibody construct and CD9 would permit ready isolation and/or detection of CD9 protein, both qualitatively and quantitatively, especially in an environment where another similar or homologous protein might be present. In embodiments where the CD9 antibody construct is not conjugated to any moiety to facilitate isolation or detection, the kits often contain a second container containing a detection agent that is able to specifically detect the presence of the anti-CD9 antibody construct.

[0089] Typically, the kits for detection or isolation of CD9 also include one or more appropriate controls, indicative of the presence of CD9 protein in a certain amount or below a detection threshold for the protein. In some cases such control(s) may be provided in the form of one or more physical samples, whereas in other cases the control(s) may be provided as a set value or values. In addition, the kits of this invention may provide instruction manuals to guide users in first processing test samples and then assessing the expression profile of CD9 protein or isolating CD9 protein from the samples.

[0090] Kits for practicing the methods described herein to treat a disease or condition caused or exacerbated by CD9 expression via administering a CD9 antibody construct or a conjugate thereof to a subject in need thereof. Both therapeutic use and prophylactic use are contemplated, *i.e.*, a subject with or without a disease diagnosis may be treated, for example, once the presence and/or risk of the disease has been assessed according to the methods described herein and the subject is deemed to likely benefit from the treatment.

[0091] Kits for therapeutic use of a CD9 antibody construct or a conjugate thereof typically include one container containing a composition comprising the CD9 antibody construct, its conjugate, or a host cell expressing a CD9 antibody construct (*e.g.*, anti-CD9 CAR T cell). Typically, such composition is formulated for delivering the anti-CD9 antibody construct, its conjugate, or a host cell expressing a CD9 antibody construct, *e.g.*, by injection such as via subcutaneous, intravenous, intramuscular, intraperitoneal, or intratumoral means. In some cases, the CD9 antibody construct is conjugated with a therapeutic agent effective for treating

conditions and diseases involving CD9 expression, including cancer, such that a targeted delivery of the therapeutic agent can be achieved. In some cases, especially when host cells expressing a CD9 antibody construct are included in the kits, at least one, possibly two or more additional containers may be included in the kits, each container containing at least one therapeutic agent known for its effectiveness in treating a condition or disease characterized by inappropriate CD9 expression or CD9 overexpression. For example, when treatment of malignancy is sought, one or more anti-cancer therapeutic agents may be included in the kits. In particular, any one or more of the anti-cancer therapeutic agents known/used in the medical field or described herein may be included, especially chemotherapeutic drugs, *e.g.*, drugs capable of killing or suppressing cells that are actively undergoing proliferation, and immunotherapeutic agents, *e.g.*, checkpoint inhibitors and therapeutic antibodies.

[0092] Further, the kits of this invention may provide instruction manuals to guide users in the proper administration of the composition comprising the CD9 antibody construct, its conjugate (*e.g.*, anti-CD9 conjugated with a therapeutic agent), or a host cell expressing a CD9 antibody construct (*e.g.*, anti-CD9 CAR T cell) to a subject deemed in need of such treatment by a physician (*e.g.*, a person suffering from a condition involving CD9 expression, including certain types of malignancy including leukemia such as B-ALL), the schedule (*e.g.*, dose and frequency of administration) and route of administration, and the like.

EXAMPLES

[0093] The following examples are provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of non-critical parameters that could be changed or modified to yield essentially similar results.

[0094] The invention relates to the fields of biology, immunology and medicine. Despite significant progress in CD19/CD22-based immunotherapies, the outcomes of patients with relapse/refractory acute lymphoblastic leukemia (ALL) remains suboptimal owing to non-durable remission and treatment resistance. Meanwhile, tetraspanin CD9 endows proven prognostic relevance in ALL that could be targeted by blocking monoclonal antibodies with profound preclinical efficacy. However, platelet toxicities associated with CD9 antibodies resulting in severe thrombocytopenia and lethal thrombosis have largely hampered their clinical translation. This may be attributed to their off-target Fc region binding and activation of the FcγRIIA receptor in close proximity to CD9 on platelet surface. This invention presents novel CD9-binding compounds and therapeutic applications thereof. The disclosure

is directed to a biological entity, either isolated, synthetic or recombinant antibody, or functional part or functional equivalent thereof specific to the tetraspanin transmembrane protein CD9, compositions, moieties and modifications comprising such antibody or functional part or functional equivalent; methods for binding and inhibiting CD9; and method of uses in the modulation, diagnosis, treatment and prevention of CD9-dependent diseases and processes.

INTRODUCTION

[0095] While intensive chemotherapy could achieve high remission rates in pediatric and adult acute lymphoblastic leukemia (ALL), poor survival outcome persists for patients who develop resistant diseases [1, 2]. The advent of CD19- or CD22-targeting immunotherapies has notably transformed the treatment landscape of relapsed/refractory B-lineage ALL (B-ALL) [3, 4]. Tumor evasion through antigen downregulation remains a significant challenge [5, 6], however, requiring the identification of additional or alternative immunotherapeutic targets.

[0096] CD9, a cell surface protein belonging to the tetraspanin superfamily, has been implicated in cancer progression, with its influence on specific disease being context-dependent [7]. Featuring four transmembrane (TM) segments with N- and C-terminal domains situated intracellularly, CD9 bears two extracellular loops: one between TM1 and TM2 known as small extracellular loop (SEL or EC1) and the other between TM3 and TM4 known as large extracellular loop (LEL or EC2) [8]. CD9 plays a crucial role in physiological cell migration, adhesion and differentiation [9-12], as well as malignant properties such as tumor metastasis [13-15]. In B-ALL, previous studies have revealed its heterogeneous and subtype-specific expression pattern [16, 17]. Subsequent investigations have shown its enrichment in leukemia-initiating cells [18-20] and its involvement in leukemia dissemination [21].

[0097] The potential of CD9 as a therapeutic target is underscored by its ubiquitous expression in >85% of B-ALL patients and the robust leukemia control by the monoclonal antibody ALB6 in patient-derived xenografts [22, 23]. Beyond hematological malignancies, numerous solid tumors also present with CD9 positivity, including but not limited to melanoma, multiple myeloma, colorectal cancer, pancreatic cancer, esophageal cancer, gastric cancer, lung cancer, breast cancer, ovarian cancer, and liver cancer [24, 25].

Additionally, CD9 has been implicated in other pathological conditions beyond malignancies, such as arthritis and colitis [26-28].

[0098] Due to the expression of CD9 on platelet surfaces, adverse effects, particularly platelet activation, aggregation and lethal thrombosis, can be induced by CD9-specific antibodies [29, 30]. This phenomenon is well evidenced by ALB6. Conversely, AT1412 binds to CD9 via a unique epitope, demonstrating no platelet aggregation upon antigen binding. Moreover, AT1412 exhibits antibody-dependent cytotoxicity (ADCC) *in vitro* and reduces tumor load in humanized mice [31].

[0099] The present invention provides Fc-engineered humanized antibodies (hAb-D and hAb-F) designed to prevent platelet activation. These antibodies features novel CDR sequences and exhibits remarkable binding affinity and specificity for CD9 as well as highly potent anti-leukemia activity. Crucially, they do not trigger platelet activation compared to existing antibodies such as ALB6. In addition, unlike AT1412, hAb-D and hAb-F does not require ADCC function for leukemia killing.

[0100] Regarding platelet toxicity, the antibody of our invention, particularly when modified with Fc variants such as N297A, displays significantly reduced platelet complications. In multiple models, our antibody does not trigger platelet activation, aggregation, and thrombocytopenia. This helps clear the largest hurdle preventing CD9 antibodies from being placed into clinical applications. Furthermore, our antibody has demonstrated limited immunogenicity compared to existing murine antibodies due to its humanized nature, which significantly lowers the risk of adverse immune reactions upon administration and enhance its safety profile in human subjects.

RESULTS

Generation of murine CD9 monoclonal antibodies

[0101] The EC2 of CD9 was selected as the antigen for subsequent experiments. The amino acid sequence of the CD9-EC2 region is set forth in **SEQ ID NO:19**. The CD9-EC2 coding sequence was cloned into the eukaryotic expression vector pSectag B. To facilitate the detection and purification of the antigen, different tags (His and Fc) were added to the expression vector. The constructed CD9 antigen expression vector was expressed in 293 Freestyle cells and purified for further experiments (**Figure 1**).

[0102] Using the expressed recombinant protein CD9-mFc, BALB/c mice were immunized. Splenocytes were extracted and fused with myeloma cells to form hybridomas.

Positive monoclonal hybridoma lines were selected for their ability to bind CD9 antigen using ELISA. Three cell lines demonstrating high CD9 antigen binding were identified (**Figure 2**).

[0103] Hybridoma cells were selected, and total RNA was extracted using Trizol reagent. Subsequently, cDNA was generated by reverse transcription. Specific PCR primers were obtained according to references [32]. The variable region sequences of the heavy or light chains of CD9 antibodies in the hybridoma were amplified and sequenced. Due to two clones having identical sequences, two sets of different heavy and light chain variable region sequences (mAb-D, mAb-F) were obtained.

Humanization of murine CD9 antibodies

[0104] The sequences of the 6 CDR regions of the murine CD9 antibody heavy and light chains were determined using the Kabat definition scheme. By aligning with sequences in the IMGT database, the human antibody variable region lineage sequence IGHV1-206+IGHJ401 was chosen as the template for humanization of the heavy chain of mAb-D and mAb-F. For the light chain of mAb-D, the template selected was IGKV1-NL101+IGKJ201, and for the light chain of mAb-F, the template selected was IGKV1-1201+IGKJ201. The CDR regions of the murine antibodies were then transplanted into the respective humanization templates, and key amino acid residues in the FR regions were reverted to the corresponding human residues. Finally, the corresponding humanized CD9 antibody sequences, named hAb-D and hAb-F, were obtained. The amino acid sequences for the V_H and V_L of hAb-D and hAb-F are set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, and SEQ ID NO:4, respectively. The amino acid sequences for the V_H and V_L of murine Ab-D and murine Ab-F are set forth in SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, and SEQ ID NO:23, respectively.

Production of murine and humanized CD9 antibodies

[0105] The hybridoma cell lines of mAb-D and mAb-F were expanded in culture medium (Shanghai BasaImedia). Supernatants were collected and subjected to Protein A (GE Healthcare) purification to obtain murine IgG proteins against CD9 (**Figure 3A**).

[0106] The variable region sequences of humanized anti-CD9 antibody (hAb-D and hAb-F) were cloned into the mammalian cell expression vector, which contains a signal peptide, human IgG1 constant regions for both heavy and light chains. The IgGs of hAb-D and hAb-

F were expressed in 293freestyle host cells using polyethylenimine (PEI; MW 25000, Polysciences). Supernatants were collected and purified using Protein A (**Figure 3B**).

[0107] The two sets of heavy and light chain variable region sequences of hAb-D and hAb-F were connected via flexible linkers (GGGGS)3 using PCR and cloned into the prokaryotic expression vector, respectively. Subsequently, the constructs were transformed into HB2151 *Escherichia coli* competent cells, and single-chain variable fragment (scFv) antibodies were expressed upon induction with IPTG (Sigma). The soluble scFvs were purified with Ni-NTA resin (QIAGEN) (**Figure 3C**).

Antigen affinity and specificity of CD9 antibodies

[0108] To test their binding to CD9 extracellular domain, soluble CD9 antigens were coated on the ELISA plates. Then, the IgGs of mAb-D, mAb-F, hAb-D and hAb-F were incubated and detected by the following the HRP-labelled secondary antibodies (Jackson ImmunoResearch). The absorbance at 450 nm was determined by a microplate reader. The four antibodies strongly bound to CD9 antigens but not human Fc fragment. (**Figure 4A**). In order to assess the specificity, different antigens (CD9, CD22, Her2, IGF-1, CD133, B7-H3) were used to detect with anti-CD9 IgGs or scFvs by ELISA, respectively. As shown in **Figure 4B**, two murine antibodies did not show cross-reactivity to other irrelevant antigens. As comparison to murine antibodies, humanized anti-CD9 scFvs exhibited the similar antigen binding in ELISA (**Figure 4C**). Meanwhile, humanized IgGs of hAb-D and hAb-F showed the CD9 binding in dose dependent manner **(Figure 4D)** and no cross-reactivity to two irrelevant antigens (**Figure 4E**). Furthermore, we measured the affinity constant values by biolayer interferometry (BLI). As shown in **Figure 5** and **Table 1**, humanized antibodies showed Kd values similar to those of the murine antibodies.

Table 1. Binding rate constants and affinities of anti-CD9 scFvs against CD9

Anti-CD9 scFv	K_D (M)	ka (1/Ms)	kdis (1/s)
mAb-D	$7.25x10^{-9}$	$4.98x10^{5}$	$3.61x10^{-3}$
mAb-F	$1.0x10^{-12}$	$5.33x10^{5}$	$1.86x10^{-7}$
hAb-D	$4.76x10^{-9}$	$3.74x10^{5}$	$1.78x10^{-3}$
hAb-F	$7.40x10^{-10}$	$7.31x10^{5}$	$5.41x10^{-4}$

[0109] Antigen specificity of anti-CD9 antibodies in the context of lymphoblasts were assessed by flow cytometry. mAb-D and mAb-F, alongside with three commercially available CD9 antibodies ML-13, ALB6 and MM2/57, showed the correct pattern of reactivity to $CD9^{low}$ Reh, $CD9^{intermediate}$ SEM and $CD9^{high}$ RS4;11 B-ALL cell lines (**Figure 6A**), where their specificity to CD9 was further confirmed by testing in CD9-overexpressing and CD9-knockout B-ALL cells (**Figure 6B**). The specificity of hAb-D and hAb-F to CD9 was also confirmed in the overexpression and knockout models (**Figure 6C**).

Platelet toxicity of CD9 antibodies is triggered by its Fc domain

[0110] CD9 antibody was reported to induce severe thrombocytopenia and lethal pulmonary thrombosis within 5 minutes post-injection in non-human primates [29]. The toxicity could be due to either on-target binding of its Fab domain to CD9 on platelets or off-target binding of its Fc domain to FcγRIIA receptor in close proximity to CD9. We assessed the degree of platelet activation, the initiating step of coagulation cascade, upon full-length hAb-D and hAb-F IgG treatment by flow cytometric measurement of the platelet activation marker CD62P (**Figure 7A**). Consistent with previous reports on CD9 antibody-induced platelet activation, virtually all platelets were activated within 30 minutes after exposure to hAb-D or hAb-F IgG. Importantly, this phenomenon was absent when we exposed platelets to their Fab or scFv fragments, indicating platelet activation is a Fc region-dependent event (**Figure 7B**). This observation provides a strong scientific rationale for commencing antibody engineering.

Platelet toxicity of CD9 antibodies can be overcome by Fc engineering

[0111] We introduced a point mutation at the Fc domain of hAb-D and hAb-F IgG to substitute the amino acid at position 297 from asparagine (N) to alanine (A) to achieve aglycosylation (N297A) (**Figure 7C**), which is expected to diminish Fc receptor binding [33]. By pinpointing the coagulation cascade, we designed a series of experiments to comprehensively assess the platelet toxicity of N297A-engineered CD9 antibodies. Surprisingly, platelet activation was completely extinguished by N297A engineering compared with non-engineered version as reflected by the absence of phenotypic CD62P expression by flow cytometry (**Figure 7D**) and physical platelet aggregation by optical aggregometry (**Figure 7E**). Utilizing a transgenic mouse model with platelets expressing human FcγRIIA (**Figure 7F**), we reproduced findings observed by Taylor *et al.* (2000) [30] that the CD9 antibody ALB6 mediated thrombocytopenia in hemizygous but not wild-type

mice (**Figure 7G**). Importantly, N297A engineering of hAb-D did not induce thrombocytopenia in this model (**Figure 7H**).

Fc modification of CD9 antibodies does not compromise specificity and antitumor activity

[0112] By CD9 overexpression and knockout models, we showed that Fc-engineering of CD9 antibodies did not compromise antigen specificity (**Figure 8**). Xenograft experiments with 697-transplanted NOD/SCID mice revealed that both mAb-D and mAb-F, similar to ALB6, demonstrated strong leukemia-suppressive activities (**Figure 9A, B**) along with a substantial extension of animal survival (**Figure 9C**). The administration of either hAb-D or hAb-F also significantly impeded leukemia progression and markedly prolonged animal survival (**Figure 9D, E**). Importantly, the anti-leukemia activity remained uncompromised even after N297A Fc engineering (**Figure 9F, G**).

Fc-engineered CD9 antibodies are effective in patient-derived xenografts of resistant B-ALL

[0113] We next examined the therapeutic relevance of Fc-engineered CD9 antibodies in a patient-derived xenograft model developed from a patient with CD19-negative relapse after chimeric antigen receptor (CAR) T therapy. Administration of Fc-engineered hAb-D or hAb-F significantly alleviated splenomegaly and hepatomegaly (**Figure 10A**). At parallel endpoint harvest, hAb-D N297A or hAb-F N297A profoundly reduced leukemia burden in major hematopoietic organs by ≥98.3% and ≥99.0%, respectively for when compared to human IgG control (**Figure 10B**).

CD9 antibodies exerts direct apoptotic effect on leukemia and does not require ADCC

[0114] To decipher mechanisms underlying the potent activities of Fc-engineered CD9 antibodies, we first assessed morphological changes upon treatment. Fluorescence microscopy revealed that CD9 antibodies induced homotypic aggregation in GFP-expressing $CD9^{high}$ 697 cell line, which were known to be associated with direct cell death [34, 35] (**Figure 11A**). Fc-engineered CD9 mAbs also inhibited proliferation of CD9-expressing B-ALL cell lines with distinct cytogenetics (697, TCF3-PBX1; RS4;11, KMT2A-AFF1, BV-173, BCR-ABL1) and induced direct apoptosis (**Figure 11B, C**). Notably, antibody-dependent NK-mediated cytotoxicity was lost after N297A Fc modification **(Figure 11D)** despite robust anti-leukemia activities. Therefore Fc-engineered CD9 antibodies act via an ADCC-independent mechanism.

REFERENCES

1. Mullighan CG. Molecular genetics of B-precursor acute lymphoblastic leukemia. J Clin Invest. 2012;122:3407-3415.

2. Bhojwani D, Pui CH. Relapsed childhood acute lymphoblastic leukemia. Lancet Oncol. 2013;14:e205-e217.

3. DasGupta RK, Marini BL, Rudoni J, Perissinotti AJ. A review of CD19-targeted immunotherapies for relapsed or refractory acute lymphoblastic leukemia. J Oncol Pharm Pract. 2018;24:453-467.

4. Aureli A, Marziani B, Venditti A, Sconocchia T, Sconocchia G. Acute Lymphoblastic Leukemia Immunotherapy Treatment: Now, Next, and Beyond. Cancers (Basel). 2023;15.

5. Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov. 2015;5(12):1282-1295.

6. Orlando EJ, Han X, Tribouley C, Wood PA, Leary RJ, Riester M, et al. Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. Nat Med. 2018;24(10):1504-1506.

7. Zoller M. Tetraspanins: push and pull in suppressing and promoting metastasis. Nat Rev Cancer. 2009;9:40-55.

8. Umeda R, Satouh Y, Takemoto M, et al. Structural insights into tetraspanin CD9 function. Nat Commun. 2020;11(1):1606.

9. Hemler ME. Tetraspanin functions and associated microdomains. Nat Rev Mol Cell Biol. 2005;6:801-811.

10. Clay D, Rubinstein E, Mishal Z, Anjo A, Prenant M, Jasmin C, et al. CD9 and megakaryocyte differentiation. Blood. 2001;97:1982-1989.

11. Qi JC, Wang J, Mandadi S, Tanaka K, Roufogalis BD, Madigan MC, et al. Human and mouse mast cells use the tetraspanin CD9 as an alternate interleukin-16 receptor. Blood. 2006;107:135-142.

12. Masellis-Smith A, Shaw AR. CD9-regulated adhesion. Anti-CD9 monoclonal antibody induces pre-B cell adhesion to bone marrow fibroblasts through de novo recognition of fibronectin. J Immunol. 1994;152:2768-2777.

13. Kischel P, Bellahcene A, Deux B, Lamour V, Dobson R, DE Pauw E, et al. Overexpression of CD9 in human breast cancer cells promotes the development of bone metastases. Anticancer Res. 2012;32:5211-5220.

14. Hori H, Yano S, Koufuji K, Takeda J, Shirouzu K. CD9 expression in gastric cancer and its significance. J Surg Res. 2004;117:208-215.

15. Kohmo S, Kijima T, Otani Y, et al. Cell surface tetraspanin CD9 mediates chemoresistance in small cell lung cancer. Cancer Res. 2010;70(20):8025-8035. doi:10.1158/0008-5472.CAN-10-0996

16. Gandemer V, Rio AG, de Tayrac M, Sibut V, Mottier S, Ly Sunnaram B, et al. Five distinct biological processes and 14 differentially expressed genes characterize TEL/AML1-positive leukemia. BMC Genomics. 2007;8:385.

17. Gandemer V, Aubry M, Roussel M, Rio AG, de Tayrac M, Vallee A, et al. CD9 expression can be used to predict childhood TEL/AML1-positive acute lymphoblastic leukemia: proposal for an accelerated diagnostic flowchart. Leuk Res. 2010;34:430–7.

18. Nishida H, Yamazaki H, Yamada T, Iwata S, Dang NH, Inukai T, et al. CD9 correlates with cancer stem cell potentials in human B-acute lymphoblastic leukemia cells. Biochem Biophys Res Commun. 2009;382:57–62.

19. Aoki Y, Watanabe T, Saito Y, Kuroki Y, Hijikata A, Takagi M, et al. Identification of CD34+ and CD34- leukemia-initiating cells in MLL-rearranged human acute lymphoblastic leukemia. Blood. 2015;125:967–80.

20. Yamazaki H, Xu CW, Naito M, Nishida H, Okamoto T, Ghani FI, et al. Regulation of cancer stem cell properties by CD9 in human B-acute lymphoblastic leukemia. Biochem Biophys Res Commun. 2011;409:14–21.

21. Arnaud MP, Vallee A, Robert G, Bonneau J, Leroy C, Varin-Blank N, et al. CD9, a key actor in the dissemination of lymphoblastic leukemia, modulating CXCR4-mediated migration via RAC1 signaling. Blood. 2015;126:1802–12.

22. Leung KT, Cai J, Liu Y, Chan KYY, Shao J, Yang H, Hu Q, Xue Y, Wu X, Guo X, et al. Prognostic implications of CD9 in childhood acute lymphoblastic leukemia: insights from a nationwide multicenter study in China. Leukemia. 2024;38:250-257.

23. Leung KT, Zhang C, Chan KYY, Li K, Cheung JTK, Ng MHL, Zhang XB, Sit T, Lee WYW, Kang W, et al. CD9 blockade suppresses disease progression of high-risk pediatric B-cell precursor acute lymphoblastic leukemia and enhances chemosensitivity. Leukemia. 2020;34:709-720.

24. Lorico A, Lorico-Rappa M, Karbanova J, Corbeil D, Pizzorno G. CD9, a tetraspanin target for cancer therapy? Exp Biol Med (Maywood). 2021;246:1121-1138.

25. Ondruššek R, Kvokačková B, Kryštofová K, Brychtová S, Souček K, Bouchal J. Prognostic value and multifaceted roles of tetraspanin CD9 in cancer. Front Oncol. 2023;13:1140738.

26. Sumiyoshi N, Ishitobi H, Miyaki S, Miyado K, Adachi N, Ochi M. The role of tetraspanin CD9 in osteoarthritis using three different mouse models. Biomed Res. 2016;37(5):283-291.

27. Rydland A, Heinicke F, Flåm ST, Mjaavatten MD, Lie BA. Small extracellular vesicles have distinct CD81 and CD9 tetraspanin expression profiles in plasma from rheumatoid arthritis patients. Clin Exp Med. 2023;23(6):2867-2875.

28. Saiz ML, Cibrian D, Ramírez-Huesca M, Torralba D, Moreno-Gonzalo O, Sánchez-Madrid F. Tetraspanin CD9 Limits Mucosal Healing in Experimental Colitis. Front Immunol. 2017;8:1854.

29. Kawakatsu T, Suzuki M, Kido H, et al. Antithrombotic effect of an anti-glycoprotein IIB/IIIA antibody in primate lethal thrombosis. Thromb Res. 1993;70(3):245-254.

30. Taylor SM, Reilly MP, Schreiber AD, Chien P, Tuckosh JR, McKenzie SE. Thrombosis and shock induced by activating antiplatelet antibodies in human Fc gamma RIIA transgenic mice: the interplay among antibody, spleen, and Fc receptor. Blood. 2000;96(13):4254-4260.

31. Remko Schotte, Julien Villaudy, Greta De Jong, Viviana Neviani, Wouter Pos, Sophie E Levie, Daniel M Go, Etsuko Yasuda, Esmay Frankin, Madalina Cercel, Susan E van Hal-van Veen, Dorien van de Berg, Anikó Szabó, Christien Fatmawati, Martijn Kedde, Yvonne Claassen, Sjeng Horbach, Anita W Rijneveld, Piet Gros, Hergen Spits, Mette D Hazenberg, Hans van Eenennaam; Preclinical Development of AT1412, a Patient Derived CD9 Antibody That Does Not Induce Thrombosis for Treatment of B ALL. Blood. 2020; 136 (Supplement 1):41–42.

32. Toleikis L, Broders O, Dubel S. Cloning single-chain antibody fragments (scFv) from hybridoma cells. Methods Mol Med. 2004;94:447-458. doi:10.1385/1-59259-679-7:447.

33. Borrok MJ, Jung ST, Kang TH, Monzingo AF, Georgiou G. Revisiting the role of glycosylation in the structure of human IgG Fc. ACS Chem Biol. 2012;7(9):1596-1602.

34. Mössner E, Brünker P, Moser S, et al. Increasing the efficacy of CD20 antibody therapy through the engineering of a new type II anti-CD20 antibody with enhanced direct and immune effector cell-mediated B-cell cytotoxicity. Blood. 2010;115(22):4393-4402.

35. Mark S. Cragg, Waleed Alduaij, Christian Klein, Pablo Umana, Martin J. Glennie, Tim M. Illidge; Response: novel lysosomal-dependent cell death following homotypic adhesion occurs within cell aggregates. Blood 2010; 116 (17): 3373–3374.

[0115] All patents, patent applications, and other publications, including GenBank Accession Numbers or similar sequence identification numbers, cited in this application are incorporated by reference in the entirety of their contents for all purposes.

SEQUENCE LISTING

SEQ ID NO:1 amino acid sequence of hAb-D V_H region (CDRs underlined)

QVQLVQSGPAVVKPGASVKIPCKASGYTFTDYNMDWVKQSHGKGLEWIGDINPNN
DVTIYNQKFKGKATLTVDKSSSTAYMELSRLRSEDTAVYYCARGDYYGTNFDYWG
QGTTLTVSS

SEQ ID NO:2 amino acid sequence of hAb-D V_L region (CDRs underlined)

DIQMTQSPSSLSASVGETVTITCRASENIYSNLAWYQQKQGKSPQLLVYAATNLAD
GVPSRFSGSGSGTQYTLTINSLQSEDFGSYYCQHFWGTPYTFGGGTKLEIK

SEQ ID NO:3 amino acid sequence of hAb-F V_H region (CDRs underlined)

QVQLVQSGPEVVKPGASVKVSCKTSGYTFTDYNMDWVRQSHGQGLEWIGNINPN
SAFTIYNQKFKGKATLTVDKSSSTAYMELSRLRSEDTAVYYCARGDYYGTNFDYW
GQGTTLTVSS

SEQ ID NO:4 amino acid sequence of hAb-F V_L region (CDRs underlined)

DIQMTQSPSSLSASVGETVTITCRASENIYSHLAWYQQKQGKSPQLLVYTATNLAD
GVPSRFSGSGSGTQYTLTINSLQSEDFGSYYCQHFWGSPYTFGGGTKLEIK

SEQ ID NO:5 amino acid sequence of hAb-D heavy chain CDR1

GYTFTDYN

SEQ ID NO:6 amino acid sequence of hAb-D heavy chain CDR2

INPNNDVT

SEQ ID NO:7 amino acid sequence of hAb-D heavy chain CDR3

ARGDYYGTNFDY

SEQ ID NO:8 amino acid sequence of hAb-D light chain CDR1

ENIYSN

SEQ ID NO:9 amino acid sequence of hAb-D light chain CDR2

AATNLAD

SEQ ID NO:10 amino acid sequence of hAb-D light chain CDR3

QHFWGTPYT

SEQ ID NO:11 amino acid sequence of hAb-F heavy chain CDR1

GYTFTDYN

SEQ ID NO:12 amino acid sequence of hAb-F heavy chain CDR2

INPNSAFT

SEQ ID NO:13 amino acid sequence of hAb-F heavy chain CDR3

ARGDYYGTNFDY

SEQ ID NO:14 amino acid sequence of hAb-F light chain CDR1

ENIYSH

SEQ ID NO:15 amino acid sequence of hAb-F light chain CDR2

TATNLAD

SEQ ID NO:16 amino acid sequence of hAb-F light chain CDR3

QHFWGSPYT

SEQ ID NO:17 amino acid sequence of hAb-D/F heavy chain constant region (N297A substitution in bold and underlined)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPC
PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQY**A**STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG
QPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:18 amino acid sequence of hAb-D/F light chain constant region

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQES
VTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:19 amino acid sequence of CD9-EC2 region

SHKDEVIKEVQEFYKDTYNKLKTKDEPQRETLKAIHYALNCCGLAGGVEQFISDICP
KKDVLETFTVKSCPDAIKEVFDNKFHI

SEQ ID NO:20 amino acid sequence of mAb-D V_H region

EVQLQQSGPALVKPGASVKIPCKASGYTFTDYNMDWVKQSHGKSLEWIGDINPNN
DVTIYNQKFKGKATLTVDKSSSTAYMELRSLTSEDTAVYYCARGDYYGTNFDYWG
QGTTLTVSS

SEQ ID NO:21 amino acid sequence of mAb-D V_L region

DIQMTQSPASLSVSVGETVTITCRASENIYSNLAWYQQKQGKSPQLLVYAATNLAD
GVPSRFSGSGSGTQYSLKINSLQSEDFGSYYCQHFWGTPYTFGGGTKLEIK

SEQ ID NO:22 amino acid sequence of mAb-F V_H region

EVQLQQSGPELVKPGASVKIPCKTSGYTFTDYNMDWVKQSHGKSLEWIGNINPNS
AFTIYNQKFKGKATLTVDKSSSTAYMELRSLTSEDTAVYYCARGDYYGTNFDYWG
QGTTLTVSS

SEQ ID NO:23 amino acid sequence of mAb-F V_L region

DIQMTQSPASLSVSVGETVTITCRASENIYSHLAWYQQKQGKSPQLLVYTATNLAD
GVPSRFSGSRSGTQFSLKINSLQSEDFGTYYCQHFWGSPYTFGGGTKLEIK

WHAT IS CLAIMED IS:

1. A CD9 antibody construct that specifically binds human CD9 and comprises antibody heavy chain and light chain complementarity determining regions (CDRs) having the amino acid sequences set forth in (1) SEQ ID NOs:5-10 or (2) SEQ ID NOs:11-16, respectively.

2. The CD9 antibody construct of claim 1, comprising an antibody heavy chain variable region (V_H) and light chain variable region (V_L), each comprising the amino acid sequence set forth in SEQ ID NO:1 or 2, respectively.

3. The CD9 antibody construct of claim 1, comprising an antibody heavy chain variable region (V_H) and light chain variable region (V_L), each comprising the amino acid sequence set forth in SEQ ID NO:3 or 4, respectively.

4. The CD9 antibody construct of any one of claims 1-3, comprising a heavy chain constant region having the amino acid sequence set forth in SEQ ID NO:17 and/or a light chain constant region having the amino acid sequence set forth in SEQ ID NO:18.

5. The CD9 antibody construct of any one of claims 1-5, comprising a heavy chain variable region, a light chain variable region, a heavy chain constant region, and a light chain constant region having the amino acid sequences set forth in (1) SEQ ID NOs:1, 2, 17, and 18; or (2) SEQ ID NOs: 3, 4, 17, and 18, respectively.

6. The CD9 antibody construct of any one of claims 1-3, which is an anti-CD9 single chain variable fragment (scFv), a full-length IgG, a bispecific T cell engager (BiTE), an antibody drug conjugate (ADC), or a chimeric antigen receptor (CAR) construct.

7. A nucleic acid comprising a polynucleotide sequence encoding the CD9 antibody construct any one of claims 1-6.

8. The nucleic acid of claim 7, which is an expression cassette comprising a promoter operably linked to the polynucleotide sequence.

9. The nucleic acid of claim 7 or 8, which is a plasmid or a viral vector.

10. A host cell comprising the nucleic acid of any one of claims 7-9 or the CD9 antibody construct of any one of claims 1-6.

11. The host cell of claim 10, which is a T cell or natural killer (NK) cell.

12. The host cell of claim 11, wherein the CD9 antibody construct is an scFv or a CAR construct.

13. A conjugate comprising the CD9 antibody construct of any one of claims 1-6 and a solid support, a detectable moiety, or a therapeutic agent.

14. The conjugate of claim 13, wherein the CD9 antibody construct is an scFv or a full-length IgG, and wherein the therapeutic agent comprises an anti-cancer therapeutic agent.

15. A composition comprising the CD9 antibody construct of any one of claims 1-6, the nucleic acid of any one of claims 7-9, the host cell of any one of claims 10-12, or the conjugate of claim 13 or 14.

16. A method for killing a cell expressing CD9, comprising contacting cancer cells with the composition of claim 15.

17. The method of claim 16, wherein the cell expressing CD9 is a cancer cell.

18. The method of claim 17, wherein the cancer is a blood cancer or a solid tumor.

19. The method of claim 17 or 18, wherein the cancer cell is located in a patient's body.

20. The method of claim 19, comprising administering the composition to the patient by subcutaneous, intravenous, intramuscular, intraperitoneal, or intratumoral injection.

21. A kit for treating cancer, comprising

(1) a first container containing a first composition comprising an effective amount of the composition of claim 15; and

(2) a second container containing a second composition comprising an effective amount of another anti-cancer therapeutic agent.

22. The kit of claim 21, wherein the cancer is a blood cancer or a solid tumor.

23. The kit of any one of claims 21 or 22, wherein the first composition is formulated for injection.

24. The kit of claim 23, wherein the injection is subcutaneous, intravenous, intramuscular, intraperitoneal, or intratumoral injection.

25. The kit of any one of claims 21-24, further comprising an instruction manual for using the kit.

ENGINEERED CD9 ANTIBODIES AND THEIR USE

ABSTRACT OF THE DISCLOSURE

This disclosure relates to antibody constructs that specifically bind CD9 with high affinity. These antibody constructs include a set of novel heavy chain and light chain complementarity determining regions (CDRs) sequences, optionally humanized variable regions, and engineered Fc region of the heavy chain constant region in order to eliminate undesirable platelet activation. The present invention also provides methods for diagnosing and treating conditions including cancer characterized by the aberrant expression of CD9 in the pertinent tissue and cells through the use of these anti-CD9 antibody constructs.

78536674

Figure 1

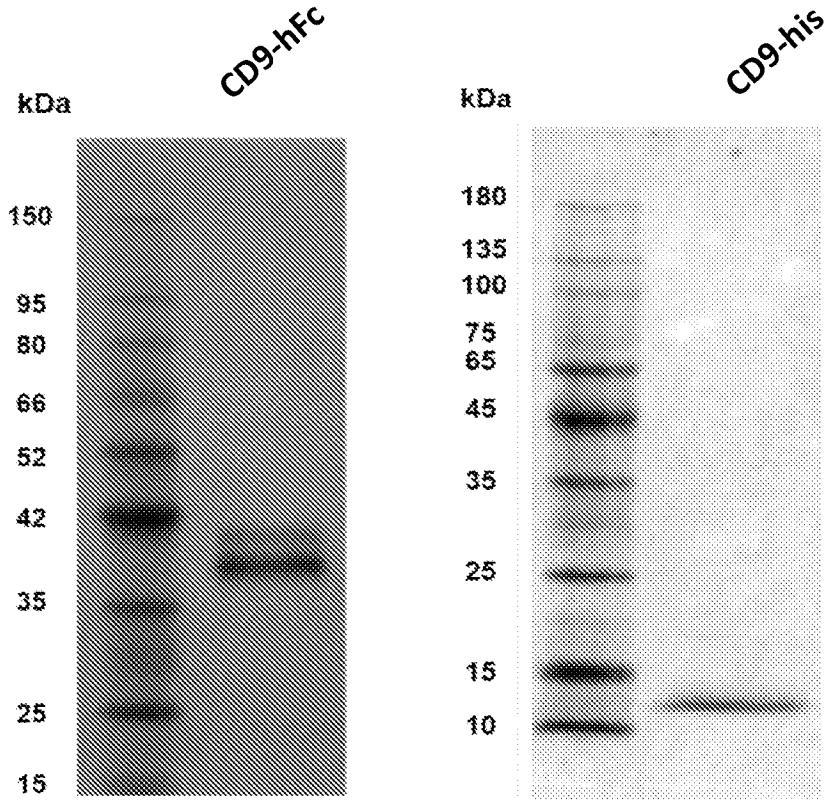

CD9-hFc
kDa
150
95
80
66
52
42
35
25
15
CD9-his
kDa
180
135
100
75
65
45
35
25
15
10

Figure 2

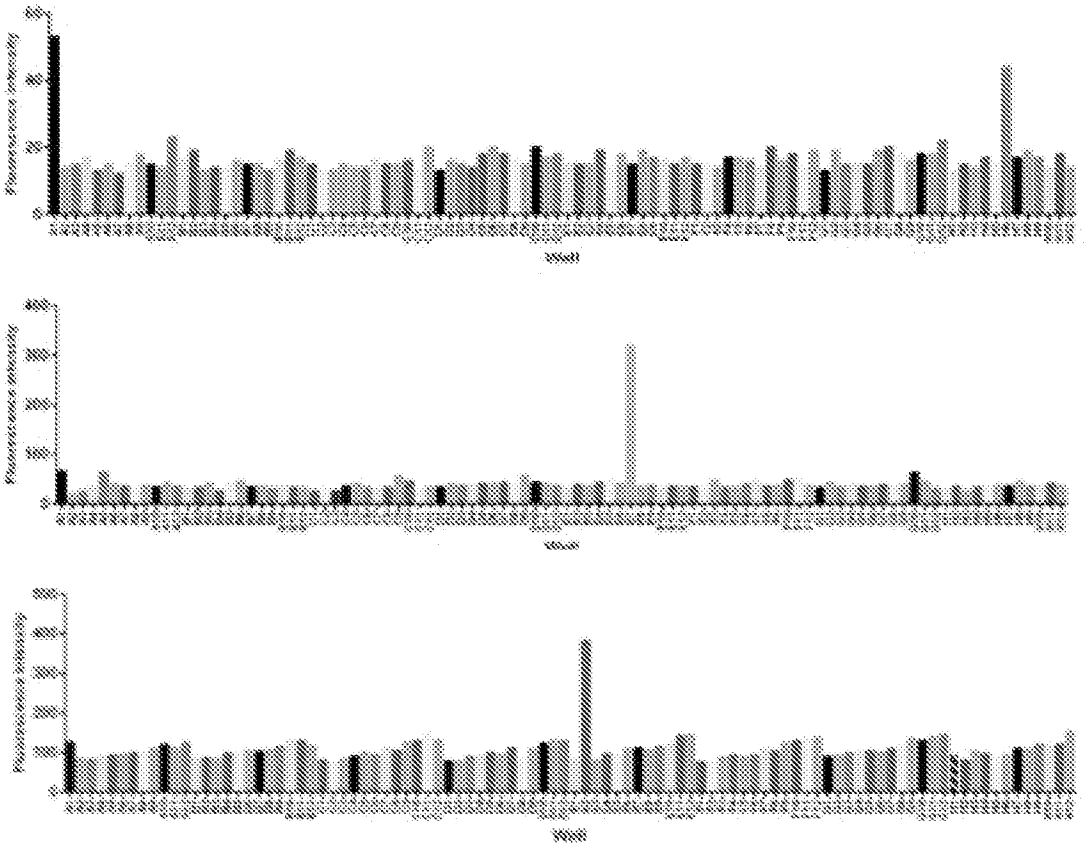


Figure 3

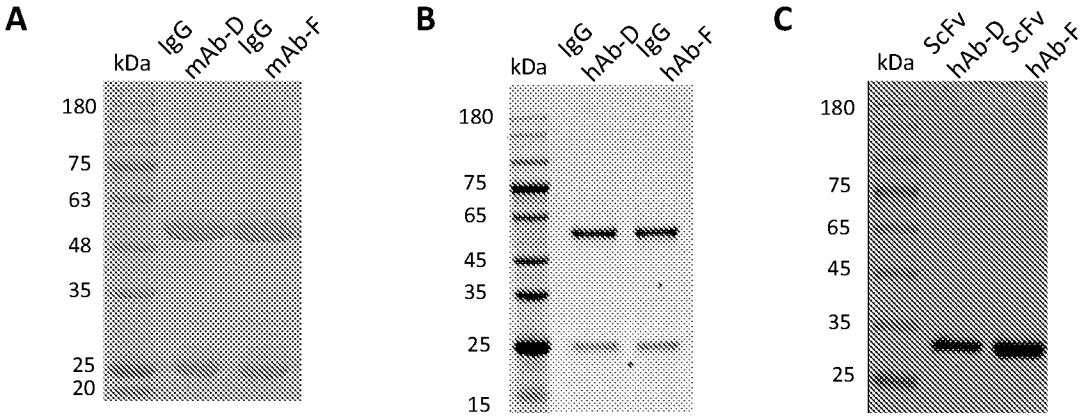

A
kDa
IgG
mAb-D
IgG
mAb-F
180
75
63
48
35
25
20
B
kDa
IgG
hAb-D
IgG
hAb-F
180
75
65
45
35
25
15
C
kDa
ScFv
hAb-D
ScFv
hAb-F
180
75
65
45
35
25

Figure 4

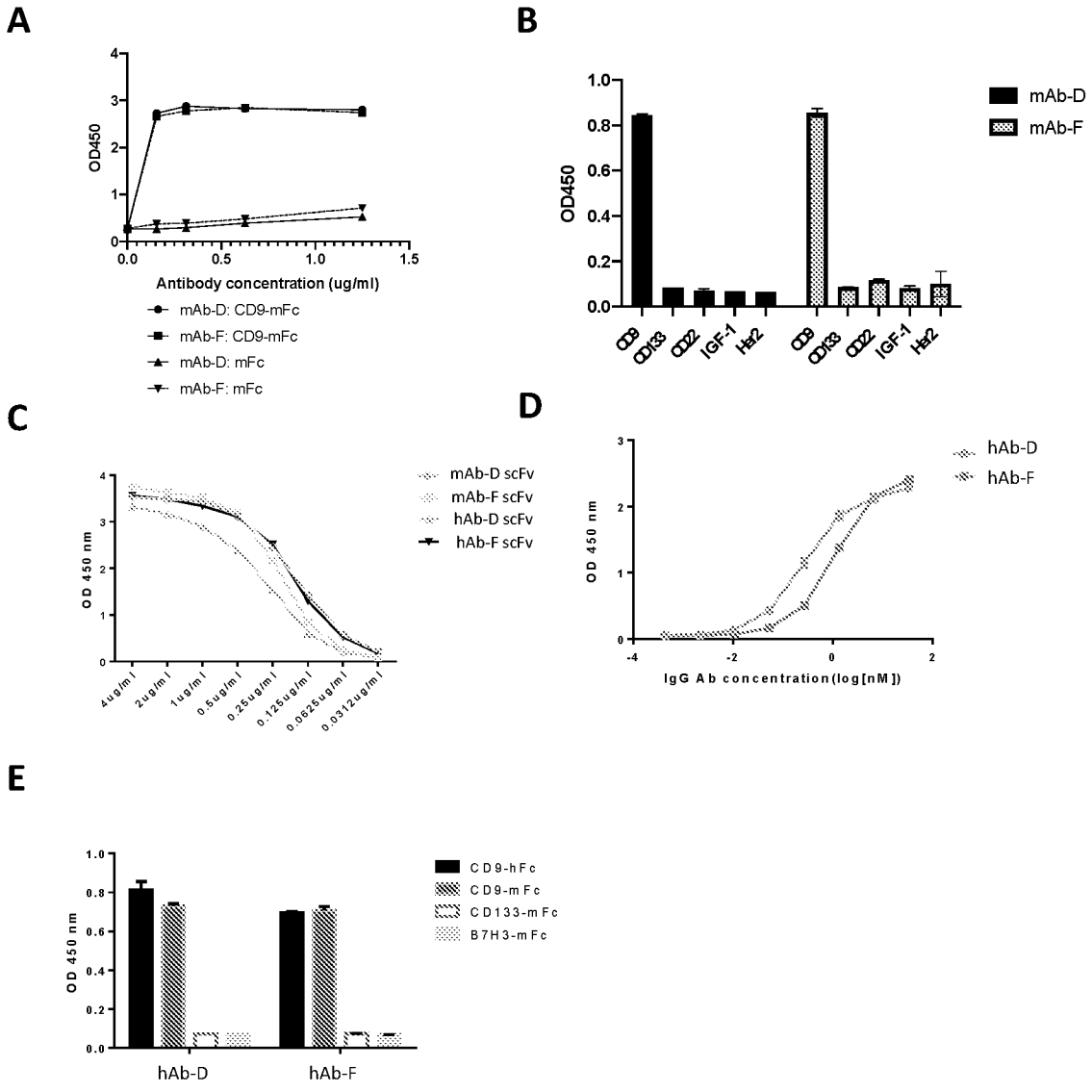

A
OD450
Antibody concentration (ug/ml)
mAb-D: CD9-mFc
mAb-F: CD9-mFc
mAb-D: mFc
mAb-F: mFc
B
OD450
mAb-D
mAb-F
CD9
CD133
CD82
IGF-1R
Her2
C
OD 450 nm
mAb-D scFv
mAb-F scFv
hAb-D scFv
hAb-F scFv
4ug/ml
2ug/ml
1ug/ml
0.5ug/ml
0.25ug/ml
0.125ug/ml
0.0625ug/ml
0.0312ug/ml
D
OD 450 nm
IgG Ab concentration(log[nM])
hAb-D
hAb-F
E
OD 450 nm
CD9-hFc
CD9-mFc
CD133-mFc
B7H3-mFc
hAb-D
hAb-F

Figure 5

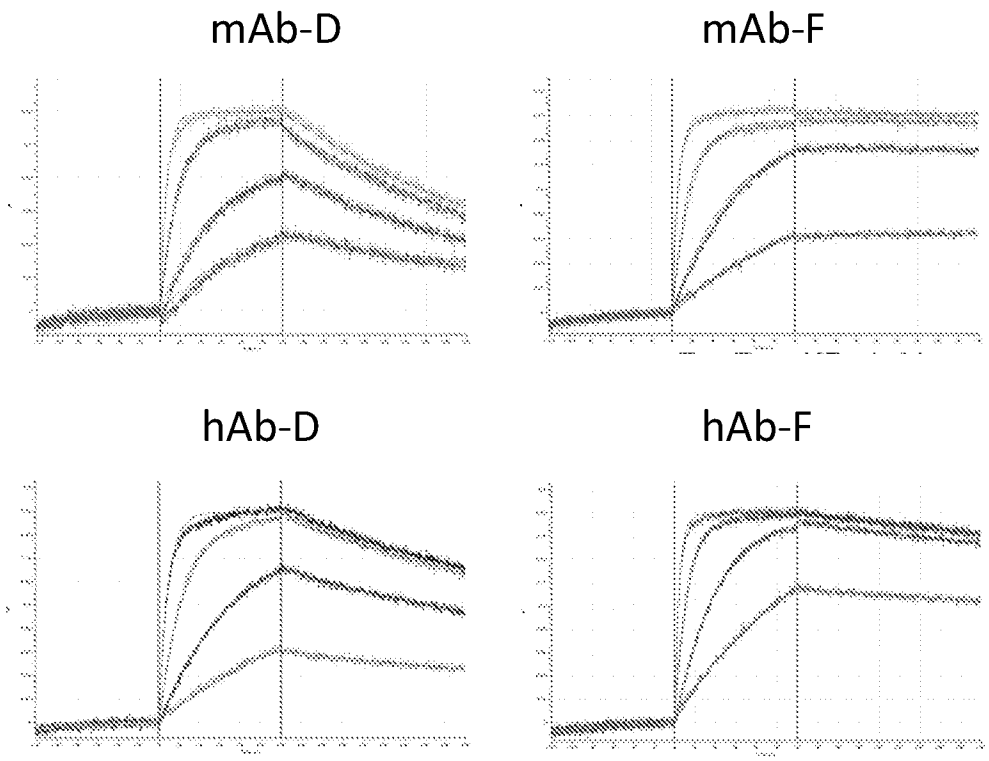
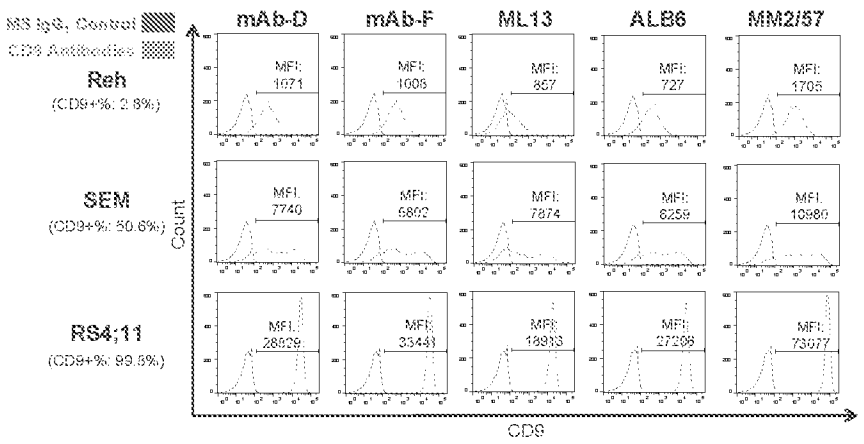
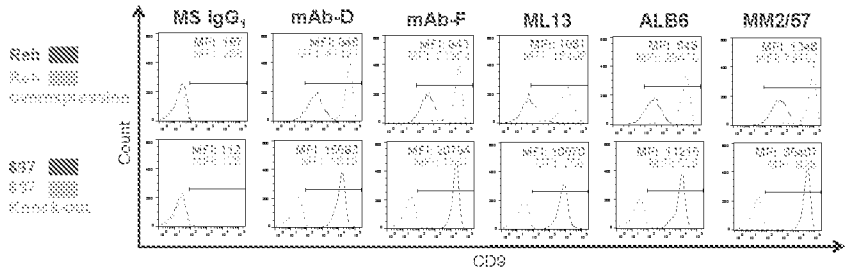


Figure 6

A


MS IgG1 Control
CD9 Antibodies
mAb-D
mAb-F
ML13
ALB6
MM2/57
Reh
(CD9+%: 2.8%)
SEM
(CD9+%: 50.6%)
RS4;11
(CD9+%: 99.5%)
MFI: 1071
MFI: 1005
MFI: 857
MFI: 727
MFI: 1705
MFI: 7740
MFI: 6802
MFI: 7874
MFI: 8259
MFI: 10980
MFI: 28829
MFI: 33443
MFI: 18933
MFI: 27208
MFI: 73977
Count
CD9

B


MS IgG1
mAb-D
mAb-F
ML13
ALB6
MM2/57
Reh
Overexpression
697
Control
Count
CD9

C

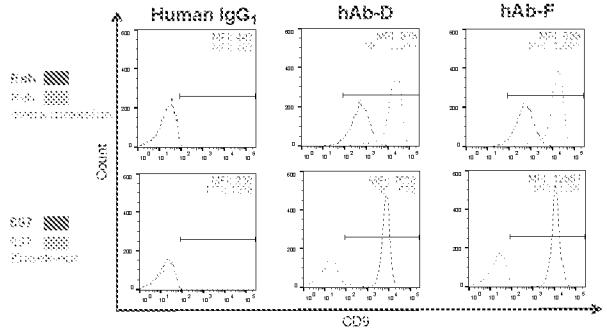

Human IgG1
hAb-D
hAb-F
Count
CD9

Figure 7

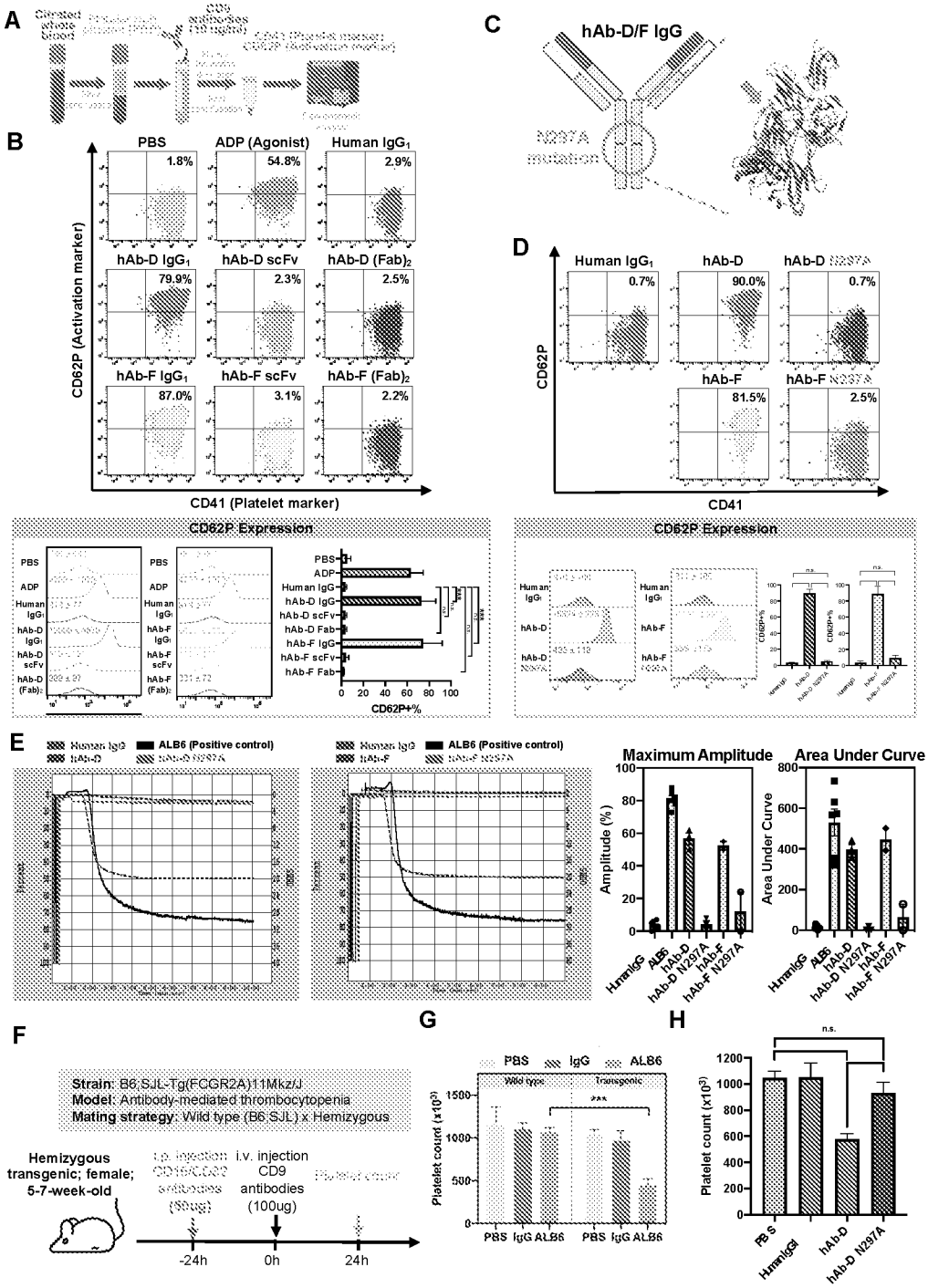

A
B
PBS
ADP (Agonist)
Human IgG1
1.8%
54.8%
2.9%
hAb-D IgG1
hAb-D scFv
hAb-D (Fab)2
79.9%
2.3%
2.5%
hAb-F IgG1
hAb-F scFv
hAb-F (Fab)2
87.0%
3.1%
2.2%
CD62P (Activation marker)
CD41 (Platelet marker)
C
hAb-D/F IgG
N297A mutation
D
Human IgG1
hAb-D
hAb-D N297A
0.7%
90.0%
0.7%
hAb-F
hAb-F N297A
81.5%
2.5%
CD62P
CD41
CD62P Expression
CD62P Expression
PBS
ADP
Human IgG1
hAb-D IgG1
hAb-D scFv
hAb-D Fab
hAb-F IgG1
hAb-F scFv
hAb-F Fab
CD62P+%
E
Human IgG
ALB6 (Positive control)
hAb-D
hAb-D N297A
hAb-F
hAb-F N297A
Maximum Amplitude
Area Under Curve
Amplitude (%)
Area Under Curve
HumanIgG
ALB6
hAb-D
hAb-D N297A
hAb-F
hAb-F N297A
F
Strain: B6.SJL-Tg(FCGR2A)11Mkz/J
Model: Antibody-mediated thrombocytopenia
Mating strategy: Wild type (B6.SJL) x Hemizygous
Hemizygous transgenic; female; 5-7-week-old
i.p. injection CD16/CD32 antibodies (50ug)
i.v. injection CD9 antibodies (100ug)
Platelet count
-24h
0h
24h
G
PBS
IgG
ALB6
Wild type
Transgenic
***
Platelet count (x10^3)
PBS
IgG
ALB6
H
n.s.
Platelet count (x10^3)
PBS
HumanIgG
hAb-D
hAb-D N297A

Figure 8

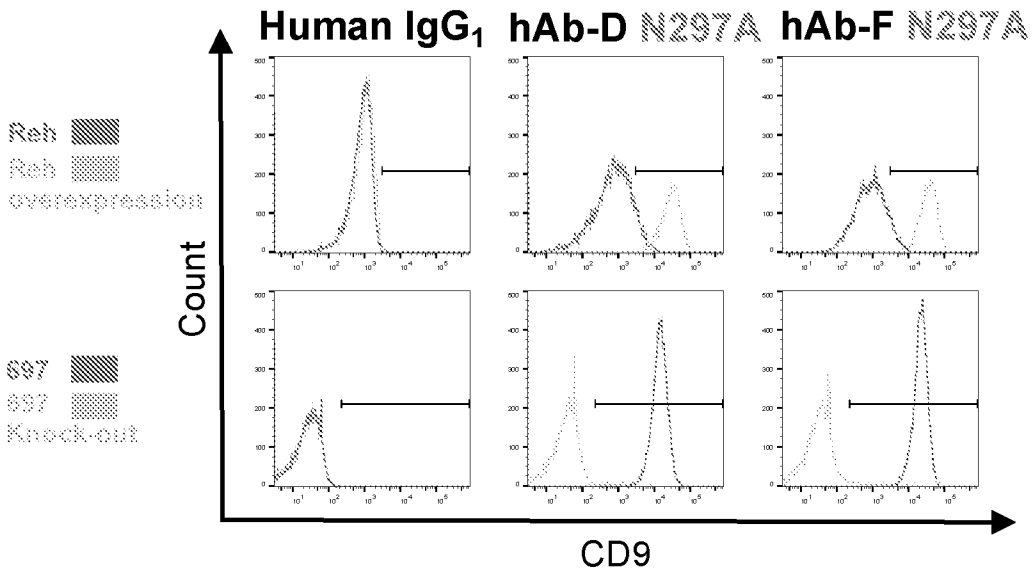

Human IgG1
hAb-D N297A
hAb-F N297A
Reh
Reh overexpression
697
697 Knock-out
Count
CD9

Figure 9

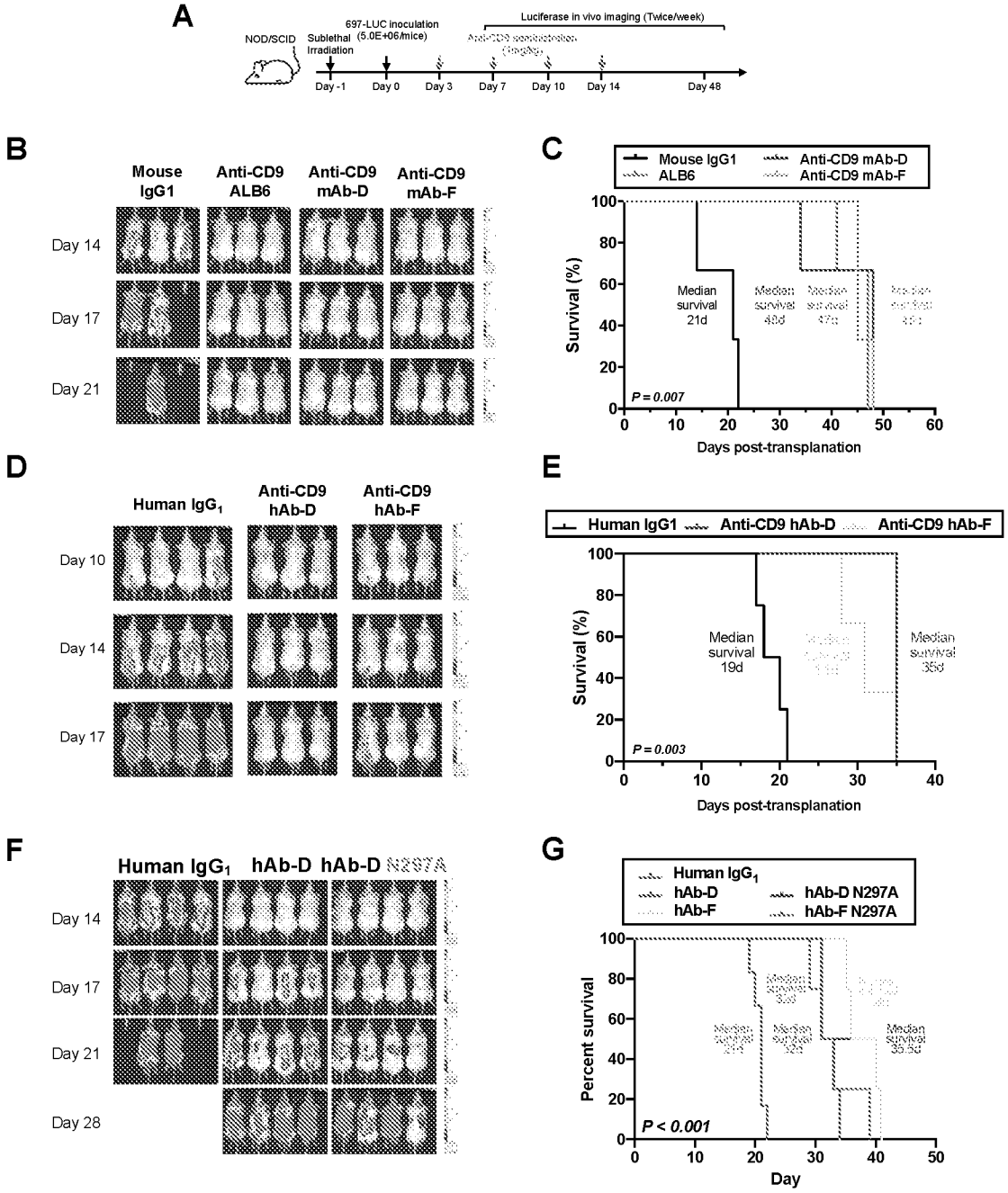

A
NOD/SCID
Sublethal Irradiation
697-LUC inoculation (5.0E+06/mice)
Luciferase in vivo imaging (Twice/week)
Day -1
Day 0
Day 3
Day 7
Day 10
Day 14
Day 48
B
Mouse IgG1
Anti-CD9 ALB6
Anti-CD9 mAb-D
Anti-CD9 mAb-F
Day 14
Day 17
Day 21
C
Mouse IgG1
ALB6
Anti-CD9 mAb-D
Anti-CD9 mAb-F
Survival (%)
Median survival 21d
P = 0.007
Days post-transplanation
D
Human IgG1
Anti-CD9 hAb-D
Anti-CD9 hAb-F
Day 10
Day 14
Day 17
E
Human IgG1
Anti-CD9 hAb-D
Anti-CD9 hAb-F
Survival (%)
Median survival 19d
Median survival 35d
P = 0.003
Days post-transplanation
F
Human IgG1
hAb-D
hAb-D N297A
Day 14
Day 17
Day 21
Day 28
G
Human IgG1
hAb-D
hAb-F
hAb-D N297A
hAb-F N297A
Percent survival
Median survival 35.5d
P < 0.001
Day

Figure 10

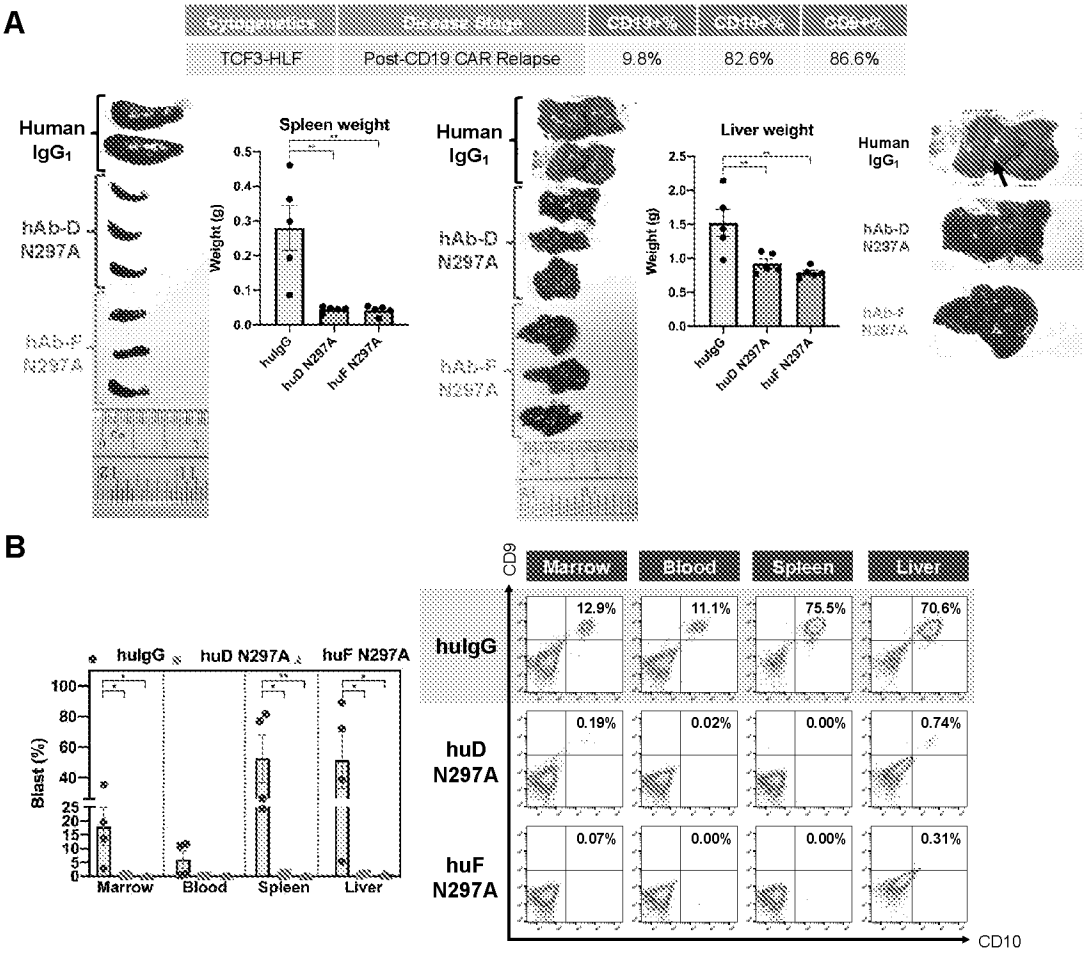
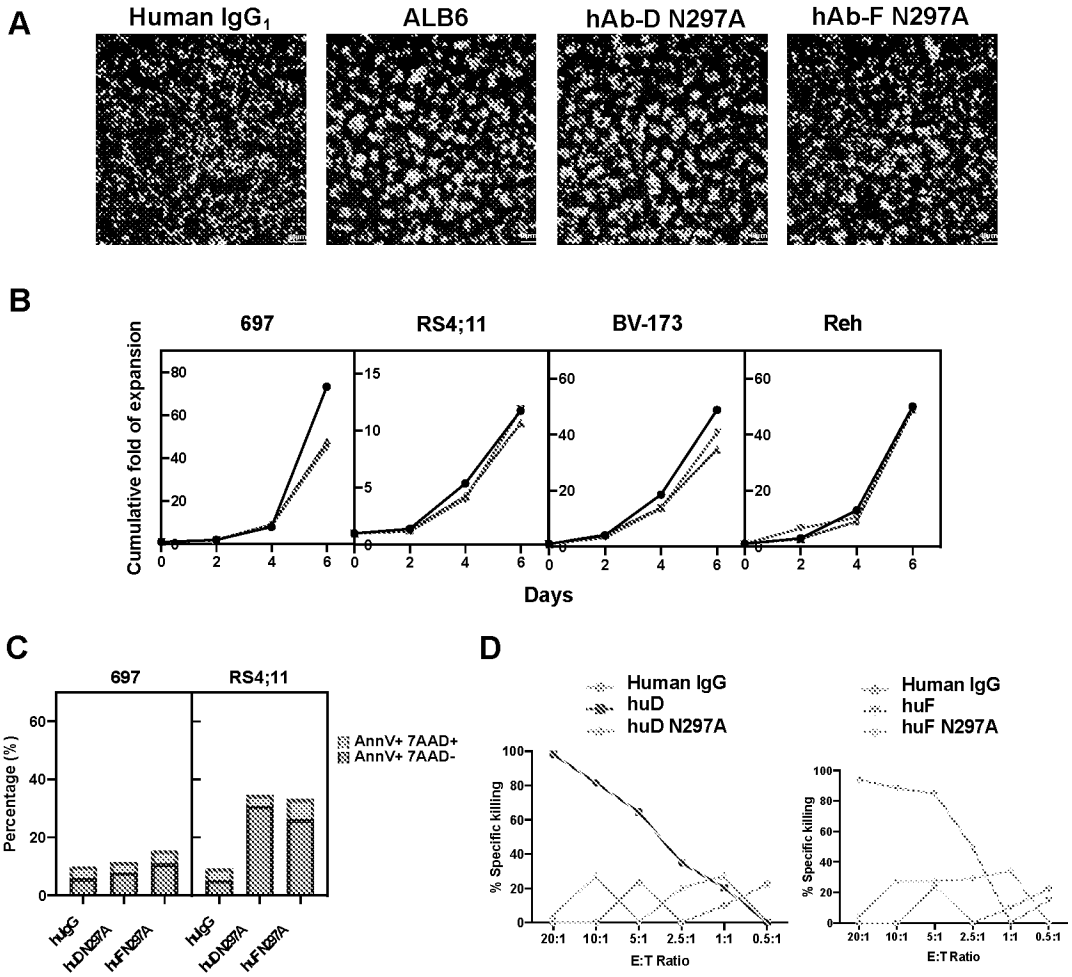

A
Cytogenetics
Sample Type
CD19 %
CD10 %
TCF3-HLF
Post-CD19 CAR Relapse
9.8%
82.6%
86.6%
Human IgG1
hAb-D N297A
hAb-F N297A
Spleen weight
Weight (g)
huIgG
huD N297A
huF N297A
Liver weight
B
huIgG
huD N297A
huF N297A
Blast (%)
Marrow
Blood
Spleen
Liver
CD9
CD10
12.9%
11.1%
75.5%
70.6%
0.19%
0.02%
0.00%
0.74%
0.07%
0.00%
0.00%
0.31%

Figure 11


A
Human IgG1
ALB6
hAb-D N297A
hAb-F N297A
B
697
RS4;11
BV-173
Reh
Cumulative fold of expansion
Days
C
697
RS4;11
Percentage (%)
AnnV+ 7AAD+
AnnV+ 7AAD-
huIgG
huDN297A
huFN297A
D
Human IgG
huD
huD N297A
huF
huF N297A
% Specific killing
E:T Ratio
20:1
10:1
5:1
2.5:1
1:1
0.5:1

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	080015-1445918-041000US
	Application Number	
Title of Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Kam Tong		LEUNG	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Kowloon Bay, Kowloon, Hong Kong SAR	Country of Residence ⓘ	CN

Mailing Address of Inventor:

Address 1	Research Office, 8/F, Tower A		
Address 2	Hong Kong Children's Hospital		
City	Kowloon Bay, Kowloon, Hong Kong SAR	**State/Province**	
Postal Code		**Country** ⓘ	CN

Inventor 2 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Qi		ZHAO	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Taipa, Macau	Country of Residence ⓘ	CN

Mailing Address of Inventor:

Address 1	E12-3022, Faculty of Health Sciences		
Address 2	The University of Macau		
City	Taipa, Macau	**State/Province**	
Postal Code		**Country** ⓘ	CN

Inventor 3 Remove

Legal Name

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	080015-1445918-041000US
	Application Number	
Title of Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE	

Prefix	Given Name	Middle Name	Family Name	Suffix
	Chi Kong		LI	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Kowloon Bay, Kowloon, Hong Kong SAR	Country of Residence	CN

Mailing Address of Inventor:

Address 1	Doctor's Office, 9/F, Tower B		
Address 2	Hong Kong Children's Hospital		
City	Kowloon Bay, Kowloon, Hong Kong SAR	State/Province	
Postal Code		Country	CN

Inventor 4 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Yuen Yee		CHAN	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Shatin, New Territories, Hong Kong SAR	Country of Residence	CN

Mailing Address of Inventor:

Address 1	Rm 84041, 6/F, Lui Che Woo Clinical Sciences Bldg		
Address 2	Prince Wales Hospital		
City	Shatin, New Territories, Hong Kong SAR	State/Province	
Postal Code		Country	CN

Inventor 5 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Wing Hei		NG	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Kowloon Bay, Kowloon, Hong Kong SAR	Country of Residence	CN

Mailing Address of Inventor:

Address 1	Lab D, 8/F, Tower A, Hong Kong Children's Hospital		
Address 2			
City	Kowloon Bay, Kowloon, Hong Kong SAR	State/Province	
Postal Code		Country	CN

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	080015-1445918-041000US
	Application Number	
Title of Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE	

Inventor 6 Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Shengyu		FU	

Residence Information (Select One) ○ US Residency ◉ Non US Residency ○ Active US Military Service

City	Taipa, Macau	Country of Residence	CN

Mailing Address of Inventor:

Address 1	E12-2069, Faculty of Health Sciences		
Address 2	The University of Macau		
City	Taipa, Macau	**State/Province**	
Postal Code		**Country**	CN

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	20350		
Email Address	ipefiling@kilpatricktownsend.com	Add Email	Remove Email

Application Information:

Title of the Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE		
Attorney Docket Number	080015-1445918-041000US	**Small Entity Status Claimed**	☒
Application Type	Provisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	11	**Suggested Figure for Publication (if any)**	

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	080015-1445918-041000US
	Application Number	
Title of Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☐ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	◉ Customer Number	○ US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	20350		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status			Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	080015-1445918-041000US
	Application Number	
Title of Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE	

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	080015-1445918-041000US
	Application Number	
Title of Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	080015-1445918-041000US
	Application Number	
Title of Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	The Chinese University of Hong Kong

Mailing Address Information For Applicant:

Address 1	Office of Research and Knowledge Transfer Services		
Address 2	Room 301, Pi Ch'iu Building		
City	Shatin, New Territories, Hong Kong S	**State/Province**	
Country	CN	Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	080015-1445918-041000US
	Application Number	
Title of Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE	

Applicant 2 Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

◉ Assignee	○ Legal Representative under 35 U.S.C. 117	○ Joint Inventor
○ Person to whom the inventor is obligated to assign.	○ Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	The University of Macau		
Mailing Address Information For Applicant:			
Address 1	Avenida da Universidade		
Address 2			
City	Taipa, Macau	**State/Province**	
Country	CN	Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

PTO/AIA/14 (02-18)
Approved for use through 11/30/2020. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Application Data Sheet 37 CFR 1.76	Attorney Docket Number	080015-1445918-041000US
	Application Number	
Title of Invention	ENGINEERED CD9 ANTIBODIES AND THEIR USE	

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Remove

Signature:

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Chuan Gao/			Date (YYYY-MM-DD)	2024-08-16
First Name	Chuan	Last Name	Gao	Registration Number	54,111

Additional Signature may be generated within this form by selecting the Add button. Add

This collection of information is required by 37 CFR 1.76. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1 The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.

2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.

3 A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.

4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).

5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent CooperationTreaty.

6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).

7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.

9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

DocCode - SCORE

Placeholder Sheet for Supplemental Content

Application Number: 63684204 Document Date: 08/16/2024

The presence of this form indicates that the following document type was received in electronic format on the date identified above. This document is stored separately from the typical case contents which are stored in image format.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded and no paper documents or physical media exist.

- Drawing

At the time of document entry (noted above):

- USPTO employees may access this supplemental content via Docket Application Viewer (DAV) or Supplemental Content Viewer (SCV).
- External customers may access this supplemental content via Patent Center.

Form Revision Date: March 1, 2024