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(54) Title: ANTI-DLL3 ANTIBODIES AND USES THEREOF

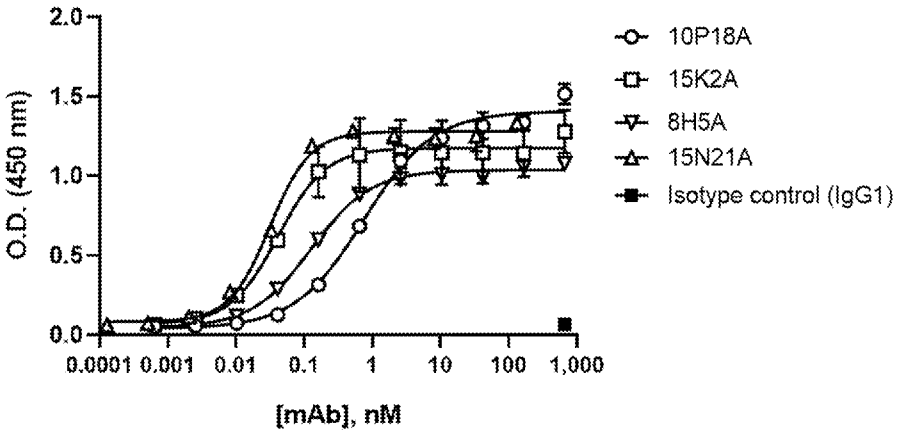

FIG. 1A

(57) Abstract: Anti-DLL3 antibodies and antigen-binding fragments thereof, anti-CD47 antibodies and antigen-binding fragments thereof, and anti-CD47/DLL3 bispecific antibodies and antigen-binding fragments thereof are described. Also described are nucleic acids encoding the antibodies, compositions comprising the antibodies, and methods of producing the antibodies and using the antibodies for treating or preventing diseases, such as cancer and/or associated complications.

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ANTI-DLL3 ANTIBODIES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This invention claims priority to U.S. Provisional Application 62/787,815, filed on January 3, 2019; U.S. Provisional Application No. 62/754,207, filed on November 1, 2018; and U.S. Provisional Application No. 62/668,427, filed on May 8, 2018. Each disclosure is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention relates to monoclonal anti-DLL3 antibodies, anti-CD47 antibodies, and anti-CD47/DLL3 bispecific antibodies, nucleic acids and expression vectors encoding the antibodies, recombinant cells containing the vectors, and compositions comprising the antibodies. Methods of making the antibodies, and methods of using the antibodies to treat diseases including cancer and/or associated complications are also provided.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0003] This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "689204.17WO Sequence Listing" and a creation date of April 29, 2019 and having a size of 70 kb. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

[0004] Delta like canonical Notch ligand 3 (DLL3), also known as delta like 3 or delta like protein 3, is required for somite segmentation during early development (Dunwoodie et al., Development 129:1795-806 (2002)). Unlike the mammalian Notch family ligands DLL1, DLL4, JAG1, and JAG2 which all activate Notch receptor signaling in trans (Ntziachristos et al., Cancer Cell 25(3):318-34 (2014)), DLL3 is predominantly localized in the Golgi apparatus and is unable to activate Notch signaling (Chapman et al., Hum Mol Genet 20(5):905-16 (2011) and Geffers et al., J Cell Biol 178(3):465-76 (2007)).

During normal development, DLL3 inhibits both cis- and trans-acting Notch pathway activation by interacting with Notch and DLL1 (Chapman et al., Hum Mol Genet 20(5):905-16(2011)). DLL3 is normally either absent or present at very low levels in adult normal tissues except brain, but is overexpressed in lung cancer, testicular cancer, glioma and melanoma samples (Uhlen et al., Science 357(6352): eaan2507 (2017)). Furthermore, DLL3 is detectable on the surface of small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC) tumor cells (Saunders et al., Sci Transl Med 7(302):302ra136 (2015) and Sharma et al., Cancer Res 77(14):3931-41 (2017)), making it a potential target of monoclonal antibodies for cancer therapy. Therefore, an anti-DLL3 monoclonal antibody could be used to specifically target DLL3-expressing tumor cells and serve as a potential anti-cancer therapeutic.

BRIEF SUMMARY OF THE INVENTION

[0005] In one general aspect, the invention relates to isolated anti-DLL3 monoclonal antibodies or antigen-binding fragments thereof that bind DLL3.

[0006] Provided are isolated anti-DLL3 monoclonal antibodies or antigen-binding fragments thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 25, 26, 27, 61, 62 and 63, respectively;

(2) SEQ ID NOs: 28, 29, 30, 64, 65 and 66, respectively;

(3) SEQ ID NOs: 31, 32, 33, 67, 68 and 69, respectively;

(4) SEQ ID NOs: 34, 35, 36, 70, 71 and 72, respectively;

(5) SEQ ID NOs: 37, 38, 39, 73, 74 and 75, respectively;

(6) SEQ ID NOs: 40, 41, 42, 76, 77 and 78, respectively;

(7) SEQ ID NOs: 43, 44, 45, 79, 80 and 81, respectively;

(8) SEQ ID NOs: 46, 47, 48, 82, 83 and 84, respectively;

(9) SEQ ID NOs: 49, 50, 51, 85, 86 and 87, respectively;

(10) SEQ ID NOs: 52, 53, 54, 88, 89 and 90, respectively;

(11) SEQ ID NOs: 55, 56, 57, 91, 92 and 93, respectively; or

(12) SEQ ID NOs: 58, 59, 60, 94, 95 and 96, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds DLL3, preferably human DLL3.

[0007] Provided are isolated anti-DLL3 monoclonal antibodies or antigen-binding fragments thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs: 97, 98, 99, 133, 134 and 135, respectively;

(2) SEQ ID NOs: 100, 101, 102, 136, 137 and 138, respectively;

(3) SEQ ID NOs: 103, 104, 105, 139, 140 and 141, respectively;

(4) SEQ ID NOs: 106, 107, 108, 142, 143 and 144, respectively;

(5) SEQ ID NOs: 109, 110, 111, 145, 146 and 147, respectively;

(6) SEQ ID NOs: 112, 113, 114, 148, 149 and 150, respectively;

(7) SEQ ID NOs: 115, 116, 117, 151, 152 and 153, respectively;

(8) SEQ ID NOs: 118, 119, 120, 154, 155 and 156, respectively;

(9) SEQ ID NOs: 121, 122, 123, 157, 158 and 159, respectively;

(10) SEQ ID NOs: 124, 125, 126, 160, 161 and 162, respectively;

(11) SEQ ID NOs: 127, 128, 129, 163, 164 and 165, respectively; or

(12) SEQ ID NOs: 130, 131, 132, 166, 167 and 168, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds DLL3, preferably human DLL3.

[0008] In certain embodiments, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 23, or a light chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24.

[0009] In certain embodiments, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

(b) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;

(c) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;

(d) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

(e) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

(f) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;

(g) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

(h) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

(i) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;

(j) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;

(k) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22; or

(l) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24.

[0010] In certain embodiments, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof is chimeric.

[0011] In certain embodiments, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof is human or humanized. In certain embodiments, the humanized monoclonal antibody or antigen-binding fragment thereof comprises:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;
b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172; or
c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173.

[0012] In certain embodiments, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof is capable of inducing effector-mediated tumor cell lysis through antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent phagocytosis (ADPC) and complement-dependent cytotoxicity (CDC), and/or mediating the recruitment of conjugated drugs, and/or forming a bispecific antibody with another mAb or antigen-binding fragment with cancer-killing effect.

[0013] Also provided are isolated anti-CD47 monoclonal antibodies or antigen-binding fragments thereof comprising the humanized heavy chain variable region of an anti-CD47 monoclonal antibody and the humanized light chain variable region of an anti-DLL3 monoclonal antibody, wherein the anti-CD47 monoclonal antibody or antigen-binding fragment thereof comprises:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;

b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;
c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173;
d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;
e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;
f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173;
g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;
h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;
i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173; or
j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:174.

[0014] In certain embodiments, the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to signal regulatory protein alpha (SIRPα).

[0015] In certain embodiments, the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells.

[0016] Provided are isolated humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 178, 179 and 180; the second antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 181, 182 and 183; and the first antigen-binding domain and the second antigen-binding domain each comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NOs: 184, 185 and 186.

[0017] Provided are isolated humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 187, 188 and 189; the second antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 190, 191 and 192; and the first antigen-binding domain and the second antigen-binding domain each comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NOs: 193, 194 and 195.

[0018] In certain embodiments, the isolated humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof comprise a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-

binding domain comprises a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:176, and a light chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:172; and wherein the second antigen-binding domain comprises a heavy chain variable region having a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:170, and a light chain variable region having the a polypeptide sequence at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:172.

[0019] In certain embodiments, the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to signal regulatory protein alpha (SIRPα) on cancer cells that express both DLL3 and CD47.

[0020] In certain embodiments, the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells that express both DLL3 and CD47.

[0021] In certain embodiments, the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof is capable of binding cancer cells that express both DLL3 and CD47 with minimal to undetectable binding to human red blood cells (RBCs).

[0022] Also provided are isolated nucleic acids encoding the anti-DLL3 monoclonal antibodies or antigen-binding fragments thereof, the anti-CD47 monoclonal antibodies or antigen-binding fragments thereof, or the humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof of the invention disclosed herein.

[0023] Also provided are vectors comprising the isolated nucleic acids encoding the anti-DLL3 monoclonal antibodies or antigen-binding fragments thereof, the anti-CD47 monoclonal antibodies or antigen-binding fragments thereof, or the humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof of the invention.

[0024] Also provided are host cells comprising the vectors comprising the isolated nucleic acids encoding the anti-DLL3 monoclonal antibodies or antigen-binding fragments thereof, the anti-CD47 monoclonal antibodies or antigen-binding fragments

thereof, or the humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof of the invention.

[0025] In certain embodiments, provided is a pharmaceutical composition comprising the isolated anti-DLL3 monoclonal antibodies or antigen-binding fragments thereof, the isolated anti-CD47 monoclonal antibodies or antigen-binding fragments thereof, or the isolated humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof of the invention and a pharmaceutically acceptable carrier.

[0026] Also provided are methods of targeting DLL3 on a cancer cell surface in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions comprising the isolated anti-DLL3 monoclonal antibodies or antigen-binding fragments thereof of the invention.

[0027] Also provided are methods of blocking binding of CD47 to signal regulatory protein alpha (SIRPα) in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions comprising the isolated anti-CD47 monoclonal antibodies or antigen-binding fragments thereof of the invention.

[0028] Also provided are methods of inducing macrophage-mediated phagocytosis of cancer cells in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions comprising the isolated anti-CD47 monoclonal antibodies or antigen-binding fragments thereof of the invention.

[0029] Also provided are methods of targeting DLL3 and CD47 that are both expressed on a cancer cell surface in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions comprising the isolated humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof of the invention.

[0030] Also provided are methods of blocking binding of CD47 to SIRPα on cancer cells that express both DLL3 and CD47 in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions comprising the isolated humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof of the invention.

[0031] Also provided are methods of inducing macrophage-mediated phagocytosis of cancer cells that express both DLL3 and CD47 in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions comprising the isolated

humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof of the invention.

[0032] Also provided are methods of binding cancer cells that express both DLL3 and CD47 by a humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment with minimal to undetectable binding to human red blood cells (RBCs) in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions comprising the isolated humanized anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof of the invention.

[0033] Also provided are methods of treating cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical compositions of the invention. The cancer can be any liquid or solid cancer, for example, it can be selected from, but not limited to, a lung cancer, such as small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[0034] Also provided are methods of producing the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of the invention, comprising culturing a cell comprising a nucleic acid encoding the antibody or antigen-binding fragment thereof under conditions to produce the antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof from the cell or culture.

[0035] Also provided are methods of producing a pharmaceutical composition comprising the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding

fragment thereof of the invention, comprising combining the antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

[0036] Also provided are methods of determining a level of DLL3 in a subject. The methods comprise (a) obtaining a sample from the subject; (b) contacting the sample with an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of the invention; and (c) determining the level of DLL3 in the subject. In certain embodiments, the sample is a tissue sample. The tissue sample can, for example, be a cancer tissue sample. In certain embodiments, the sample is a blood sample.

BRIEF DESCRIPTION OF THE DRAWINGS

[0037] The foregoing summary, as well as the following detailed description of preferred embodiments of the present application, will be better understood when read in conjunction with the appended drawings. It should be understood, however, that the application is not limited to the precise embodiments shown in the drawings.

[0038] FIGs. 1A-1C show the binding of purified anti-DLL3 chimeric mAbs to coated recombinant DLL3 protein by ELISA.

[0039] FIGs. 2A-2C show the binding of chimeric anti-DLL3 mAbs to HEK293-huDLL3 cells by FACS. Three different mAb concentrations were used in the assay (666.67 nM (FIG. 2A); 333.33 nM (FIG. 2B); and 66.67 nM (FIG. 2C)).

[0040] FIG. 3 shows the binding of humanized anti-DLL3 mAb H1L2 to SHP-77 cells by FACS.

[0041] FIGs. 4A-4B show the bridging ELISA data of the bispecific antibodies BA1 and BA1(C) (FIG. 4A) and BA4(C) (FIG. 4B).

[0042] FIG. 5 shows the binding of the bispecific antibodies BA1, BA1(C) and BA4(C) to immobilized CD47 by ELISA assay.

[0043] FIGs. 6A-6B show the binding of the bispecific antibodies BA1 and BA1(C) (FIG. 6A) and BA4(C) (FIG. 6B) to immobilized DLL3 by ELISA assay.

[0044] FIG. 7 shows the binding of the bispecific antibodies BA1, BA1(C) and BA4(C) to simultaneously immobilized CD47 and DLL3 in a 1:1 concentration ratio by ELISA assay.

[0045] FIGs. 8A-8B show the activity of the bispecific antibodies BA1, BA1(C) and BA4(C) in blocking the CD47/SIRPα interaction in an ELISA assay. Only CD47 was immobilized in the assay in FIG. 8A and both CD47 and DLL3 were immobilized in a 2:1 concentration ratio in the assay in FIG. 8B.

[0046] FIG. 9 shows the binding of the bispecific antibodies BA1, BA1(C) and BA4(C) to Raji cells.

[0047] FIG. 10 shows the inhibitory effect of the anti-CD47 or anti-DLL3 F(ab')2 on the binding of the bispecific antibody BA1(C) to SHP-77 cells.

[0048] FIGs. 11A-11C show the binding of the bispecific antibodies to human red blood cells (RBCs) at different mAb concentrations (1,600 nM (FIG. 11A), 533 nM (FIG. 11B), and 178 nM (FIG. 11C)).

DETAILED DESCRIPTION OF THE INVENTION

[0049] Various publications, articles and patents are cited or described in the background and throughout the specification; each of these references is herein incorporated by reference in its entirety. Discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is for the purpose of providing context for the invention. Such discussion is not an admission that any or all of these matters form part of the prior art with respect to any inventions disclosed or claimed.

[0050] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention pertains. Otherwise, certain terms used herein have the meanings as set forth in the specification.

[0051] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise.

[0052] Unless otherwise stated, any numerical values, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term "about." Thus, a numerical value typically includes ± 10% of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1

mg/mL. Likewise, a concentration range of 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v). As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0053] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

[0054] As used herein, the terms "comprises," "comprising," "includes," "including," "has," "having," "contains" or "containing," or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers and are intended to be non-exclusive or open-ended. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those elements but can include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, "or" refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present).

[0055] As used herein, the conjunctive term "and/or" between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by "and/or," a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or" as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term "and/or."

[0056] As used herein, the term "consists of," or variations such as "consist of" or "consisting of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, but that no additional integer or group of integers can be added to the specified method, structure, or composition.

[0057] As used herein, the term "consists essentially of," or variations such as "consist essentially of" or "consisting essentially of," as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, and the optional inclusion of any recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure or composition. See M.P.E.P. § 2111.03.

[0058] As used herein, "subject" means any animal, preferably a mammal, most preferably a human. The term "mammal" as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys, humans, etc., more preferably a human.

[0059] The words "right," "left," "lower," and "upper" designate directions in the drawings to which reference is made.

[0060] It should also be understood that the terms "about," "approximately," "generally," "substantially" and like terms, used herein when referring to a dimension or characteristic of a component of the preferred invention, indicate that the described dimension/characteristic is not a strict boundary or parameter and does not exclude minor variations therefrom that are functionally the same or similar, as would be understood by one having ordinary skill in the art. At a minimum, such references that include a numerical parameter would include variations that, using mathematical and industrial principles accepted in the art (e.g., rounding, measurement or other systematic errors, manufacturing tolerances, etc.), would not vary the least significant digit.

[0061] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences (e.g., anti-DLL3 antibodies, anti-CD47 antibodies, anti-CD47/DLL3 bi-specific antibodies, DLL3 polypeptides, and polynucleotides that encode them), refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when

compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

[0062] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0063] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally, Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1995 Supplement) (Ausubel)).

[0064] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) J. Mol. Biol. 215: 403-410 and Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al, supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased.

[0065] Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)).

[0066] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0067] A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

Antibodies

[0068] The invention generally relates to isolated anti-DLL3 antibodies, anti-CD47 antibodies, anti-CD47/DLL3 bispecific antibodies, nucleic acids and expression vectors encoding the antibodies, recombinant cells containing the vectors, and compositions comprising the antibodies. Methods of making the antibodies, and methods of using the antibodies to treat diseases, including cancer, are also provided. The antibodies of the invention possess one or more desirable functional properties, including but not limited to high-affinity binding to DLL3 and/or CD47, high specificity to DLL3 and/or CD47, the ability to induce effector-mediated tumor cell lysis, the ability to stimulate complement-dependent cytotoxicity (CDC), antibody-dependent phagocytosis (ADPC), and/or antibody-dependent cellular-mediated cytotoxicity (ADCC) against cells expressing DLL3 and/or CD47, the ability to mediate the recruitment of conjugated drugs, the ability to form a bispecific antibody with another mAb or antigen-binding fragment with cancer-killing effect, and the ability to inhibit tumor growth in subjects and animal models when administered alone or in combination with other anti-cancer therapies.

[0069] In a general aspect, the invention relates to isolated anti-DLL3 monoclonal antibodies or antigen-binding fragments thereof that bind DLL3.

[0070] As used herein, the term "antibody" is used in a broad sense and includes immunoglobulin or antibody molecules including human, humanized, composite and chimeric antibodies and antibody fragments that are monoclonal or polyclonal. In general, antibodies are proteins or peptide chains that exhibit binding specificity to a specific antigen. Antibody structures are well known. Immunoglobulins can be assigned to five major classes (i.e., IgA, IgD, IgE, IgG and IgM), depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Accordingly, the antibodies of the invention can be of any of the five major classes or corresponding sub-classes. Preferably, the antibodies of the invention are IgG1, IgG2, IgG3 or IgG4. Antibody light chains of vertebrate species can be assigned to one of two clearly distinct types, namely kappa and lambda, based on the amino acid sequences of their constant domains. Accordingly, the antibodies of the invention can contain a kappa or lambda light chain constant domain. According to particular embodiments, the antibodies of the invention

include heavy and/or light chain constant regions from rat or human antibodies. In addition to the heavy and light constant domains, antibodies contain an antigen-binding region that is made up of a light chain variable region and a heavy chain variable region, each of which contains three domains (i.e., complementarity determining regions 1-3; CDR1, CDR2, and CDR3). The light chain variable region domains are alternatively referred to as LCDR1, LCDR2, and LCDR3, and the heavy chain variable region domains are alternatively referred to as HCDR1, HCDR2, and HCDR3.

[0071] As used herein, the term an "isolated antibody" refers to an antibody which is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds to DLL3 is substantially free of antibodies that do not bind to DLL3, an isolated antibody that specifically binds to CD47 is substantially free of antibodies that do not bind to CD47, a bispecific antibody that specifically binds to CD47 and DLL3 is substantially free of antibodies that do not bind to CD47 and DLL3). In addition, an isolated antibody is substantially free of other cellular material and/or chemicals.

[0072] As used herein, the term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. The monoclonal antibodies of the invention can be made by the hybridoma method, phage display technology, single lymphocyte gene cloning technology, or by recombinant DNA methods. For example, the monoclonal antibodies can be produced by a hybridoma which includes a B cell obtained from a transgenic nonhuman animal, such as a transgenic mouse or rat, having a genome comprising a human heavy chain transgene and a light chain transgene.

[0073] As used herein, the term "antigen-binding fragment" refers to an antibody fragment such as, for example, a diabody, a Fab, a Fab', a F(ab')2, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a $(dsFv)_2$, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), a single domain antibody (sdab) an scFv dimer (bivalent diabody), a multispecific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, or any

other antibody fragment that binds to an antigen but does not comprise a complete antibody structure. An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment binds. According to particular embodiments, the antigen-binding fragment comprises a light chain variable region, a light chain constant region, and an Fd segment of the heavy chain. According to other particular embodiments, the antigen-binding fragment comprises Fab and F(ab').

[0074] As used herein, the term "single-chain antibody" refers to a conventional single-chain antibody in the field, which comprises a heavy chain variable region and a light chain variable region connected by a short peptide of about 15 to about 20 amino acids. As used herein, the term "single domain antibody" refers to a conventional single domain antibody in the field, which comprises a heavy chain variable region and a heavy chain constant region or which comprises only a heavy chain variable region.

[0075] As used herein, the term "human antibody" refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any technique known in the art. This definition of a human antibody includes intact or full-length antibodies, fragments thereof, and/or antibodies comprising at least one human heavy and/or light chain polypeptide.

[0076] As used herein, the term "humanized antibody" refers to a non-human antibody that is modified to increase the sequence homology to that of a human antibody, such that the antigen-binding properties of the antibody are retained, but its antigenicity in the human body is reduced.

[0077] As used herein, the term "chimeric antibody" refers to an antibody wherein the amino acid sequence of the immunoglobulin molecule is derived from two or more species. The variable region of both the light and heavy chains often corresponds to the variable region of an antibody derived from one species of mammal (e.g., mouse, rat, rabbit, etc.) having the desired specificity, affinity, and capability, while the constant regions correspond to the sequences of an antibody derived from another species of mammal (e.g., human) to avoid eliciting an immune response in that species.

[0078] As used herein, the term "multispecific antibody" refers to an antibody that comprises a plurality of immunoglobulin variable domain sequences, wherein a first immunoglobulin variable domain sequence of the plurality has binding specificity for a

first epitope and a second immunoglobulin variable domain sequence of the plurality has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, *e.g.,* the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap or substantially overlap. In an embodiment, the first and second epitopes do not overlap or do not substantially overlap. In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, a multispecific antibody comprises a third, fourth, or fifth immunoglobulin variable domain. In an embodiment, a multispecific antibody is a bispecific antibody molecule, a trispecific antibody molecule, or a tetraspecific antibody molecule.

[0079] As used herein, the term "bispecifc antibody" refers to a multispecific antibody that binds no more than two epitopes or two antigens. A bispecific antibody is characterized by a first immunoglobulin variable domain sequence which has binding specificity for a first epitope and a second immunoglobulin variable domain sequence that has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment, the first and second epitopes overlap or substantially overlap. In an embodiment, the first and second epitopes are on different antigens, *e.g.*, the different proteins (or different subunits of a multimeric protein). In an embodiment, a bispecific antibody comprises a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a first epitope and a heavy chain variable domain sequence and a light chain variable domain sequence which have binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment, a bispecific antibody comprises a scFv, or fragment thereof, having binding specificity for a first epitope, and a scFv, or fragment thereof, having binding specificity for a second epitope. In an embodiment, the first epitope is located on DLL3 and the second epitope is located on PD-1, PD-L1, TIM-3, LAG-3, CD73, apelin, CTLA-4, EGFR, HER-2, CD3, CD19, CD20, CD33, CD47, TIP-1, CLDN18.2, FOLR1, and/or other tumor associated immune suppressors or surface antigens.

[0080] As used herein, the term "DLL3" refers to Delta like canonical Notch ligand 3 (DLL3), also known as delta like 3 or delta like protein 3, is required for somite segmentation during early development (Dunwoodie et al., Development 129:1795-806 (2002)). Unlike the mammalian Notch family ligands DLL1, DLL4, JAG1, and JAG2, which all activate Notch receptor signaling in trans (Ntziachristos et al., Cancer Cell 25(3):318-34 (2014)), DLL3 is predominantly localized in the Golgi apparatus and is unable to activate Notch signaling (Chapman et al., Hum Mol Genet 2011;20(5):905-16 and Geffers et al., J Cell Biol 178(3):465-76 (2007)). During normal development, DLL3 inhibits both cis- and trans-acting Notch pathway activation by interacting with Notch and DLL1 (Chapman et al., Hum Mol Genet 20(5):905-16 (2011)). DLL3 is normally either absent or present at very low levels in adult normal tissues except brain, but is overexpressed in lung cancer, testicular cancer, glioma and melanoma samples (Uhlen et al., Science 357(6352):eaan2507 (2017)). Further, DLL3 is detectable on the surface of small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC) tumor cells (Saunders et al., Sci Transl Med 7(302):302ra136 (2015) and Sharma et al., Cancer Res 77(14):3931-3941 (2017)), making it a potential target of monoclonal antibodies for cancer therapy. The term "human DLL3" refers to a DLL3 originated from a human. An exemplary amino acid sequence of a human DLL3 is represented in GenBank Accession No. NP_058637.1 (SEQ ID NO:169).

[0081] As used herein, the term "CD47" refers to a multi-spanning transmembrane receptor belonging to the immunoglobulin superfamily, which has been indicated to be involved in multiple cellular process, including cell migration, adhesion, and T cell function. CD47, also known as integrin-associated protein (IAP), ovarian cancer antigen (OA3), Rh-related antigen, and MER6, was originally identified as a tumor antigen on human ovarian cancer and was subsequently shown to be expressed on multiple human tumor types, including both hematologic and solid tumors. The interaction between CD47 and signal regulatory protein alpha (SIRPα), an inhibitory protein expressed on macrophages, prevents phagocytosis of CD47-expressing cells. CD47 is additionally expressed at low levels on virtually all non-malignant cells. The term "human CD47" refers to a CD47 originated from a human. An exemplary amino acid sequence of a human CD47 is represented in GenBank Accession No. NP_001768.1.

[0082] As used herein, an antibody that "specifically binds to DLL3," "specifically binds to CD47," or an antibody that "specifically binds to CD47 and DLL3" refers to an antibody that binds to a DLL3, preferably a human DLL3; binds to CD47, preferably a human CD47; or DLL3 and CD47, preferably human DLL3 and human CD47, with a KD of 1×10^{-7} M or less, preferably 1×10^{-8} M or less, more preferably 5×10^{-9} M or less, 1×10^{-9} M or less, 5×10^{-10} M or less, or 1×10^{-10} M or less. The term "KD" refers to the dissociation constant, which is obtained from the ratio of Kd to Ka (i.e., Kd/Ka) and is expressed as a molar concentration (M). KD values for antibodies can be determined using methods in the art in view of the present disclosure. For example, the KD of an antibody can be determined by using surface plasmon resonance, such as by using a biosensor system, e.g., a Biacore® system, or by using bio-layer interferometry technology, such as an Octet RED96 system.

[0083] The smaller the value of the KD of an antibody, the higher affinity that the antibody binds to a target antigen.

[0084] According to a particular aspect, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs:25, 26, 27, 61, 62 and 63, respectively;

(2) SEQ ID NOs:28, 29, 30, 64, 65 and 66, respectively;

(3) SEQ ID NOs:31, 32, 33, 67, 68 and 69, respectively;

(4) SEQ ID NOs:34, 35, 36, 70, 71 and 72, respectively;

(5) SEQ ID NOs:37, 38, 39, 73, 74 and 75, respectively;

(6) SEQ ID NOs:40, 41, 42, 76, 77 and 78, respectively;

(7) SEQ ID NOs:43, 44, 45, 79, 80 and 81, respectively;

(8) SEQ ID NOs:46, 47, 48, 82, 83 and 84, respectively;

(9) SEQ ID NOs:49, 50, 51, 85, 86 and 87, respectively;

(10) SEQ ID NOs:52, 53, 54, 88, 89 and 90, respectively;

(11) SEQ ID NOs:55, 56, 57, 91, 92 and 93, respectively; or

(12) SEQ ID NOs:58, 59, 60, 94, 95 and 96, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds DLL3, preferably human DLL3.

[0085] According to a particular aspect, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2, and a LCDR3, having the polypeptide sequences of:

(1) SEQ ID NOs:97, 98, 99, 133, 134 and 135, respectively;
(2) SEQ ID NOs:100, 101, 102, 136, 137 and 138, respectively;
(3) SEQ ID NOs:103, 104, 105, 139, 140 and 141, respectively;
(4) SEQ ID NOs:106, 107, 108, 142, 143 and 144, respectively;
(5) SEQ ID NOs:109, 110, 111, 145, 146 and 147, respectively;
(6) SEQ ID NOs:112, 113, 114, 148, 149 and 150, respectively;
(7) SEQ ID NOs:115, 116, 117, 151, 152 and 153, respectively;
(8) SEQ ID NOs:118, 119, 120, 154, 155 and 156, respectively;
(9) SEQ ID NOs:121, 122, 123, 157, 158 and 159, respectively;
(10) SEQ ID NOs:124, 125, 126, 160, 161 and 162, respectively;
(11) SEQ ID NOs:127, 128, 129, 163, 164 and 165, respectively; or
(12) SEQ ID NOs:130, 131, 132, 166, 167 and 168, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds DLL3, preferably human DLL3.

[0086] According to another particular aspect, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 23, or a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to one of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24. According to one preferred embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of the invention comprises a heavy chain variable region having the polypeptide sequence at least 85%, preferably

90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 23, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24, respectively.

[0087] According to another particular aspect, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of the invention, comprising:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;
b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;
c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;
d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;
e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;
f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;
g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;

j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;

k. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22; or

l. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24.

[0088] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 25, 26, 27, 61, 62 and 63, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:2. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1; and a light chain variable region having the polypeptide sequence of SEQ ID NO:2.

[0089] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 28, 29,

30, 64, 65 and 66, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:3, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:4. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3; and a light chain variable region having the polypeptide sequence of SEQ ID NO:4.

[0090] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 31, 32, 33, 67, 68 and 69, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:5, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:6. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5; and a light chain variable region having the polypeptide sequence of SEQ ID NO:6.

[0091] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 34, 35, 36, 70, 71 and 72, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:7, and a light chain variable region having a polypeptide sequence at least 85%,

preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:8. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7; and a light chain variable region having the polypeptide sequence of SEQ ID NO:8.

[0092] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 37, 38, 39, 73, 74 and 75, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:9, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:10. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9; and a light chain variable region having the polypeptide sequence of SEQ ID NO:10.

[0093] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 40, 41, 42, 76, 77 and 78, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:11, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:12. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11; and a light chain variable region having the polypeptide sequence of SEQ ID NO:12.

[0094] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 43, 44, 45, 79, 80 and 81, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:13, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:14. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13; and a light chain variable region having the polypeptide sequence of SEQ ID NO:14.

[0095] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 46, 47, 48, 82, 83 and 84, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:15, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:16. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15; and a light chain variable region having the polypeptide sequence of SEQ ID NO:16.

[0096] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 49, 50, 51, 85, 86 and 87, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain

variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:17, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:18. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17; and a light chain variable region having the polypeptide sequence of SEQ ID NO:18.

[0097] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 52, 53, 54, 88, 89 and 90, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:19, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:20. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19; and a light chain variable region having the polypeptide sequence of SEQ ID NO:20.

[0098] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 55, 56, 57, 91, 92 and 93, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:21, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:22. Preferably, the isolated anti-DLL3 monoclonal antibody or

antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21; and a light chain variable region having the polypeptide sequence of SEQ ID NO:22.

[0099] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 58, 59, 60, 94, 95 and 96, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:23, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:24. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23; and a light chain variable region having the polypeptide sequence of SEQ ID NO:24.

[00100] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 97, 98, 99, 133, 134 and 135, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:1, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:2. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1; and a light chain variable region having the polypeptide sequence of SEQ ID NO:2.

[00101] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3,

LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 100, 101, 102, 136, 137 and 138, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:3, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:4. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3; and a light chain variable region having the polypeptide sequence of SEQ ID NO:4.

[00102] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 103, 104, 105, 139, 140 and 141, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:5, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:6. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5; and a light chain variable region having the polypeptide sequence of SEQ ID NO:6.

[00103] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 106, 107, 108, 142, 143 and 144, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID

NO:7, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:8. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7; and a light chain variable region having the polypeptide sequence of SEQ ID NO:8.

[00104] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 109, 110, 111, 145, 146 and 147, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:9, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:10. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9; and a light chain variable region having the polypeptide sequence of SEQ ID NO:10.

[00105] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 112, 113, 114, 148, 149 and 150, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:11, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:12. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the

polypeptide sequence of SEQ ID NO:11; and a light chain variable region having the polypeptide sequence of SEQ ID NO:12.

[00106] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 115, 116, 117, 151, 152 and 153, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:13, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:14. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13; and a light chain variable region having the polypeptide sequence of SEQ ID NO:14.

[00107] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 118, 119, 120, 154, 155 and 156, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:15, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:16. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15; and a light chain variable region having the polypeptide sequence of SEQ ID NO:16.

[00108] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 121,

122, 123, 157, 158 and 159, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:17, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:18. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17; and a light chain variable region having the polypeptide sequence of SEQ ID NO:18.

[00109] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 124, 125, 126, 160, 161 and 162, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:19, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:20. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19; and a light chain variable region having the polypeptide sequence of SEQ ID NO:20.

[00110] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 127, 128, 129, 163, 164 and 165, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:21, and a light chain variable region having a polypeptide sequence at least 85%,

preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:22. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21; and a light chain variable region having the polypeptide sequence of SEQ ID NO:22.

[00111] In one embodiment, the invention relates to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3, having the polypeptide sequences of SEQ ID NOs: 130, 131, 132, 166, 167 and 168, respectively. In another embodiment, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:23, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:24. Preferably, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprises a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23; and a light chain variable region having the polypeptide sequence of SEQ ID NO:24.

[00112] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof is chimeric.

[00113] According to another particular aspect, the invention relates to an isolated monoclonal antibody or antigen-binding fragment thereof of the invention, wherein the antibody or antigen-binding fragment thereof is human or humanized.

[00114] According to another particular aspect, the invention relates to an isolated humanized anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, wherein the isolated humanized antibody or antigen-binding fragment thereof comprises:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;

b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172; or

c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173.

[00115] According to another particular aspect, the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof is capable of inducing effector-mediated tumor cell lysis through antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent phagocytosis (ADPC) and complement-dependent cytotoxicity (CDC), and/or mediating the recruitment of conjugated drugs, and/or forming a bispecific antibody with another mAb or antigen-binding fragment with cancer-killing effect.

[00116] According to another particular aspect, the invention relates to an isolated anti-CD47 monoclonal antibody or antigen-binding fragment comprising the humanized heavy chain variable region of an anti-CD47 monoclonal antibody and the humanized light chain variable region of an anti-DLL3 monoclonal antibody, wherein the anti-CD47 monoclonal antibody or antigen-binding fragment thereof comprises:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;

b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;

c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173;

d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;

e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;
f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173;
g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;
h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;
i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173; or
j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:174.

[00117] According to another particular aspect, the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to signal regulatory protein alpha (SIRPα).

[00118] According to another particular aspect, the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells.

[00119] According to another particular aspect, the invention relates to an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 178, 179 and 180; the second antigen-binding domain comprises a heavy

chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 181, 182 and 183; and the first antigen-binding domain and the second antigen-binding domain each comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NOs: 184, 185 and 186.

[00120] According to another particular aspect, the invention relates to an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 187, 188 and 189; the second antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 190, 191 and 192; and the first antigen-binding domain and the second antigen-binding domain each comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NOs: 193, 194 and 195.

[00121] According to another particular aspect, the invention relates to an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-binding domain comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:176, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:172; and wherein the second antigen-binding domain comprises a heavy chain variable region having a polypeptide sequence at least 85%, preferably 90%, more preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:170, and a light chain variable region having a polypeptide sequence at least 85%, preferably 90%, more

preferably 95% or more, such as 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO:172.

[00122] According to another particular aspect, the invention relates to an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to SIRPα on cancer cells that express both DLL3 and CD47.

[00123] According to another particular aspect, the invention relates to an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells that express both DLL3 and CD47.

[00124] According to another particular aspect, the invention relates to an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof is capable of binding cancer cells that express both DLL3 and CD47 with minimal to undetectable binding to human red blood cells (RBCs).

[00125] In another general aspect, the invention relates to an isolated nucleic acid encoding an anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, an anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or a humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of the invention. It will be appreciated by those skilled in the art that the coding sequence of a protein can be changed (e.g., replaced, deleted, inserted, etc.) without changing the amino acid sequence of the protein. Accordingly, it will be understood by those skilled in the art that

nucleic acid sequences encoding antibodies or antigen-binding fragments thereof of the invention can be altered without changing the amino acid sequences of the proteins.

[00126] In another general aspect, the invention relates to a vector comprising an isolated nucleic acid encoding an anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, an anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or a humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of the invention. Any vector known to those skilled in the art in view of the present disclosure can be used, such as a plasmid, a cosmid, a phage vector or a viral vector. In some embodiments, the vector is a recombinant expression vector such as a plasmid. The vector can include any element to establish a conventional function of an expression vector, for example, a promoter, ribosome binding element, terminator, enhancer, selection marker, and origin of replication. The promoter can be a constitutive, inducible or repressible promoter. A number of expression vectors capable of delivering nucleic acids to a cell are known in the art and can be used herein for production of an antibody or antigen-binding fragment thereof in the cell. Conventional cloning techniques or artificial gene synthesis can be used to generate a recombinant expression vector according to embodiments of the invention. Such techniques are well known to those skilled in the art in view of the present disclosure.

[00127] In another general aspect, the invention relates to a host cell comprising an isolated nucleic acid encoding an anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, an anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or a humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of the invention. Any host cell known to those skilled in the art in view of the present disclosure can be used for recombinant expression of antibodies or antigen-binding fragments thereof of the invention. In some embodiments, the host cells are E. coli TG1 or BL21 cells (for expression of, e.g., an scFv or Fab antibody), CHO-DG44 or CHO-K1 cells or HEK293 cells (for expression of, e.g., a full-length IgG antibody). According to particular embodiments, the recombinant expression vector is transformed into host cells by conventional methods such as chemical transfection, heat shock, or electroporation, where it is stably integrated into the host cell genome such that the recombinant nucleic acid is effectively expressed.

[00128] In another general aspect, the invention relates to a method of producing an anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, an anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or a humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of the invention, comprising culturing a cell comprising a nucleic acid encoding the antibody or antigen-binding fragment thereof under conditions to produce an antibody or antigen-binding fragment thereof of the invention, and recovering the antibody or antigen-binding fragment thereof from the cell or cell culture (e.g., from the supernatant). Expressed antibodies or antigen-binding fragments thereof can be harvested from the cells and purified according to conventional techniques known in the art and as described herein.

Pharmaceutical Compositions

[00129] In another general aspect, the invention relates to a pharmaceutical composition, comprising an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, an isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of the invention and a pharmaceutically acceptable carrier. The term "pharmaceutical composition" as used herein means a product comprising an antibody of the invention together with a pharmaceutically acceptable carrier. Antibodies of the invention and compositions comprising them are also useful in the manufacture of a medicament for therapeutic applications mentioned herein.

[00130] As used herein, the term "carrier" refers to any excipient, diluent, filler, salt, buffer, stabilizer, solubilizer, oil, lipid, lipid containing vesicle, microsphere, liposomal encapsulation, or other material well known in the art for use in pharmaceutical formulations. It will be understood that the characteristics of the carrier, excipient or diluent will depend on the route of administration for a particular application. As used herein, the term "pharmaceutically acceptable carrier" refers to a non-toxic material that does not interfere with the effectiveness of a composition according to the invention or the biological activity of a composition according to the invention. According to particular embodiments, in view of the present disclosure, any pharmaceutically acceptable carrier suitable for use in an antibody pharmaceutical composition can be used in the invention.

[00131] The formulation of pharmaceutically active ingredients with pharmaceutically acceptable carriers is known in the art, e.g., Remington: The Science and Practice of Pharmacy (e.g. 21st edition (2005), and any later editions). Non-limiting examples of additional ingredients include: buffers, diluents, solvents, tonicity regulating agents, preservatives, stabilizers, and chelating agents. One or more pharmaceutically acceptable carrier can be used in formulating the pharmaceutical compositions of the invention.

[00132] In one embodiment of the invention, the pharmaceutical composition is a liquid formulation. A preferred example of a liquid formulation is an aqueous formulation, i.e., a formulation comprising water. The liquid formulation can comprise a solution, a suspension, an emulsion, a microemulsion, a gel, and the like. An aqueous formulation typically comprises at least 50% w/w water, or at least 60%, 70%, 75%, 80%, 85%, 90%, or at least 95% w/w of water.

[00133] In one embodiment, the pharmaceutical composition can be formulated as an injectable which can be injected, for example, via an injection device (e.g., a syringe or an infusion pump). The injection can be delivered subcutaneously, intramuscularly, intraperitoneally, intravitreally, or intravenously, for example.

[00134] In another embodiment, the pharmaceutical composition is a solid formulation, e.g., a freeze-dried or spray-dried composition, which can be used as is, or whereto the physician or the patient adds solvents, and/or diluents prior to use. Solid dosage forms can include tablets, such as compressed tablets, and/or coated tablets, and capsules (e.g., hard or soft gelatin capsules). The pharmaceutical composition can also be in the form of sachets, dragees, powders, granules, lozenges, or powders for reconstitution, for example.

[00135] The dosage forms can be immediate release, in which case they can comprise a water-soluble or dispersible carrier, or they can be delayed release, sustained release, or modified release, in which case they can comprise water-insoluble polymers that regulate the rate of dissolution of the dosage form in the gastrointestinal tract or under the skin.

[00136] In other embodiments, the pharmaceutical composition can be delivered intranasally, intrabuccally, or sublingually.

[00137] The pH in an aqueous formulation can be between pH 3 and pH 10. In one embodiment of the invention, the pH of the formulation is from about 7.0 to about 9.5. In

another embodiment of the invention, the pH of the formulation is from about 3.0 to about 7.0.

[00138] In another embodiment of the invention, the pharmaceutical composition comprises a buffer. Non-limiting examples of buffers include: arginine, aspartic acid, bicine, citrate, disodium hydrogen phosphate, fumaric acid, glycine, glycylglycine, histidine, lysine, maleic acid, malic acid, sodium acetate, sodium carbonate, sodium dihydrogen phosphate, sodium phosphate, succinate, tartaric acid, tricine, and tris(hydroxymethyl)-aminomethane, and mixtures thereof. The buffer can be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific buffers constitute alternative embodiments of the invention.

[00139] In another embodiment of the invention, the pharmaceutical composition comprises a preservative. Non-limiting examples of preservatives include: benzethonium chloride, benzoic acid, benzyl alcohol, bronopol, butyl 4-hydroxybenzoate, chlorobutanol, chlorocresol, chlorohexidine, chlorphenesin, o-cresol, m-cresol, p-cresol, ethyl 4-hydroxybenzoate, imidurea, methyl 4-hydroxybenzoate, phenol, 2-phenoxyethanol, 2-phenylethanol, propyl 4-hydroxybenzoate, sodium dehydroacetate, thiomerosal, and mixtures thereof. The preservative can be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific preservatives constitute alternative embodiments of the invention.

[00140] In another embodiment of the invention, the pharmaceutical composition comprises an isotonic agent. Non-limiting examples of the isotonic agents include a salt (such as sodium chloride), an amino acid (such as glycine, histidine, arginine, lysine, isoleucine, aspartic acid, tryptophan, and threonine), an alditol (such as glycerol, 1,2-propanediol propyleneglycol), 1,3-propanediol, and 1,3-butanediol), polyethyleneglycol (e.g. PEG400), and mixtures thereof. Another example of an isotonic agent includes a sugar. Non-limiting examples of sugars may be mono-, di-, or polysaccharides, or water-soluble glucans, including for example fructose, glucose, mannose, sorbose, xylose, maltose, lactose, sucrose, trehalose, dextran, pullulan, dextrin, cyclodextrin, alpha and

beta- HPCD, soluble starch, hydroxyethyl starch, and sodium carboxymethylcellulose. Another example of an isotonic agent is a sugar alcohol, wherein the term "sugar alcohol" is defined as a C(4-8) hydrocarbon having at least one -OH group. Non-limiting examples of sugar alcohols include mannitol, sorbitol, inositol, galactitol, dulcitol, xylitol, and arabitol. Pharmaceutical compositions comprising each isotonic agent listed in this paragraph constitute alternative embodiments of the invention. The isotonic agent can be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific isotonic agents constitute alternative embodiments of the invention.

[00141] In another embodiment of the invention, the pharmaceutical composition comprises a chelating agent. Non-limiting examples of chelating agents include citric acid, aspartic acid, salts of ethylenediaminetetraacetic acid (EDTA), and mixtures thereof. The chelating agent can be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific chelating agents constitute alternative embodiments of the invention.

[00142] In another embodiment of the invention, the pharmaceutical composition comprises a stabilizer. Non-limiting examples of stabilizers include one or more aggregation inhibitors, one or more oxidation inhibitors, one or more surfactants, and/or one or more protease inhibitors.

[00143] In another embodiment of the invention, the pharmaceutical composition comprises a stabilizer, wherein said stabilizer is carboxy-/hydroxycellulose and derivates thereof (such as HPC, HPC-SL, HPC-L and HPMC), cyclodextrins, 2-methylthioethanol, polyethylene glycol (such as PEG 3350), polyvinyl alcohol (PVA), polyvinyl pyrrolidone, salts (such as sodium chloride), sulphur-containing substances such as monothioglycerol), or thioglycolic acid. The stabilizer can be present individually or in the aggregate, in a concentration from about 0.01 mg/ml to about 50 mg/ml, for example from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific stabilizers constitute alternative embodiments of the invention.

[00144] In further embodiments of the invention, the pharmaceutical composition comprises one or more surfactants, preferably a surfactant, at least one surfactant, or two different surfactants. The term "surfactant" refers to any molecules or ions that are comprised of a water-soluble (hydrophilic) part, and a fat-soluble (lipophilic) part. The surfactant can, for example, be selected from the group consisting of anionic surfactants, cationic surfactants, nonionic surfactants, and/or zwitterionic surfactants. The surfactant can be present individually or in the aggregate, in a concentration from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific surfactants constitute alternative embodiments of the invention.

[00145] In a further embodiment of the invention, the pharmaceutical composition comprises one or more protease inhibitors, such as, e.g., EDTA, and/or benzamidine hydrochloric acid (HCl). The protease inhibitor can be present individually or in the aggregate, in a concentration from about 0.1 mg/ml to about 20 mg/ml. Pharmaceutical compositions comprising each one of these specific protease inhibitors constitute alternative embodiments of the invention.

[00146] In another general aspect, the invention relates to a method of producing a pharmaceutical composition comprising an anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, an anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or a humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of the invention, comprising combining an antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

Methods of use

[00147] In another general aspect, the invention relates to a method of targeting DLL3 on a cancer cell surface in a subject in need thereof, the method comprises administering to the subject in need thereof an isolated anti-DLL3 monoclonal antibody or antigen binding fragment thereof that specifically binds DLL3 or a pharmaceutical composition of the invention. Binding of the monoclonal antibody or antigen-binding fragment thereof to DLL3 can mediate complement-dependent cytotoxicity (CDC), antibody-dependent phagocytosis (ADPC), and/or antibody-dependent cellular cytotoxicity (ADCC) or other effects that result in the death of the targeted cancer cell. The

monoclonal antibody or antigen-binding fragment thereof can, for example, serve to recruit conjugated drugs, and/or can form a bispecific antibody with another monoclonal antibody or antigen-binding fragment thereof to mediate the death of the targeted cancer cell.

[00148] In another general aspect, the invention relates to a method of blocking binding of CD47 to signal regulatory protein alpha (SIRPα) in a subject in need thereof, the method comprises administering to the subject in need thereof an anti-CD47 monoclonal antibody or antigen-binding fragment thereof or a pharmaceutical composition of the invention.

[00149] In another general aspect, the invention relates to a method of inducing macrophage-mediated phagocytosis of cancer cells in a subject in need thereof, the method comprises administering to the subject in need thereof an anti-CD47 monoclonal antibody or antigen-binding fragment thereof or a pharmaceutical composition of the invention.

[00150] In another general aspect, the invention relates to a method of targeting DLL3 and CD47 on a cancer cell surface in a subject in need thereof, the method comprises administering to the subject in need thereof a humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, or a pharmaceutical composition of the invention.

[00151] In another general aspect, the invention relates to a method of blocking binding of CD47 to signal regulatory protein alpha (SIRPα) on cancer cells that express both DLL3 and CD47 in a subject in need thereof, the method comprises administering to the subject in need thereof an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, or a pharmaceutical composition of the invention. Binding of the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof to the cancer cells can mediate blocking the binding of CD47 to SIRPα.

[00152] In another general aspect, the invention relates to a method of inducing macrophage-mediated phagocytosis of cancer cells that express both DLL3 and CD47 in a subject in need thereof, the method comprises administering to the subject in need thereof an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, or a pharmaceutical composition of the invention. Binding of the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment to the cancer cells can induce macrophage-mediated phagocytosis of the cancer cells.

[00153] In another general aspect, the invention relates to a method of binding cancer cells that express both DLL3 and CD47 with minimal to undetectable binding to human red blood cells (RBCs) in a subject in need thereof, the method comprises administering to the subject in need thereof an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, or a pharmaceutical composition of the invention. The humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of the invention has high selectivity for cancer cells with minimal to undetectable binding to human red blood cells (RBCs).

[00154] The functional activity of antibodies and antigen-binding fragments thereof that bind DLL3 or CD47, or bispecific antibodies and antigen binding fragments thereof that bind DLL3 and CD47 can be characterized by methods known in the art and as described herein. Methods for characterizing antibodies and antigen-binding fragments thereof that bind DLL3 or CD47, or bispecific antibodies and antigen binding fragments thereof that bind DLL3 and CD47 include, but are not limited to, affinity and specificity assays including Biacore, ELISA, FACS and OctetRed analysis. According to particular embodiments, the methods for characterizing antibodies and antigen-binding fragments thereof that bind DLL3 or CD47, or bispecific antibodies and antigen binding fragments thereof that bind DLL3 and CD47 include those described below.

[00155] In another general aspect, the invention relates to a method of treating a cancer in a subject in need thereof, comprising administering to the subject in need thereof an isolated anti-DLL3 monoclonal antibody or antigen binding fragment thereof, an isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof or a pharmaceutical composition of the invention. The cancer can, for example, be selected from but not limited to, a lung cancer, such as small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[00156] According to embodiments of the invention, the pharmaceutical composition comprises a therapeutically effective amount of an anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, an anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or a humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of the invention. As used herein, the term "therapeutically effective amount" refers to an amount of an active ingredient or component that elicits the desired biological or medicinal response in a subject. A therapeutically effective amount can be determined empirically and in a routine manner, in relation to the stated purpose.

[00157] As used herein with reference to anti-DLL3 antibodies or antigen-binding fragments thereof, anti-CD47 antibodies or antigen-binding fragments thereof, or anti-CD47/DLL3 bispecific antibodies or antigen-binding fragments thereof, a therapeutically effective amount means an amount of the anti-DLL3 antibody or antigen-binding fragment thereof, anti-CD47 antibody or antigen-binding fragment thereof, or anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof that modulates an immune response in a subject in need thereof. Also as used herein with reference to anti-DLL3 antibodies or antigen-binding fragments thereof, anti-CD47 antibody or antigen-binding fragment thereof, or anti-CD47/DLL3 bispecific antibodies or antigen-binding

fragments thereof, a therapeutically effective amount means an amount of the anti-DLL3 antibody or antigen-binding fragment thereof, anti-CD47 antibody or antigen-binding fragment thereof, or anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof that results in treatment of a disease, disorder, or condition; prevents or slows the progression of the disease, disorder, or condition; or reduces or completely alleviates symptoms associated with the disease, disorder, or condition.

[00158] According to particular embodiments, the disease, disorder or condition to be treated is cancer, preferably a cancer selected from the group consisting of a lung cancer, such as small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[00159] According to particular embodiments, a therapeutically effective amount refers to the amount of therapy which is sufficient to achieve one, two, three, four, or more of the following effects: (i) reduce or ameliorate the severity of the disease, disorder or condition to be treated or a symptom associated therewith; (ii) reduce the duration of the disease, disorder or condition to be treated, or a symptom associated therewith; (iii) prevent the progression of the disease, disorder or condition to be treated, or a symptom associated therewith; (iv) cause regression of the disease, disorder or condition to be treated, or a symptom associated therewith; (v) prevent the development or onset of the disease, disorder or condition to be treated, or a symptom associated therewith; (vi) prevent the recurrence of the disease, disorder or condition to be treated, or a symptom associated therewith; (vii) reduce hospitalization of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (viii) reduce hospitalization length of a subject having the disease, disorder or condition to be treated, or a symptom associated therewith; (ix) increase the survival of a subject with the disease, disorder or condition to be treated, or a symptom associated therewith; (xi) inhibit or

reduce the disease, disorder or condition to be treated, or a symptom associated therewith in a subject; and/or (xii) enhance or improve the prophylactic or therapeutic effect(s) of another therapy.

[00160] The therapeutically effective amount or dosage can vary according to various factors, such as the disease, disorder or condition to be treated, the means of administration, the target site, the physiological state of the subject (including, e.g., age, body weight, health), whether the subject is a human or an animal, other medications administered, and whether the treatment is prophylactic or therapeutic. Treatment dosages are optimally titrated to optimize safety and efficacy.

[00161] According to particular embodiments, the compositions described herein are formulated to be suitable for the intended route of administration to a subject. For example, the compositions described herein can be formulated to be suitable for intravenous, subcutaneous, or intramuscular administration.

[00162] As used herein, the terms "treat," "treating," and "treatment" are all intended to refer to an amelioration or reversal of at least one measurable physical parameter related to a cancer, which is not necessarily discernible in the subject, but can be discernible in the subject. The terms "treat," "treating," and "treatment," can also refer to causing regression, preventing the progression, or at least slowing down the progression of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an alleviation, prevention of the development or onset, or reduction in the duration of one or more symptoms associated with the disease, disorder, or condition, such as a tumor or more preferably a cancer. In a particular embodiment, "treat," "treating," and "treatment" refer to prevention of the recurrence of the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to an increase in the survival of a subject having the disease, disorder, or condition. In a particular embodiment, "treat," "treating," and "treatment" refer to elimination of the disease, disorder, or condition in the subject.

[00163] According to particular embodiments, a composition used in the treatment of a cancer. For cancer therapy, it can be used in combination with another treatment including, but not limited to, a chemotherapy, an anti-TIM-3 mAb, an anti-LAG-3 mAb, an anti-CD73 mAb, an anti-apelin mAb, an anti-CTLA-4 antibody, an anti-EGFR mAb,

an anti-HER-2 mAb, an anti-CD19 mAb, an anti-CD20 mAb, an anti-CD33 mAb, an anti-CD47 mAb, an anti-TIP-1 mAb, an anti-CLDN18.2 mAb, an anti-FOLR1 mAb, an anti-PD-L1 antibody, an anti-PD-1 antibody, a PD-1/PD-L1 therapy, other immuno-oncology drugs, an antiangiogenic agent, a radiation therapy, an antibody-drug conjugate (ADC), a targeted therapy, or other anticancer drugs.

[00164] As used herein, the term "in combination," in the context of the administration of two or more therapies to a subject, refers to the use of more than one therapy. The use of the term "in combination" does not restrict the order in which therapies are administered to a subject. For example, a first therapy (e.g., a composition described herein) can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 16 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second therapy to a subject.

[00165] In another general aspect, the invention relates to a method of determining a level of DLL3 in a subject. The methods comprise (a) obtaining a sample from the subject; (b) contacting the sample with an anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of the invention; and (c) determining a level of DLL3 in the subject.

[00166] As used herein, "sample" refers to a biological sample isolated from a subject and can include, but is not limited to, whole blood, serum, plasma, blood cells, endothelial cells, tissue biopsies (e.g., a cancer tissue), lymphatic fluid, ascites fluid, interstitial fluid, bone marrow, cerebrospinal fluid, saliva, mucous, sputum, sweat, urine, or any other secretion, excretion, or other bodily fluids. A "blood sample" refers to whole blood or any fraction thereof, including blood cells, serum, and plasma.

[00167] In certain embodiments, the level of DLL3 in the subject can be determined utilizing assays selected from, but not limited to, a Western blot assay, an ELISA assay, and/or an immunohistochemistry (IHC) assay. Relative protein levels can be determined by utilizing Western blot analysis and IHC, and absolute protein levels can be determined

by utilizing an ELISA assay. When determining the relative levels of DLL3, the levels of DLL3 can be determined between at least two samples, e.g., between samples from the same subject at different time points, between samples from different tissues in the same subject, and/or between samples from different subjects. Alternatively, when determining absolute levels of DLL3, such as by an ELISA assay, the absolute level of DLL3 in the sample can be determined by creating a standard for the ELISA assay prior to testing the sample. A person skilled in the art would understand which analytical techniques to utilize to determine the level of DLL3 in a sample from the subject utilizing the antibodies or antigen-binding fragments thereof of the invention.

[00168] Utilizing methods of determining a level of DLL3 in a sample from a subject can lead to the diagnosis of abnormal (elevated, reduced, or insufficient) DLL3 levels in a disease and making appropriate therapeutic decisions. Such a disease can be, but not limited to, a cancer. Additionally, by monitoring the levels of DLL3 in a subject, the risk of developing a disease as indicated above can be determined based on the knowledge of the level of DLL3 in a particular disease and/or during the progression of the particular disease.

EMBODIMENTS

[00169] The invention provides also the following non-limiting embodiments.

[00170] Embodiment 1 is an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of

(1) SEQ ID NOs:25, 26, 27, 61, 62 and 63, respectively;

(2) SEQ ID NOs:28, 29, 30, 64, 65 and 66, respectively;

(3) SEQ ID NOs:31, 32, 33, 67, 68 and 69, respectively;

(4) SEQ ID NOs:34, 35, 36, 70, 71 and 72, respectively;

(5) SEQ ID NOs:37, 38, 39, 73, 74 and 75, respectively;

(6) SEQ ID NOs:40, 41, 42, 76, 77 and 78, respectively;

(7) SEQ ID NOs:43, 44, 45, 79, 80 and 81, respectively;

(8) SEQ ID NOs:46, 47, 48, 82, 83 and 84, respectively;

(9) SEQ ID NOs:49, 50, 51, 85, 86 and 87, respectively;

(10) SEQ ID NOs:52, 53, 54, 88, 89 and 90, respectively;

(11) SEQ ID NOs:55, 56, 57, 91, 92 and 93, respectively; or

(12) SEQ ID NOs:58, 59, 60, 94, 95 and 96, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds DLL3, preferably specifically binds human DLL3.

[00171] Embodiment 2 is an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of

(1) SEQ ID NOs:97, 98, 99, 133, 134 and 135, respectively;

(2) SEQ ID NOs:100, 101, 102, 136, 137 and 138, respectively;

(3) SEQ ID NOs:103, 104, 105, 139, 140 and 141, respectively;

(4) SEQ ID NOs:106, 107, 108, 142, 143 and 144, respectively;

(5) SEQ ID NOs:109, 110, 111, 145, 146 and 147, respectively;

(6) SEQ ID NOs:112, 113, 114, 148, 149 and 150, respectively;

(7) SEQ ID NOs:115, 116, 117, 151, 152 and 153, respectively;

(8) SEQ ID NOs:118, 119, 120, 154, 155 and 156, respectively;

(9) SEQ ID NOs:121, 122, 123, 157, 158 and 159, respectively;

(10) SEQ ID NOs:124, 125, 126, 160, 161 and 162, respectively;

(11) SEQ ID NOs:127, 128, 129, 163, 164 and 165, respectively; or

(12) SEQ ID NOs:130, 131, 132, 166, 167 and 168, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds DLL3, preferably specifically binds human DLL3.

[00172] Embodiment 3 is the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment of embodiment 1 or 2, comprising a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 23, or a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24.

[00173] Embodiment 4 is the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment of any one of embodiments 1-3, comprising

(a) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;

(b) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;

(c) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;

(d) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;

(e) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

(f) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;

(g) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;

(h) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;

(i) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;

(j) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;

(k) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22; or

(l) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24.

[00174] Embodiment 5 is the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment of any one of embodiments 1-4, wherein the antibody or antigen-binding fragment thereof is chimeric.

[00175] Embodiment 6 is the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment of any one of embodiments 1-4, wherein the antibody or antigen-binding fragment thereof is human or humanized.

[00176] Embodiment 7 is the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of embodiment 6, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;

(b) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172; or

(c) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173.

[00177] Embodiment 8 is the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 1-7, wherein the anti-DLL3 monoclonal antibody or antigen-binding fragment thereof is capable of inducing effector-mediated tumor cell lysis.

[00178] Embodiment 9 is an isolated anti-CD47 monoclonal antibody or antigen-binding fragment comprising a humanized heavy chain variable region of an anti-CD47 monoclonal antibody and a humanized light chain variable region of an anti-DLL3

monoclonal antibody, wherein the anti-CD47 monoclonal antibody or antigen-binding fragment thereof comprises:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;
b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;
c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173;
d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;
e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;
f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173;
g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;
h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;
i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173; or

j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:174.

[00179] Embodiment 10 is the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof of embodiment 9, wherein the anti-CD47 monoclonal antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to signal regulatory protein alpha (SIRPα).

[00180] Embodiment 11 is the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof of embodiment 9, wherein the anti-CD47 monoclonal antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells.

[00181] Embodiment 12 is an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 178, 179 and 180; the second antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 181, 182 and 183; and the first antigen-binding domain and the second antigen-binding domain each comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NOs: 184, 185 and 186.

[00182] Embodiment 13 is an isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 187, 188 and 189; the second antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs:

190, 191 and 192; and the first antigen-binding domain and the second antigen-binding domain each comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NOs: 193, 194 and 195.

[00183] Embodiment 14 is the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of embodiment 12 or 13, wherein the first antigen-binding domain comprises a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:176, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:172; and wherein the second antigen-binding domain comprises a heavy chain variable region a polypeptide sequence at least 95% identical to SEQ ID NO:170, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:172.

[00184] Embodiment 15 is the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 12-14, wherein the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to SIRPα on cancer cells that express both DLL3 and CD47.

[00185] Embodiment 16 is the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 12-14, wherein the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells that express both DLL3 and CD47.

[00186] Embodiment 17 is the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 12-14, wherein the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof is capable of binding cancer cells that express both DLL3 and CD47 with minimal to undetectable binding to human red blood cells (RBCs).

[00187] Embodiment 18 is an isolated nucleic acid encoding the anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment of any one of embodiments 1-17.

[00188] Embodiment 19 is a vector comprising the isolated nucleic acid of embodiment 18.

[00189] Embodiment 20 is a host cell comprising the vector of embodiment 19.

[00190] Embodiment 21 is a pharmaceutical composition, comprising the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1-17 and a pharmaceutically acceptable carrier.

[00191] Embodiment 22 is a method of targeting DLL3 on a cancer cell surface in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition comprising the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 1-8.

[00192] Embodiment 23 is a method of blocking binding of CD47 to signal regulatory protein alpha (SIRPα) in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition comprising the anti-CD47 monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 9-11.

[00193] Embodiment 24 is a method of inducing macrophage-mediated phagocytosis of cancer cells in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition comprising the anti-CD47 monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 9-11.

[00194] Embodiment 25 is a method of targeting DLL3 and CD47 that are both expressed on a cancer cell surface in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition comprising the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 12-17.

[00195] Embodiment 26 is a method of blocking binding of CD47 to SIRPα on cancer cells that express both DLL3 and CD47 in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition comprising the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 12-17.

[00196] Embodiment 27 is a method of inducing macrophage-mediated phagocytosis of cancer cells that express both DLL3 and CD47 in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition comprising the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 12-17.

[00197] Embodiment 28 is a method of binding cancer cells that express both DLL3 and CD47 with minimal to undetectable binding to human red blood cells (RBCs) in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition comprising the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 12-17.

[00198] Embodiment 29 is a method of treating cancer in a subject in need thereof, comprising administering to the subject in need thereof the pharmaceutical composition of embodiment 21.

[00199] Embodiment 30 is the method of embodiment 29, wherein the cancer is selected from the group consisting of a lung cancer, such as small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

[00200] Embodiment 31 is a method of producing the anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1-17, comprising culturing a cell comprising a nucleic acid encoding the antibody or antigen-binding fragment thereof under conditions to produce the antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof from the cell or culture.

[00201] Embodiment 32 is a method of producing a pharmaceutical composition comprising the anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the

anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of embodiments 1-17, comprising combining the antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

[00202] Embodiment 33 is a method of determining a level of DLL3 in a subject, the method comprising:

a. obtaining a sample from the subject;
b. contacting the sample with the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of any one of embodiments 1-8; and
c. determining a level of DLL3 in the subject.

[00203] Embodiment 34 is the method of embodiment 33, wherein the sample is a tissue sample.

[00204] Embodiment 35 is the method of embodiment 34, wherein the tissue sample is a cancer tissue sample.

[00205] Embodiment 36 is the method of embodiment 33, wherein the sample is a blood sample.

EXAMPLES

[00206] Example 1: Identification of anti-DLL3 monoclonal antibodies

[00207] Mice were immunized with recombinant FLAG-huDLL3 (Adipogen, Cat#: AG40B-0151), a fusion protein of human DLL3 extracellular domain (ECD) with FLAG tag at the N-terminus. Plasma titer was determined by ELISA. After euthanization, spleens and lymph nodes were collected to produce hybridomas. Hybridomas were grown in 384-well tissue culture plates and supernatants from individual wells were screened by ELISA using FLAG-huDLL3, FACS using stable pools of HEK293-huDLL3 and HEK293-cyDLL3, and Octet off-rate analysis using FLAG-huDLL3. Top positive clones were isolated and sequenced.

[00208] Sequences of heavy and light chain variable regions for anti-DLL3 monoclonal antibodies are provided in Tables 1 and 2, and the CDR regions for the anti-DLL3 monoclonal antibodies are provided in Tables 3-6.

[00209] Table 1: Sequences of heavy chain variable regions for anti-DLL3 mAbs

mAb clones	VH	SEQ ID NO:
13P9A	EVQLQQSGPELVKPGASVKMSCKASGYTFTSYVMHWVKQKPGQGPDWIGYI NPYNDATKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARGGYDY DGDYWGQGTTLTVSS	1
5A16A	EVQLQQSGPELVKPGASVKMSCKASGYTFTRYILHWVKLKPGQGLEWIGYIN PYNDGTKYNEKFKGKATLTSDKSSSTAYMELSRLTSYDSAVYYCARDSSGYG GAYAMDFWGQGTSVTVSS	3
14L22A	EVQLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVAAIN SNGGNTYYPDTVKDRFTISRDNAKNTLYLQMSSLRSEDTALYYCARHRGGFY YAVDYWGQGTSVTVSS	5
10P18A	EVQLQQSGPELVKPGASVKISCKASGYSFTGYYIDWVKQSPGKSLEWIGYIYP SNGETSYNQKFKGKATLTVDKSSSTVNMQLNSLTSEDSAVYYCARESYAMD YWGQGTSVTVSS	7
13P11A	DVQLQESGPGLVKPSQTVSLTCTVTGYSITNGNHWWSWIRQVSGSKLEWMG YISSSGSTDSNPSLKSRISITRDTSKNQLFLHLNSVTTEDIATYYCATTGTWGYF DYWGQGTTLTVSS	9
3C16A	EVQLQQSGPELVKPGTSVKMSCKASGYTFTSYVMHWVKQKPGQGLEWIGYV IPYNDGTKYNEKFKGKATLTSDKSSSTAYMELSSLTSEDSAVYYCARPSNWDE FDYWGQGTTLTVSS	11
3I21A	QVQLQQPGAELVKPGASVKLSCKASGYTFTNYWMNWVKQRPGRGLEWIGRI HPSDSETHYNQKFKTKATLTVDKSSSTAYIQLSSLTSEDSAVYYCARYDGYFA YWGQGTLVTVSA	13
8H5A	QVTLKESGPGILQPSQTLSLTCSFSGFSLSTFGMGVGWIRQPSGKGLEWLAHIW WDDDKYYNPALKSRLTISKDTSKNQVFLKIANVDIADTATYYCARTYDYDEY FDYWGQGTTLTVSS	15
15K2A	QVQLQQPGAELVQPGASVKLSCKASGYTFTSYWMNWMKQRPGRGLEWIGRI HPSDSETHYNQKFRTKATLTVDKSSSTAYIQLSSLTSEDSAVYYCAREDGYYW YFDVWGAGTTVTVSS	17
5A24A	EVQLQQSGAELVKPGASVKIPCKASGYKFTDFNMDWVKQSHGKSLEWIGDIN PNSGGTIYNQKFKGKATLTVDKSLSTAYMELGSLTSEDTAVYYCARWDYGNF AYWGQGTLVTVSA	19
15P17A	QVQLQQPGAELVKPGASVKLSCKASGYTFTNYWMNWVKQRPGRGLEWIGRI HPSDSETHYNQKFKSKATLTVDKSSSTAYIQLSSLTSEDSAVYYCAREDGYYW YFDVWGAGTTVTVSS	21
15N21A	EVQLVESGGGLVKPGGSLKLSCAASGFTFSSYAMSWVRQTPEKRLEWVAAIN SNGGRNYYPDTVKDRFTISRDNAKNTLYLQMSSLRSEDTALYYCARHRGGYY YAMDYWGQGTSVTVSS	23

VH: heavy chain variable region

[00210] Table 2: Sequences of light chain variable regions for anti-DLL3 mAbs

mAb clones	VL	SEQ ID NO:
13P9A	DIQMNQSPSSLSASLGDSITITCHASQNINVWLSWYQQKPGNIPKLLIYKASNLH TGVPSRFSGSGSGTGFTLTISSLQPEDIATYYCQQGQSYPFTFGSGTKLEIK	2
5A16A	DIQMTQSPASLSASVGETVTITCRASGNIHNYLAWYQQKQGRSPQLLVYNAKT LPYGVPSRFSGSGSGTQYSLKINSLQPEDFGSYYCQHFWTTPWTFGGGTKLEIK	4
14L22A	NIMMTQSPSSLAVSAGEKVTMSCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLL IYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCHQYLSSRTFGGGT	6

	KLEIK	
10P18A	DIVLTQSPASLAVSLGQRATISCRASKSVSTSGYSYMHWYQQKPGQPPKLLIYL ASNLESGVPARFSGSGSGTDFTLNIHPVEEEDAATYYCQHSRELPYTFGGGTKL EIK	8
13P11A	NIVMTQSPKSMSMSVGERVTLSCKASENVGTYVSWYQQKPEQSPKLLIYGASN RFTGVPDRFTGSGSATDFTLTISSVQAEDLADYHCGQSYSYPFTFGSGTKLEIK	10
3C16A	DIVMTQSQKFMSTSVGDRVSITCKASQNVRTAVAWYQQKPGQSPKALIYLASN RHTGVPDRFTGSGSGTDFTLTISNVQSEDLADYFCLQHWNYPLTFGAGTKLELK	12
3I21A	DIQMTQSSSYLSVSLGGRVTITCKASDHINNWLAWYQQKPGNAPRLLISGATSL ETGDPSRFSGSGSGKDYTLSITSLQIEDVATYYCQQYWSIPFTFGAGTKLELK	14
8H5A	DIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITYFYWYLQKPGQSPQLLIYQ MSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQNLELPFTFGSGTKL EIK	16
15K2A	NIVLTQSPASLAVSLGQRATISCRASESVDIYGNSFMHWYQQKPGQPPKLLIYL ASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNNEDPWTFGGGTK LEIK	18
5A24A	DIVMTQAAFSNPVTLGTSASISCRSSKSLLHSNGITYLYWYLQKPGQSPQLLIYQ MSNLASGVPDRFSSSGSGTDFTLRISRVEAEDVGVYYCAQNLELPLTFGAGTKL ELK	20
15P17A	NIVLTQSPASLAVSLGQRATISCRASESVDSYGNSFMHWYQQKPGQPPKLLIYL ASNLESGVPARFSGSGSRTDFTLTIDPVEADDAATYYCQQNHEDPWTFGGGTK LEIK	22
15N21A	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQKPGQSPKLLI YWASTRESGVPDRFTGSGSGTDFTLTISSVKAEDLAVYYCQQYYTYLTFGAGT KLELK	24

VL: light chain variable region

[00211] Table 3: CDR regions 1-3 of heavy chain for anti-DLL3 mAbs

mAb clones	**HC CDR1**	**SEQ ID NO:**	**HC CDR2**	**SEQ ID NO:**	**HC CDR3**	**SEQ ID NO:**
13P9A	GYTFTSYV	25	INPYNDAT	26	ARGGYDYDGDY	27
5A16A	GYTFTRYI	28	INPYNDGT	29	ARDSSGYGGAYAMDF	30
14L22A	GFTFSSYA	31	INSNGGNT	32	ARHRGGFYYAVDY	33
10P18A	GYSFTGYY	34	IYPSNGET	35	ARESYAMDY	36
13P11A	GYSITNGNHW	37	ISSSGST	38	ATTGTWGYFDY	39
3C16A	GYTFTSYV	40	VIPYNDGT	41	ARPSNWDEFDY	42
3I21A	GYTFTNYW	43	IHPSDSET	44	ARYDGYFAY	45
8H5A	GFSLSTFGMG	46	IWWDDDK	47	ARTYDYDEYFDY	48
15K2A	GYTFTSYW	49	IHPSDSET	50	AREDGYYWYFDV	51
5A24A	GYKFTDFN	52	INPNSGGT	53	ARWDYGNFAY	54
15P17A	GYTFTNYW	55	IHPSDSET	56	AREDGYYWYFDV	57
15N21A	GFTFSSYA	58	INSNGGRN	59	ARHRGGYYYAMDY	60

HC: heavy chain; CDR: complementarity determining region

The HC CDRs for the anti-DLL3 mAbs were determined utilizing the IMGT method (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212).

[00212] Table 4: CDR regions 1-3 of light chain for anti-DLL3 mAbs

mAb clones	LC CDR1	SEQ ID NO:	LC CDR2	SEQ ID NO:	LC CDR3	SEQ ID NO:
13P9A	QNINVW	61	KAS	62	QQGQSYPFT	63
5A16A	GNIHNY	64	NAK	65	QHFWTTPWT	66
14L22A	QSVLYSSNQKNY	67	WAS	68	HQYLSSRT	69
10P18A	KSVSTSGYSY	70	LAS	71	QHSRELPYT	72
13P11A	ENVGTY	73	GAS	74	GQSYSYPFT	75
3C16A	QNVRTA	76	LAS	77	LQHWNYPLT	78
3I21A	DHINNW	79	GAT	80	QQYWSIPFT	81
8H5A	KSLLHSNGITY	82	QMS	83	AQNLELPFT	84
15K2A	ESVDIYGNSF	85	LAS	86	QQNNEDPWT	87
5A24A	KSLLHSNGITY	88	QMS	89	AQNLELPLT	90
15P17A	ESVDSYGNSF	91	LAS	92	QQNHEDPWT	93
15N21A	QSLLYSSNQKNY	94	WAS	95	QQYYTYLT	96

LC: light chain; CDR: complementarity determining region

The LC CDRs for the anti-DLL3 mAbs were determined utilizing the IMGT method (Lefranc, M.-P. et al., Nucleic Acids Res. 1999; 27:209-212).

[00213] Table 5: CDR regions 1-3 of heavy chain for anti-DLL3 mAbs

mAb clones	HC CDR1	SEQ ID NO:	HC CDR2	SEQ ID NO:	HC CDR3	SEQ ID NO:
13P9A	SYVMH	97	YINPYNDATKYNEKFKG	98	GGYDYDGDY	99
5A16A	RYILH	100	YINPYNDGTKYNEKFKG	101	DSSGYGGAYAMDF	102
14L22A	SYAMS	103	AINSNGGNTYYPDTVKD	104	HRGGFYYAVDY	105
10P18A	GYYID	106	YIYPSNGETSYNQKFKG	107	ESYAMDY	108
13P11A	NGNHWWS	109	YISSSGSTDSNPSLKS	110	TGTWGYFDY	111
3C16A	SYVMH	112	YVIPYNDGTKYNEKFKG	113	PSNWDEFDY	114
3I21A	NYWMN	115	RIHPSDSETHYNQKFKT	116	YDGYFAY	117
8H5A	TFGMGVG	118	HIWWDDDKYYNPALKS	119	TYDYDEYFDY	120
15K2A	SYWMN	121	RIHPSDSETHYNQKFRT	122	EDGYYWYFDV	123
5A24A	DFNMD	124	DINPNSGGTIYNQKFKG	125	WDYGNFAY	126
15P17A	NYWMN	127	RIHPSDSETHYNQKFKS	128	EDGYYWYFDV	129
15N21A	SYAMS	130	AINSNGGRNYYPDTVKD	131	HRGGYYYAMDY	132

HC: heavy chain; CDR: complementarity determining region

The HC CDRs for the anti-DLL3 mAbs were determined utilizing the Kabat method (Elvin A. Kabat et al, Sequences of Proteins of Immunological Interest 5th ed. (1991)).

[00214] Table 6: CDR regions 1-3 of light chain for anti-DLL3 mAbs

mAb clones	LC CDR1	SEQ ID NO:	LC CDR2	SEQ ID NO:	LC CDR3	SEQ ID NO:
13P9A	HASQNINVWLS	133	KASNLHT	134	QQGQSYPFT	135
5A16A	RASGNIHNYLA	136	NAKTLPY	137	QHFWTTPWT	138
14L22A	KSSQSVLYSSNQKNYLA	139	WASTRES	140	HQYLSSRT	141
10P18A	RASKSVSTSGYSYMH	142	LASNLES	143	QHSRELPYT	144
13P11A	KASENVGTYVS	145	GASNRFT	146	GQSYSYPFT	147
3C16A	KASQNVRTAVA	148	LASNRHT	149	LQHWNYPLT	150
3I21A	KASDHINNWLA	151	GATSLET	152	QQYWSIPFT	153
8H5A	RSSKSLLHSNGITYFY	154	QMSNLAS	155	AQNLELPFT	156
15K2A	RASESVDIYGNSFMH	157	LASNLES	158	QQNNEDPWT	159
5A24A	RSSKSLLHSNGITYLY	160	QMSNLAS	161	AQNLELPLT	162
15P17A	RASESVDSYGNSFMH	163	LASNLES	164	QQNHEDPWT	165
15N21A	KSSQSLLYSSNQKNYLA	166	WASTRES	167	QQYYTYLT	168

LC: light chain; CDR: complementarity determining region

The LC CDRs for the anti-DLL3 mAbs were determined utilizing the Kabat method (Elvin A. Kabat et al, Sequences of Proteins of Immunological Interest 5th ed. (1991)).

[00215] Example 2: Production and purification of chimeric mAbs from the culture media of transfected cells

[00216] To obtain the recombinant anti-DLL3 chimeric mAbs, the expression vectors containing the mouse variable regions (VH and VL) fused to the constant regions of human IgG1 heavy chain and kappa light chain, respectively, were transiently transfected into 293E, ExpiCHO-S, or Expi293F cells. The recombinant antibodies produced in the suspension of the cells were purified using Protein A affinity chromatography.

[00217] Example 3: ELISA binding analysis of purified chimeric antibodies

[00218] FLAG-huDLL3 in carbonate coating buffer (50 μL/well at 0.25 μg/mL) was coated on an ELISA plate for 1 hour at room temperature. After washing, the ELISA plate was blocked in 5% BSA in TBST for 1 hour at room temperature and washed again. An anti-DLL3 mAb was added, mixed, and incubated for 1 hour at room temperature. The plate was washed and the binding of anti-DLL3 mAb to the immobilized FLAG-huDLL3 was detected by adding a secondary antibody, an anti-human IgG conjugated to horseradish peroxidase (hIgG-HRP) (ThermoFisher Scientific, Cat#: H10007) in 5% BSA in TBST, incubating for 1 hour, and then washing the plate. The ELISA was

developed using One-step Detection Solution (ThermoFisher Scientific, Cat#: 34028) and measured as the absorbance at 450 nm. An isotype control IgG1 mAb that does not cross-react with DLL3 was used as negative control to ensure assay specificity. Binding results of the chimeric anti-DLL3 mAbs are provided in FIGs. 1A-1C.

[00219] Example 4: FACS analysis of chimeric anti-DLL3 mAbs

[00220] A HEK293 cell line stably expressing human DLL3 (HEK-huDLL3) was used in a FACS assay. The cells (100,000 cells per well on a 96-well plate) were incubated with either a solution of the purified mAbs at various concentrations (e.g., 666.67 nM, 333.33 nM, or 66.67 nM) or an isotype control in Hanks' Balanced Salt Solution (HBSS) containing 0.05% sodium azide and 0.1% BSA. Using an Alexa Fluor 488-conjugated anti-human IgG secondary antibody (ThermoFisher, Cat#: H10120), the presence of the mAb on HEK293-huDLL3 cells was measured by FACS (Attune NxT; ThermoFisher, Carlsbad, CA). The result of the FACS binding analysis is provided in FIGs. 2A-2C.

[00221] Example 5: Humanization of an anti-DLL3 mAb

[00222] The mouse anti-DLL3 13P9A was humanized to reduce the potential of immunogenicity when used in human patients. The sequences of the variable regions of the heavy and light chains (VH and VL) were compared with the human antibody sequences in the Protein Data Bank (PDB) database and homology models were built. The CDRs in both the heavy and light chains of the mouse mAbs were grafted into human frameworks that have the highest possibility of maintaining the proper structure likely required for antigen binding. Backmutations from human residues to mouse residues or other mutations were designed when necessary. The sequences of the humanized VH and VL regions are shown in Tables 7 and 8. The humanized VH and VL regions were fused to the constant regions of human IgG4 heavy chain and kappa light chain, respectively. Antibodies were purified from transiently transfected 293E cells and analyzed for their ability to bind recombinant human FLAG-huDLL3 on a plate using ELISA. The EC_{50} values for DLL3 binding by the humanized mAbs are provided in Table 9.

[00223] Table 7: Sequence of the humanized heavy chain variable region of anti-DLL3 mAb 13P9A

Design	VH	SEQ ID NO:
H1	EVRLSQSGGQMKKPGESMRLSCRASGYTFTSYVMHWVRQAPGRRPEWIG YINPYNDATKYARKFQGRATLTSDKYSDTAFLELRSLTSDDTAVYYCARG GYDYDGDYWGRGAPVTVSS	170

[00224] Table 8: Sequences of humanized light chain variable regions of anti-DLL3 mAb 13P9A

Design	VL	SEQ ID NO:
L1	EIVMTQSPGTLSLSPGERATLSCHASQNINVWLSWYQQKPGQAPRLLIYKA SNLHTGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGQSYPFTFGQGTK VEIK	171
L2	EIVLTQSPGTLSLSPGERATLSCHASQNINVWLSWYQQKPGQAPRLLIYKA SNLHTGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQGQSYPFTFGQGTK VEIK	172
L3	EIVMTQSPATLSLSPGETAIISCHASQNINVWLSWYQQRPGQAPRLLIYKAS NLHTGIPDRFSGSGWGTDFNLSISNLESGDFGVYYCQQGQSYPFTFGQGTK VEIK	173
L4	EIVMTQSPATLSLSPGETAIISCHASQNINVWLSWYQQRPGQAPRLLIYKAS NLHTGIPDRFSGSGWGTDFNLSISNLESGDFGVYYCQQGQSYPWTFGQGT KVEIK	174

[00225] Table 9: EC_{50} values for DLL3 binding by humanized anti-DLL3 mAbs in an ELISA assay

mAb ID	EC50 (nM)
H1L1	0.13
H1L2	0.12
H1L3	0.15

H1L1 refers to the mAb with the H1 heavy chain variable region and the L1 light chain variable region; all the other humanized mAbs in the table adopt the same naming rule.

[00226] The humanized anti-DLL3 mAb H1L2 was analyzed by flow cytometry for its ability to bind cell surface DLL3. SHP-77 cells have been reported to express DLL3 (Barretina et al., Nature 483(7391):603-7 (2012)) and therefore were used in the assay. SHP-77 (ATCC# CRL-2195) cells (14,000 cells per well on a 96-well plate) were incubated with either a solution of the purified mAb at various concentrations or an isotype control in Hanks' Balanced Salt Solution (HBSS) containing 0.05% sodium azide

and 0.1% BSA. Using a PE-Cy7-conjugated anti-human IgG secondary antibody, the presence of the mAb on SHP-77 cells was measured by FACS (Attune NxT; ThermoFisher, Carlsbad, CA). The result of the FACS binding analysis is provided in FIG. 3.

[00227] Example 6: Assembly of anti-CD47 mAbs using the humanized heavy chain variable region of an anti-CD47 mAb and the humanized light chain variable region of an anti-DLL3 mAb

[00228] To assess whether the humanized light chain variable (VL) regions of the anti-DLL3 mAb in Table 8 can form mAbs with the heavy chain variable (VH) region of anti-CD47 mAb 17C6A (described in International Patent Application No. PCT/US18/44384), humanized sequences of the VH region of 17C6A provided in Table 10 were used for antibody expression. The humanized VL regions of the anti-DLL3 mAb in Table 8 and the humanized VH regions of 17C6A in Table 10 were fused to the constant regions of human kappa light chain and IgG4 heavy chain, respectively, and the mAbs were expressed in 293E cells or ExpiCHO-S cells. The recombinant antibodies produced in the suspension of the 293E cell or ExpiCHO-S cultures were purified using Protein A affinity chromatography.

[00229] The purified mAbs were analyzed for their ability to bind to the extracellular domain of CD47 (CD47(ECD)) in an ELISA assay as follows: human CD47(ECD) (AcroBio, Cat#: CD7-HA2E9-50μg) in carbonate coating buffer (50 μL/well at 1 μg/mL) was coated on a 96-well ELISA plate for 1 hour at room temperature. After washing, the plate was blocked in 5% BSA in TBST for 1 hour at room temperature and washed again. In each well of the plate, an antibody at 50 μL/well at various concentrations was added and incubated for 1 hour at room temperature. The plate was washed and the binding of the antibody to the immobilized CD47 was detected by incubating with anti-human IgG conjugated to horseradish peroxidase (hIgG-HRP) (ThermoFisher Scientific, Cat#: H10007) for 60 minutes at room temperature. Then after washing, the ELISA was developed using One-step Detection Solution (ThermoFisher Scientific, Cat#: 34028) and measured as the absorbance at 450 nm. The binding EC_{50} values are shown in Table 11.

These data indicate that the humanized anti-DLL3 light chains can pair with the humanized heavy chains of 17C6A to form mAbs with strong affinity for CD47.

[00230] Table 10: Sequences of humanized heavy chain variable regions of an anti-CD47 mAb

Design	VH	SEQ ID NO:
KH1	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNIDPSDSETHYAQKFQGRATLTVDKSTSTAYMELSSLRSEDTAVYYCAGTDLAYWGQGTLVTVSS	175
KH2	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQGLEWIGNIDPSDSETHYAQKFQGRVTLTVDKSTSTVYMELSSLRSEDTAVYYCAGTDLAYWGQGTLVTVSS	176
KH3	EVRLSQSGGQMKKPGESMRLSCRASGYTFTSYWMHWVRQAPGRRLEWIGNIDPSDSETHYARKFQGRATLTVDKYSDTAFLELRSLTSDDTAVYYCAGTDLAYWGRGAPVTVSS	177

[00231] Table 11: EC_{50} values for CD47 binding by humanized anti-CD47 mAbs in an ELISA assay

mAb ID	EC50 (nM)
KH1L1	1.17
KH1L2	0.64
KH1L3	0.76
KH2L1	0.19
KH2L2	0.18
KH2L3	0.09
KH3L1	0.09
KH3L2	0.08
KH3L3	0.18
KH3L4	0.08

KH1L1 refers to the mAb with the KH1 heavy chain variable region and the L1 light chain variable region; all the other humanized mAbs in the table adopt the same naming rule.

[00232] Example 7: Expression and purification of bispecific antibodies carrying a common light chain

[00233] As mentioned above, DLL3 is detectable on the surface of small cell lung cancer (SCLC) and large cell neuroendocrine carcinoma (LCNEC) tumor cells (Saunders et al., Sci Transl Med 7(302):302ra136 (2015) and Sharma et al., Cancer Res 77(14):3931-41 (2017)). Further, CD47 which mediates the “don’t eat me” signal is overexpressed in many tumors such as SCLC (Weiskopf et al., J Clin Invest 126(7):2610–2620 (2016)). A

bispecific antibody with one arm binding to CD47 and the other arm binding to DLL3 (termed anti-CD47/DLL3 bispecific antibody) can be used to selectively target a cell that expresses both antigens. Binding of the bispecific antibody to both antigens on the same cell can result in increased affinity compared with either arm due to avidity. The bispecific antibody is expected to have weaker activity against cells that express only CD47 (but not DLL3) due to lack of avidity. This helps avoid targeting normal cells that express certain levels of CD47 by the bispecific antibody and increase its safety and/or tolerability. An anti-CD47/DLL3 bispecific antibody can selectively block the CD47/SIRPα interaction on a cell that express both CD47 and DLL3 and activate the innate immune system against the cell, such as a cancer cell. Thus, an anti-CD47/DLL3 bispecific antibody can be an effective therapy for SCLC, LCNEC and other tumors that express significant levels of both CD47 and DLL3 on the cell surface.

[00234] A bispecific antibody against CD47 and DLL3 was constructed with the humanized heavy chain variable region H1 in Table 7, the humanized heavy chain variable region KH2 in Table 10, and the humanized light chain variable region L2 in Table 8. The CDR regions for the bispecific mAb KH2/H1/L2 are provided in Table 12 and Table 13. The VH and VL regions of the bispecific antibody were fused to the constant regions of IgG1 heavy chain and kappa light chain, respectively. The HC containing KH2 has the T366W mutation to form a "knob" and the HC containing H1 has the mutations T366S, L368A, and Y407V to form a "hole", so that the two heavy chains were favored to form a bispecific antibody with heterodimeric HCs (KH2/H1) rather than homodimeric HCs (KH2/KH2 or H1/H1). The resulting bispecific antibody (KH2/H1/L2), termed BA1, was further modified by introducing a S354C cysteine mutation on the anti-CD47 HC (KH2) and a Y349C cysteine mutation on the anti-DLL3 HC (H1) to stabilize the heterodimeric pairing of the heavy chains KH2 and H1 (Merchant et al. Nat. Biotechnol. 16(7):677-81 (1998)). The resulting bispecific antibody was termed BA1(C). The variable regions of BA1 were also fused to the IgG4 frame work with the same cysteine mutations in BA1(C) to produce a bispecific antibody termed BA4(C). The simultaneous expression of the two heavy chains and the light chain in the same cell led to the expression and assembly of a desired bispecific antibody, which contains the anti-CD47 arm and the anti-DLL3 arm. Different ratios of the heavy chain DNAs were used

to optimize the expression. The bispecific antibodies were produced in the suspension of ExpiCHO-S cells and purified using Protein A affinity and ion exchange chromatography.

[00235] Table 12: CDR regions 1-3 of the heavy chains and the common light chain for the bispecific antibodies

	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
KH2	GYTFTSYW (178)	IDPSDSET (179)	AGTDLAY (180)
H1	GYTFTSYV (181)	INPYNDAT (182)	ARGGYDYDGDY (183)
L2	QNINVW (184)	KAS (185)	QQGQSYPFT (186)

The CDRs were determined utilizing the IMGT method.

[00236] Table 13: CDR regions 1-3 of the heavy chains and the common light chain for the bispecific antibodies

	CDR1 (SEQ ID NO:)	CDR2 (SEQ ID NO:)	CDR3 (SEQ ID NO:)
KH2	SYWMH (187)	NIDPSDSETHYAQKFQG (188)	TDLAY (189)
H1	SYVMH (190)	YINPYNDATKYARKFQG (191)	GGYDYDGDY (192)
L2	HASQNINVWLS (193)	KASNLHT (194)	QQGQSYPFT (195)

The HC and LC CDRs were determined utilizing the Kabat method.

[00237] Example 8: Characterization of bispecific antibodies

[00238] The purified bispecific antibodies were analyzed in a bridging ELISA assay to demonstrate that a bispecific antibody can bind both antigens at the same time. Human FLAG-huDLL3 in carbonate coating buffer (50 µL/well at 0.25 µg/mL) was coated on a 96-well ELISA plate for 1 hour at room temperature. The plate was blocked by 5% BSA in TBST for 1 hour at room temperature. In each well of the plate, a bispecific mAb (50 µL/well at various concentrations) was added and incubated for 1 hour at room temperature. After washing, biotinylated human CD47 extracellular domain (ECD) (AcroBio, Cat#: CD7-H82E9) was added to each well (50 µL/well at 0.05 µg/mL) and incubated at room temperature for 60 minutes. The plate was washed and the binding of biotinylated CD47 to the immobilized bispecific antibody was detected by adding horseradish peroxidase-conjugated streptavidin (JIR, Cat#: 016-030-084) and incubating at room temperature for 60 minutes. Then, after washing, the ELISA was developed using One-step Detection Solution (ThermoFisher Scientific, Cat#: 34028) and measured as the absorbance at 450 nm. The results of the bridging ELISA assay for the bispecific

antibodies BA1 and BA1(C) are provided in FIG. 4A; the result of the bridging ELISA assay for BA4(C) is provided in FIG. 4B.

[00239] The bispecific antibodies were analyzed for their ability to bind CD47(ECD) or DLL3(ECD)-6His (R&D, Cat#: 9749-DL-050) immobilized on regular or nickel-coated plates in an ELISA assay. The results of the CD47 binding assay for the bispecific antibodies BA1, BA1(C), and BA4C are provided in FIG. 5 (CD47(ECD) was coated on regular plates). The anti-CD47 parental mAb KH2L2 (labeled as anti-CD47 parental mAb in the FIG.) on the IgG4 framework was used as a control. The results of the DLL3 binding assay for the bispecific antibodies BA1 and BA1(C) are provided in FIG. 6A (DLL3(ECD)-6His was coated on a regular plate); the result of the DLL3 binding assay for BA4(C) is provided in FIG. 6B (DLL3(ECD)-6His was immobilized on a nickel-coated plate). The anti-DLL3 parental mAb H1L2 (labeled as anti-DLL3 parental mAb in the FIG.) on the IgG4 framework was used as a control. The results in FIG. 5 indicated that the bispecific antibodies, which have one anti-CD47 arm, were capable of binding CD47, but at a lower affinity than the parental mAb KH2L2 which has two anti-CD47 arms (FIG. 5). Similarly, the results in FIGs. 6A and 6B demonstrated that the bispecific antibodies, which have one anti-DLL3 arm, had weaker binding to DLL3 when compared with the parental anti-DLL3 mAb H1L2 which has two anti-DLL3 arms.

[00240] The bispecific antibodies were analyzed for their ability to bind both CD47(ECD) and DLL3(ECD)-6His when they were simultaneously immobilized on the same plate (50 μL/well at 20 nM for both antigens). The results of the CD47/DLL3 binding assay for the bispecific antibodies BA1, BA1(C) and BA4(C) are provided in FIG. 7.

[00241] The bispecific antibodies were also analyzed for their ability to block the CD47/SIRPα interaction in an ELISA assay. Human CD47 (ECD) (AcroBio, Cat#: CD7-HA2E9-50ug) at 1 μg/mL in carbonate coating buffer was coated on a 96-well plate (50 μL/well) at 4°C overnight. The plate was blocked by 5% BSA in TBST for 1 hour at room temperature. A bispecific and parental mAb at various concentrations were added to the plate and incubated at room temperature for 30 minutes. SIRPα extracellular domain (ECD) fused to mouse Fc (AcroBio, Cat#: SIA-H52A8-100ug) was added to each well (50 μL/well at 1 μg/mL) and incubated at room temperature for 1 hour. The

plate was washed and the binding of SIRPα to the immobilized CD47 was detected by anti-mouse IgG conjugated to horseradish peroxidase (mIgG-HRP) (ThermoFisher Scientific, Cat#: A16084) with incubation for 1 hour at room temperature. Then after washing, the ELISA was developed using One-step Detection Solution (ThermoFisher Scientific, Cat#: 34028) and measured as the absorbance at 450 nm. Consistent with the data from the CD47 binding assay on ELISA, the bispecific Abs have reduced ability to block the CD47/SIRPα interaction in this context (FIG. 8A). The assay was also carried out when both CD47(ECD) and DLL3(ECD)-6His were simultaneously immobilized on the same plate at a CD47:DLL3 ratio of 2:1 (50 μL/well with CD47 at 40 nM and DLL3 at 20 nM) for 1 hour at room temperature and the plate was blocked by 5% BSA in DPBS. Under such a condition, the bispecific Abs have similar ability to block the CD47/SIRPα interaction when compared with the anti-CD47 parental mAb (FIG. 8B), suggesting that binding to the immobilized DLL3 on the plate by the anti-DLL3 arm of each of the bispecific Abs contributes to the blockade of the CD47/SIRPα interaction.

[00242] The bispecific antibodies were analyzed for their ability to bind Raji cells which have no detectable DLL3 expression. Raji cells (14,000 cells per well on a 96-well plate) were incubated with either a solution of a purified mAb at various concentrations or an isotype control in Hanks' Balanced Salt Solution (HBSS) containing 0.05% sodium azide and 0.1% BSA at room temperature for 30 minutes. After incubation, the plates were washed three times with the same buffer. Using a PE-Cy7-conjugated anti-human IgG secondary antibody, the presence of the mAb on Raji cells was measured by FACS (Attune NxT; ThermoFisher, Carlsbad, CA). The result of the FACS binding analysis is provided in FIG. 9.

[00243] The binding of the bispecific mAb BA1(C) and the two parental control mAbs to SHP-77 cells were tested in the presence or absence of one of the competing F(ab')2's: anti-DLL3 parental F(ab')2 (final concentration in the assay was 25 μM) and anti-CD47 parental F(ab')2 (final concentration in the assay was 10 μM). F(ab')2's were generated from mAbs using immobilized pepsin (Thermo Fisher Scientific, Cat: #20343) according to the instruction and purified. The result of the binding analysis is provided in FIG. 10. Inhibition of the binding of BA1(C) to SHP-77 cells by both F(ab')2's indicates that both

the anti-CD47 and anti-DLL3 arms of the bispecific antibody contribute to its binding to SHP-77 cells.

[00244] The bispecific antibodies were tested for their ability to bind to human and cynomolgus red blood cells (RBCs). 14,000 RBCs were resuspended into 20 μL FACS buffer (1X HBSS (Thermo Fisher Scientific, Cat#: 14175079) with 0.1% BSA and 0.05% sodium azide) containing diluted mAbs and incubated at room temperature for 30 minutes. After incubation, the plates were washed three times with FACS buffer and the cells were resuspended with PE-Cy7 conjugated anti-human IgG-Fc secondary antibody (BioLegend, Cat#: 409316) and incubated for 15 minutes at room temperature in dark. Plates were washed 2x with FACS buffer. Cells were resuspended and transferred to a 384-well plate for analysis with Attune NxT Flow Cytometer. FIGs. 11A-11C show the binding of the bispecific antibodies to human red blood cells (RBCs) at the following mAb concentrations: 1,600 nM (FIG. 11A), 533 nM (FIG. 11B), and 178 nM (FIG. 11C). Anti-CD47 and anti-DLL3 parental antibodies and IgG1 and IgG4 isotypes were used as controls.

[00245] It will be appreciated by those skilled in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but it is intended to cover modifications within the spirit and scope of the present invention as defined by the present description.

CLAIMS

It is claimed:

1. An isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

a. SEQ ID NOs: 25, 26, 27, 61, 62 and 63, respectively;
b. SEQ ID NOs: 28, 29, 30, 64, 65 and 66, respectively;
c. SEQ ID NOs: 31, 32, 33, 67, 68 and 69, respectively;
d. SEQ ID NOs: 34, 35, 36, 70, 71 and 72, respectively;
e. SEQ ID NOs: 37, 38, 39, 73, 74 and 75, respectively;
f. SEQ ID NOs: 40, 41, 42, 76, 77 and 78, respectively;
g. SEQ ID NOs: 43, 44, 45, 79, 80 and 81, respectively;
h. SEQ ID NOs: 46, 47, 48, 82, 83 and 84, respectively;
i. SEQ ID NOs: 49, 50, 51, 85, 86 and 87, respectively;
j. SEQ ID NOs: 52, 53, 54, 88, 89 and 90, respectively;
k. SEQ ID NOs: 55, 56, 57, 91, 92 and 93, respectively; or
l. SEQ ID NOs: 58, 59, 60, 94, 95 and 96, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds DLL3, preferably human DLL3.

2. An isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprising a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of:

a. SEQ ID NOs: 97, 98, 99, 133, 134 and 135, respectively;
b. SEQ ID NOs: 100, 101, 102, 136, 137 and 138, respectively;
c. SEQ ID NOs: 103, 104, 105, 139, 140 and 141, respectively;
d. SEQ ID NOs: 106, 107, 108, 142, 143 and 144, respectively;
e. SEQ ID NOs: 109, 110, 111, 145, 146 and 147, respectively;
f. SEQ ID NOs: 112, 113, 114, 148, 149 and 150, respectively;
g. SEQ ID NOs: 115, 116, 117, 151, 152 and 153, respectively;

h. SEQ ID NOs: 118, 119, 120, 154, 155 and 156, respectively;
i. SEQ ID NOs: 121, 122, 123, 157, 158 and 159, respectively;
j. SEQ ID NOs: 124, 125, 126, 160, 161 and 162, respectively;
k. SEQ ID NOs: 127, 128, 129, 163, 164 and 165, respectively; or
l. SEQ ID NOs: 130, 131, 132, 166, 167 and 168, respectively;

wherein the antibody or antigen-binding fragment thereof specifically binds DLL3, preferably human DLL3.

3. The isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of claim 1 or 2, comprising a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21 or 23, or a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22 or 24.

4. The isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-3, comprising:

a. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:1, and a light chain variable region having the polypeptide sequence of SEQ ID NO:2;
b. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:3, and a light chain variable region having the polypeptide sequence of SEQ ID NO:4;
c. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:5, and a light chain variable region having the polypeptide sequence of SEQ ID NO:6;
d. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:7, and a light chain variable region having the polypeptide sequence of SEQ ID NO:8;
e. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:9, and a light chain variable region having the polypeptide sequence of SEQ ID NO:10;

f. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:11, and a light chain variable region having the polypeptide sequence of SEQ ID NO:12;
g. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:13, and a light chain variable region having the polypeptide sequence of SEQ ID NO:14;
h. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:15, and a light chain variable region having the polypeptide sequence of SEQ ID NO:16;
i. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:17, and a light chain variable region having the polypeptide sequence of SEQ ID NO:18;
j. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:19, and a light chain variable region having the polypeptide sequence of SEQ ID NO:20;
k. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:21, and a light chain variable region having the polypeptide sequence of SEQ ID NO:22; or
l. a heavy chain variable region having the polypeptide sequence of SEQ ID NO:23, and a light chain variable region having the polypeptide sequence of SEQ ID NO:24.

5. The isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-4, wherein the antibody or antigen-binding fragment thereof is chimeric.

6. The isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-4, wherein the antibody or antigen-binding fragment thereof is human or humanized.

7. The isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of claim 6, wherein the antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;

(b) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172; or

(c) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:170, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173.

8. The isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-7, wherein the monoclonal antibody or antigen-binding fragment thereof is capable of inducing effector-mediated tumor cell lysis, mediating the recruitment of conjugated drugs, and/or forms a bispecific antibody with another monoclonal antibody or antigen-binding fragment with a cancer-killing effect.

9. An isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof comprising a humanized heavy chain variable region of an anti-CD47 monoclonal antibody and a humanized light chain variable region of an anti-DLL3 monoclonal antibody, wherein the anti-CD47 monoclonal antibody or antigen-binding fragment thereof comprises:

(a) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;

(b) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;

(c) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:175, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173;

(d) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;

(e) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;

(f) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:176, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173;

(g) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:171;

(h) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:172;

(i) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:173; or

(j) a heavy chain variable region having the polypeptide sequence of SEQ ID NO:177, and a light chain variable region having the polypeptide sequence of SEQ ID NO:174.

10. The isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof of claim 9, wherein the antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to SIRPα.

11. The isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof of claim 9, wherein the antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells.

12. An isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 178, 179 and 180; the second antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1),

HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 181, 182 and 183; and the first antigen-binding domain and the second antigen-binding domain each comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NOs: 184, 185 and 186.

13. An isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, wherein the first antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 187, 188 and 189; the second antigen-binding domain comprises a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, having the polypeptide sequences of SEQ ID NOs: 190, 191 and 192; and the first antigen-binding domain and the second antigen-binding domain each comprises a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NOs: 193, 194 and 195.

14. The isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment of claim 12 or 13, wherein the first antigen-binding domain comprises a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:176, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:172; and wherein the second antigen-binding domain comprises a heavy chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:170, and a light chain variable region having a polypeptide sequence at least 95% identical to SEQ ID NO:172.

15. The isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 12-14, wherein the antibody or antigen-binding fragment thereof is capable of blocking binding of CD47 to SIRPα on cancer cells that express both CD47 and DLL3.

16. The isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 12-14, wherein the antibody or antigen-binding fragment thereof is capable of inducing macrophage-mediated phagocytosis of cancer cells that express both DLL3 and CD47.

17. The isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 12-14, wherein the antibody or antigen-binding fragment thereof is capable of binding cancer cells that express both DLL3 and CD47 with minimal to undetectable binding to human red blood cells.

18. An isolated nucleic acid encoding the anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 1-17.

19. A vector comprising the isolated nucleic acid of claim 18.

20. A host cell comprising the vector of claim 19.

21. A pharmaceutical composition, comprising the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 1-17 and a pharmaceutically acceptable carrier.

22. A method of targeting DLL3 on a cancer cell surface in a subject in need thereof, comprising administering to the subject in need thereof a pharmaceutical composition comprising the isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-8.

23. A method of blocking binding of CD47 to signal regulatory protein alpha (SIRPα) in a subject in need thereof, comprising administering to the subject in need thereof a pharmaceutical composition comprising the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof of any one of claims 9-11.

24. A method of inducing macrophage-mediated phagocytosis of cancer cells in a subject in need thereof, comprising administering to the subject in need thereof a pharmaceutical composition comprising the isolated anti-CD47 monoclonal antibody or antigen-binding fragment thereof of any one of claims 9-11.

25. A method of targeting DLL3 and CD47 on a cancer cell surface in a subject in need thereof, comprising administering to the subject in need thereof a pharmaceutical composition comprising the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 12-17.

26. A method of blocking binding of CD47 to SIRPα on a cancer cell that expresses both CD47 and DLL3 on the cell surface in a subject in need thereof, comprising administering to the subject in need thereof a pharmaceutical composition comprising the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 12-17.

27. A method of inducing macrophage-mediated phagocytosis of cancer cells that express both CD47 and DLL3 on the cell surface in a subject in need thereof, comprising administering to the subject in need thereof a pharmaceutical composition comprising the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 12-17.

28. A method of binding cancer cells that express both DLL3 and CD47 with minimal to undetectable binding to human red blood cells in a subject in need thereof, comprising administering to the subject in need thereof a pharmaceutical composition comprising the isolated humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 12-17.

29. A method of treating cancer in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of claim 21.

30. The method of claim 29, wherein the cancer is selected from the group consisting of a lung cancer, such as small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNEC), a gastric cancer, a colon cancer, a hepatocellular carcinoma, a renal cell carcinoma, a bladder urothelial carcinoma, a metastatic melanoma, a breast cancer, an ovarian cancer, a cervical cancer, a head and neck cancer, a pancreatic cancer, a glioma, a glioblastoma, and other solid tumors, and a non-Hodgkin's lymphoma (NHL), an acute lymphocytic leukemia (ALL), a chronic lymphocytic leukemia (CLL), a chronic myelogenous leukemia (CML), a multiple myeloma (MM), an acute myeloid leukemia (AML), and other liquid tumors.

31. A method of producing the anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 1-17, comprising culturing a cell comprising a nucleic acid encoding the antibody or antigen-binding fragment thereof under conditions

to produce the antibody or antigen-binding fragment thereof, and recovering the antibody or antigen-binding fragment thereof from the cell or culture.

32. A method of producing a pharmaceutical composition comprising the anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, the anti-CD47 monoclonal antibody or antigen-binding fragment thereof, or the humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment thereof of any one of claims 1-17, comprising combining the antibody or antigen-binding fragment thereof with a pharmaceutically acceptable carrier to obtain the pharmaceutical composition.

33. A method of determining a level of DLL3 in a subject, the method comprising:
    a. obtaining a sample from the subject;
    b. contacting the sample with an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof of any one of claims 1-8; and
    c. determining a level of DLL3 in the subject.

34. The method of claim 33, wherein the sample is a tissue sample

35. The method of claim 34, wherein the tissue sample is a cancer tissue sample.

36. The method of claim 33, wherein the sample is a blood sample.

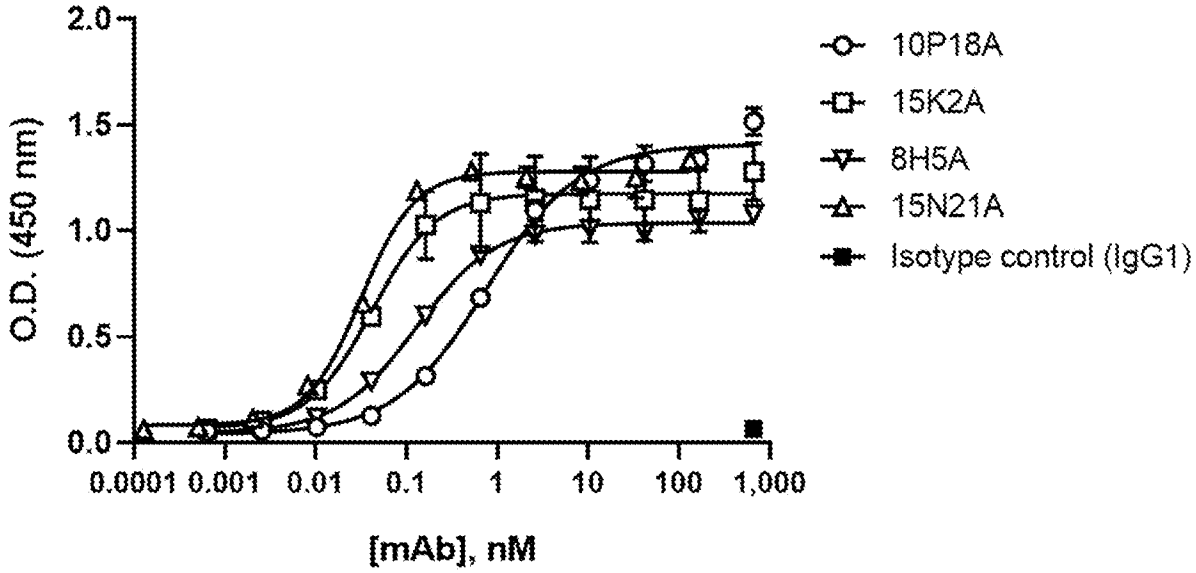

2.0
1.5
1.0
0.5
0.0
O.D. (450 nm)
0.0001 0.001 0.01 0.1 1 10 100 1,000
[mAb], nM
10P18A
15K2A
8H5A
15N21A
Isotype control (IgG1)

FIG. 1A

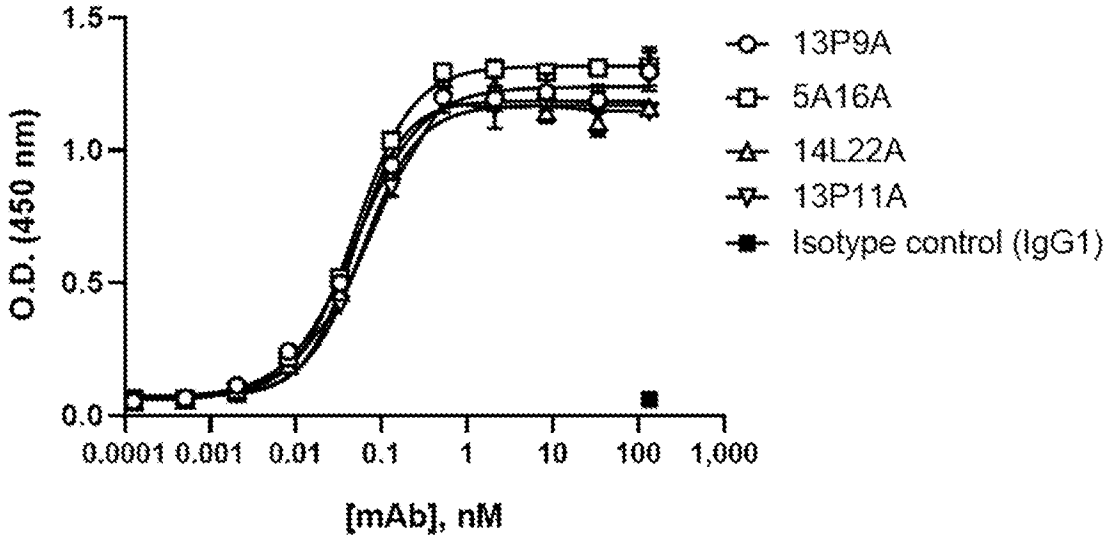

1.5
1.0
0.5
0.0
O.D. (450 nm)
0.0001 0.001 0.01 0.1 1 10 100 1,000
[mAb], nM
13P9A
5A16A
14L22A
13P11A
Isotype control (IgG1)

FIG. 1B

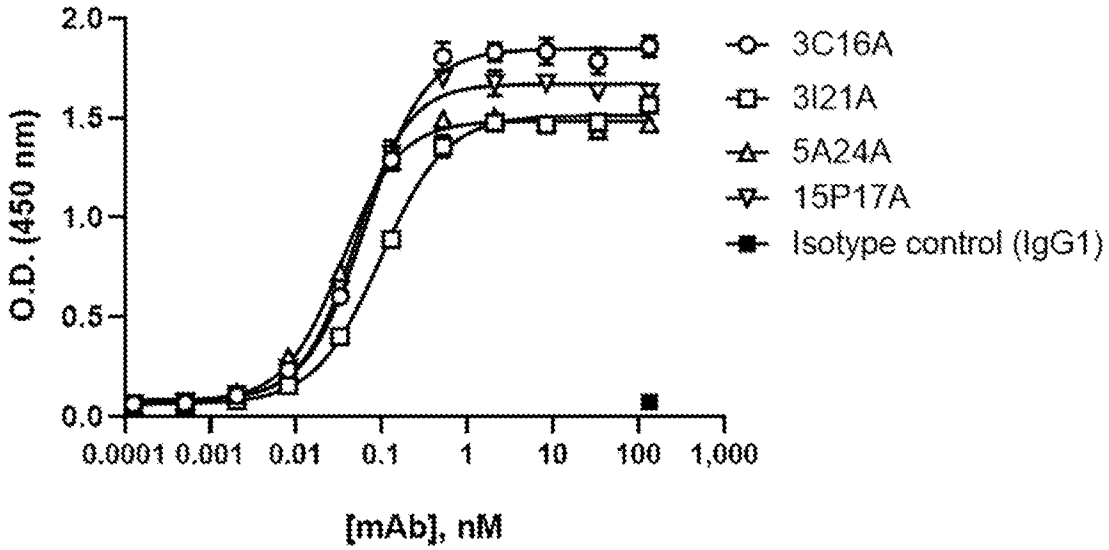

2.0
1.5
1.0
0.5
0.0
O.D. (450 nm)
0.0001 0.001 0.01 0.1 1 10 100 1,000
[mAb], nM
3C16A
3I21A
5A24A
15P17A
Isotype control (IgG1)

FIG. 1C

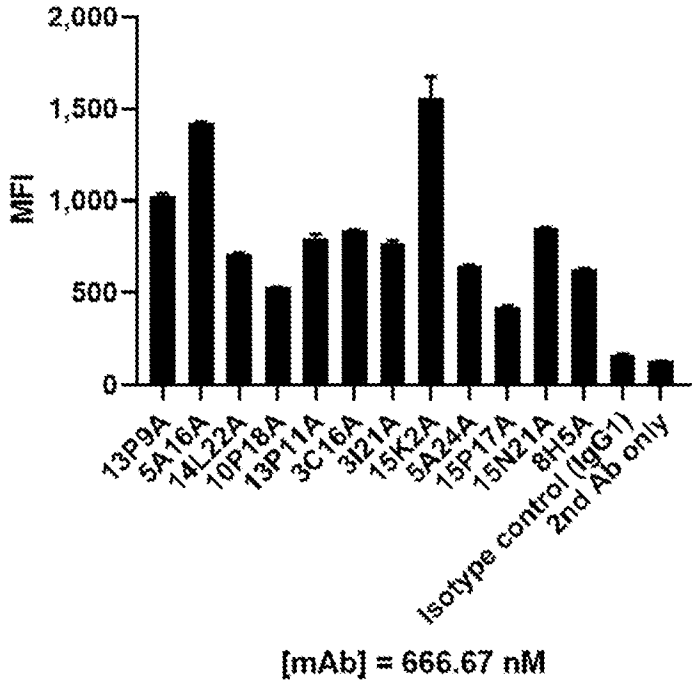

2,000
1,500
1,000
500
0
MFI
13P9A
5A16A
14L22A
10P18A
13P11A
3C16A
3I21A
15K2A
5A24A
15P17A
15N21A
8H5A
Isotype control (IgG1)
2nd Ab only
[mAb] = 666.67 nM

FIG. 2A

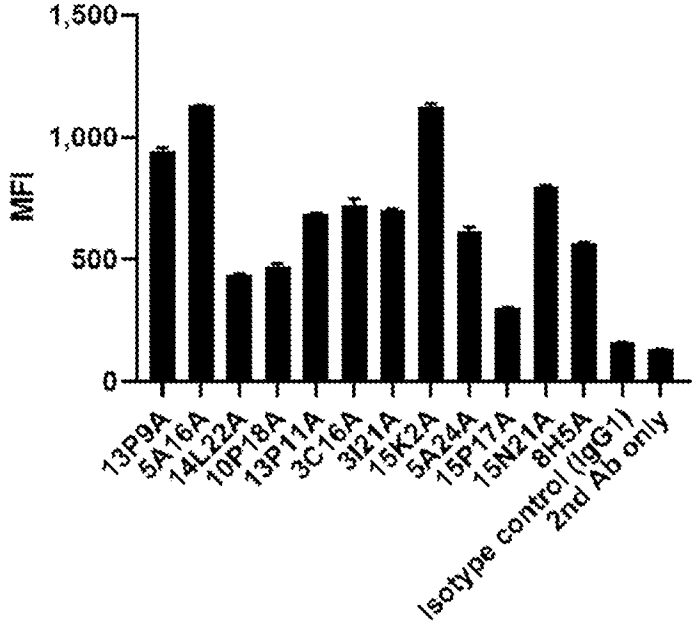

1,500
1,000
500
0
MFI
13P9A
5A16A
14L22A
10P18A
13P11A
3C16A
3I21A
15K2A
5A24A
15P17A
15N21A
8H5A
Isotype control (IgG1)
2nd Ab only
[mAb] = 333.33 nM

FIG. 2B

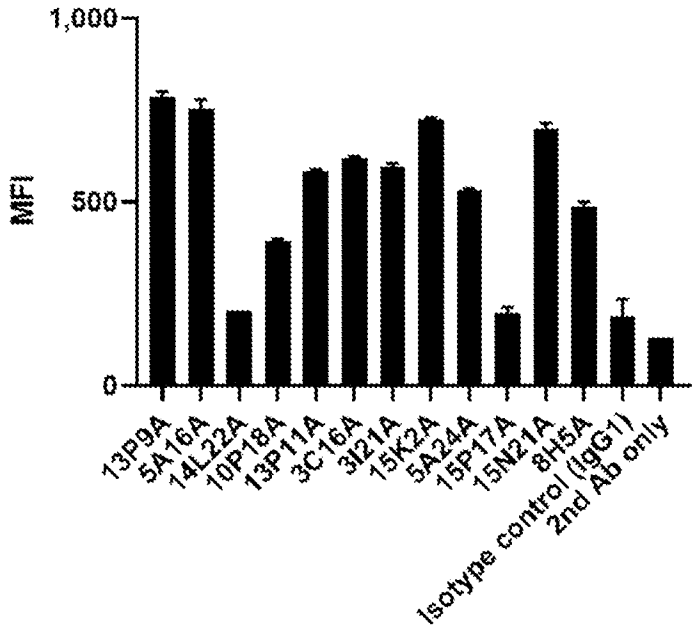

1,000
500
0
MFI
13P9A
5A16A
14L22A
10P18A
13P11A
3C16A
3I21A
15K2A
5A24A
15P17A
15N21A
8H5A
Isotype control (IgG1)
2nd Ab only
[mAb] = 66.67 nM

FIG. 2C

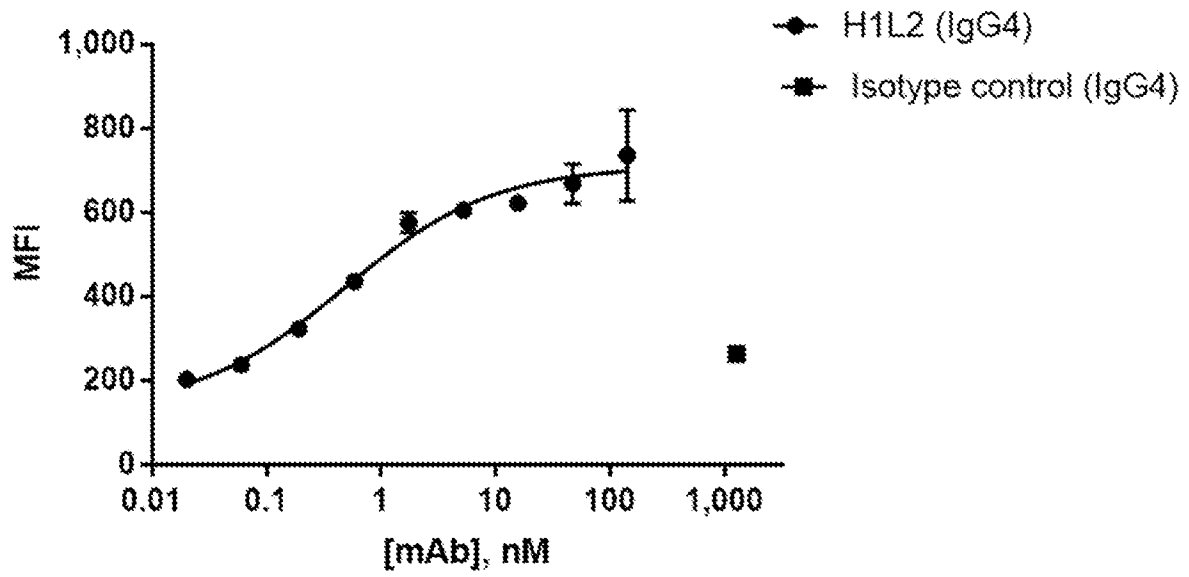

H1L2 (IgG4)
Isotype control (IgG4)
MFI
1,000
800
600
400
200
0
0.01
0.1
1
10
100
1,000
[mAb], nM

FIG. 3

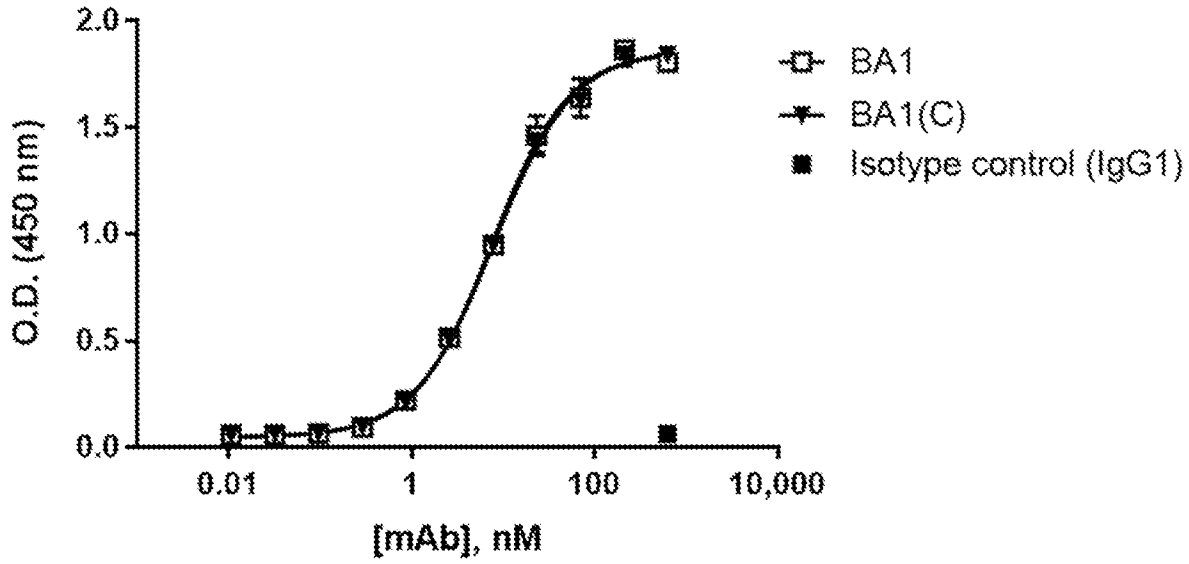

BA1
BA1(C)
Isotype control (IgG1)
O.D. (450 nm)
2.0
1.5
1.0
0.5
0.0
0.01
1
100
10,000
[mAb], nM

FIG. 4A

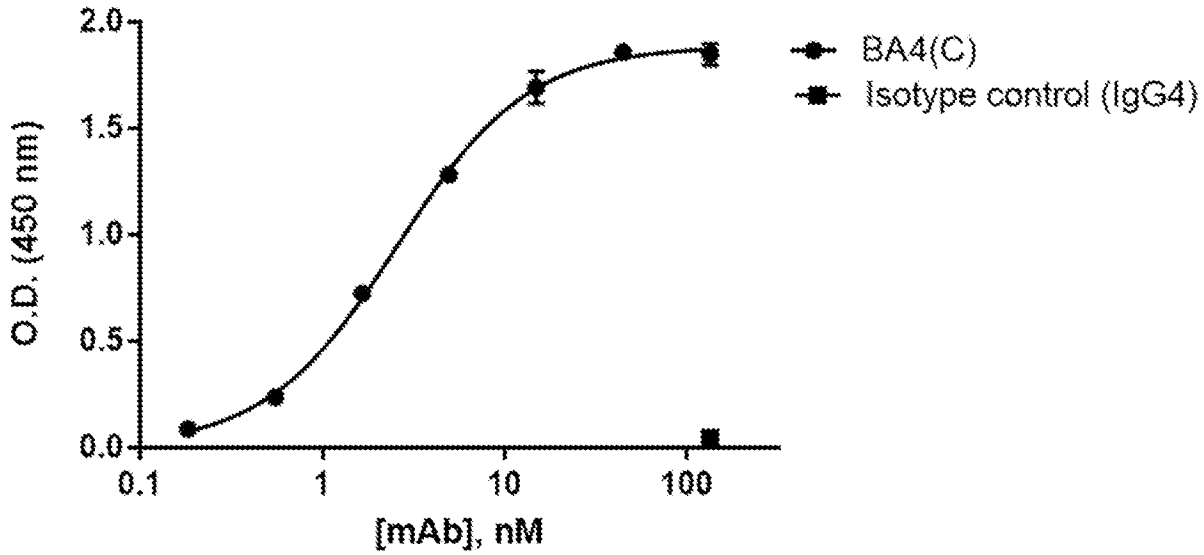

2.0
1.5
1.0
0.5
0.0
O.D. (450 nm)
0.1
1
10
100
[mAb], nM
BA4(C)
Isotype control (IgG4)

FIG. 4B

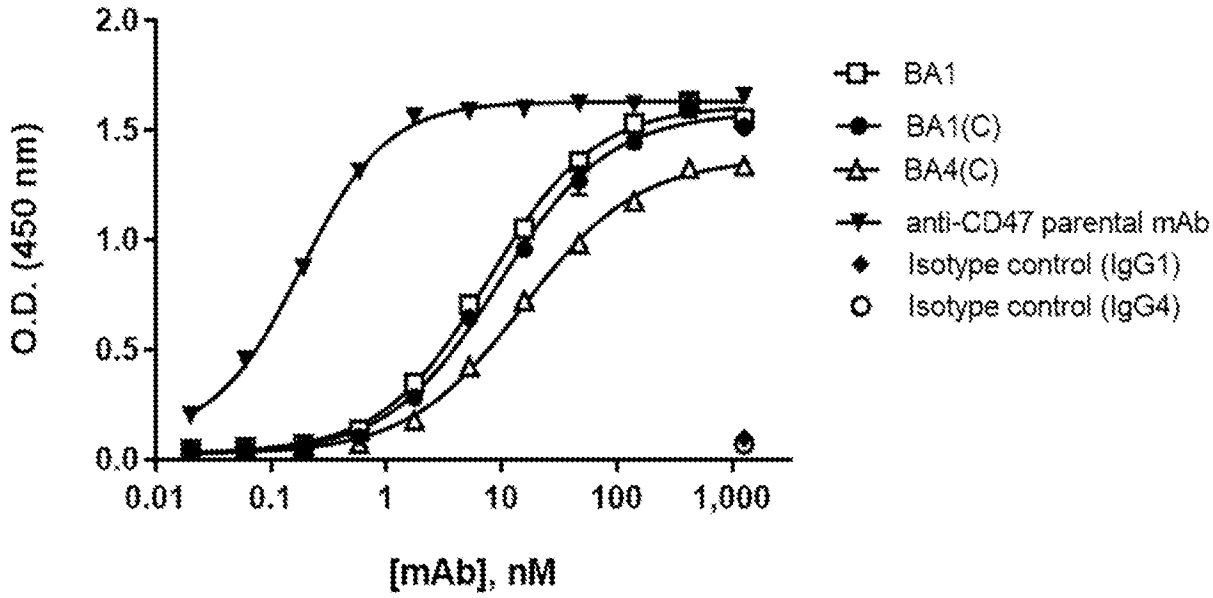

2.0
1.5
1.0
0.5
0.0
O.D. (450 nm)
0.01
0.1
1
10
100
1,000
[mAb], nM
BA1
BA1(C)
BA4(C)
anti-CD47 parental mAb
Isotype control (IgG1)
Isotype control (IgG4)

FIG. 5

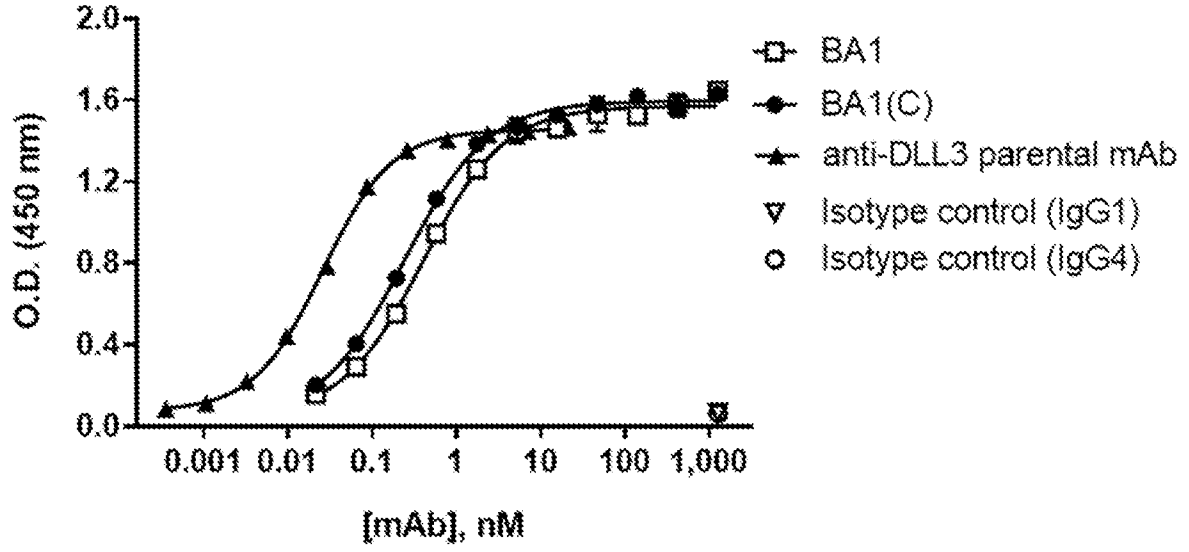

2.0
1.6
1.2
0.8
0.4
0.0
O.D. (450 nm)
0.001 0.01 0.1 1 10 100 1,000
[mAb], nM
BA1
BA1(C)
anti-DLL3 parental mAb
Isotype control (IgG1)
Isotype control (IgG4)

FIG. 6A

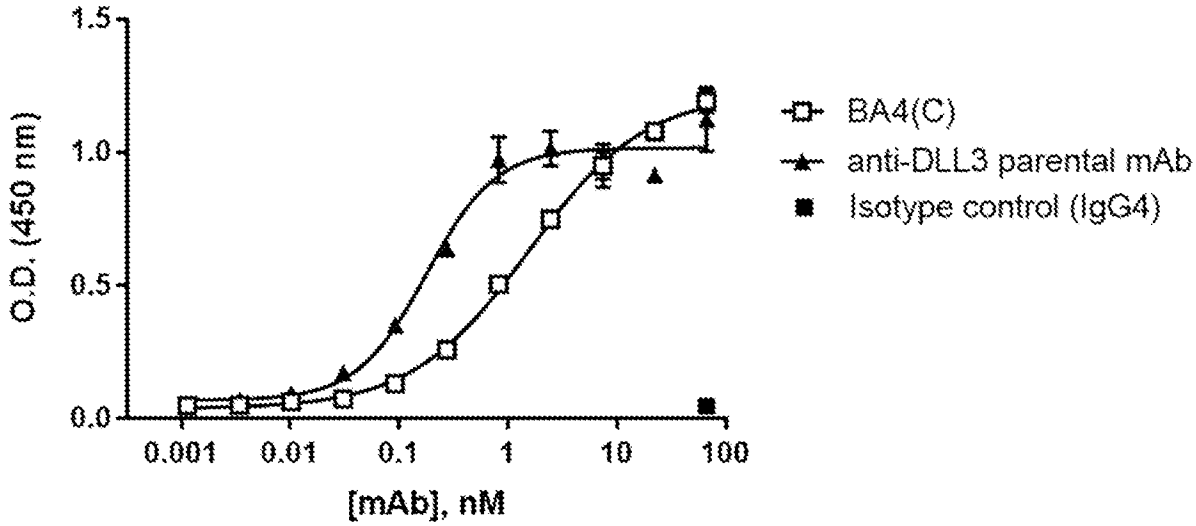

1.5
1.0
0.5
0.0
O.D. (450 nm)
0.001 0.01 0.1 1 10 100
[mAb], nM
BA4(C)
anti-DLL3 parental mAb
Isotype control (IgG4)

FIG. 6B

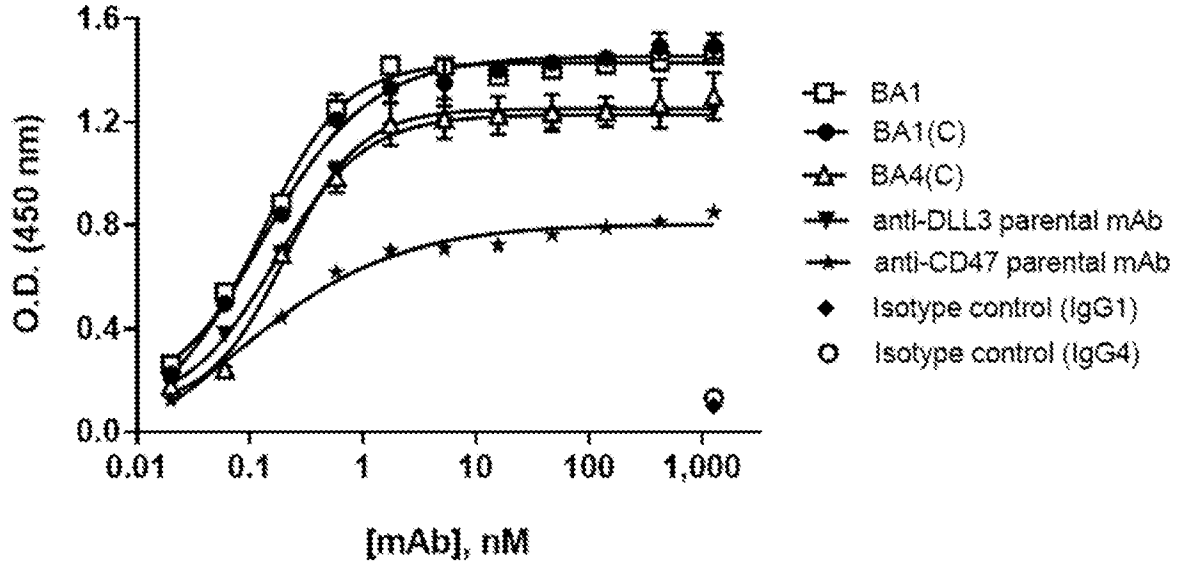

1.6
1.2
0.8
0.4
0.0
O.D. (450 nm)
0.01
0.1
1
10
100
1,000
[mAb], nM
BA1
BA1(C)
BA4(C)
anti-DLL3 parental mAb
anti-CD47 parental mAb
Isotype control (IgG1)
Isotype control (IgG4)

FIG. 7

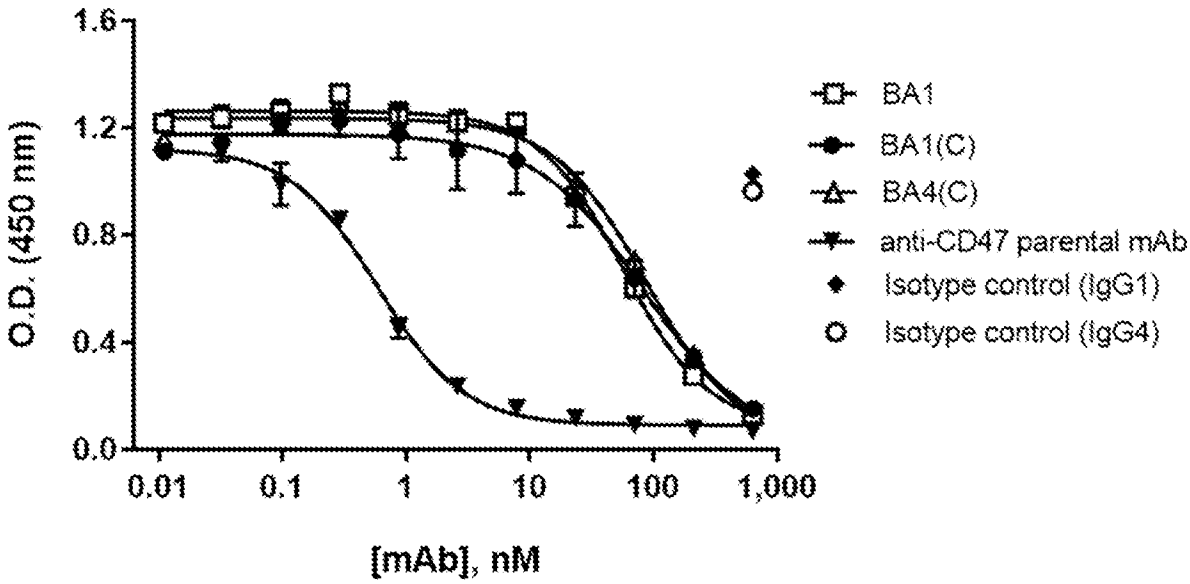

1.6
1.2
0.8
0.4
0.0
O.D. (450 nm)
0.01
0.1
1
10
100
1,000
[mAb], nM
BA1
BA1(C)
BA4(C)
anti-CD47 parental mAb
Isotype control (IgG1)
Isotype control (IgG4)

FIG. 8A

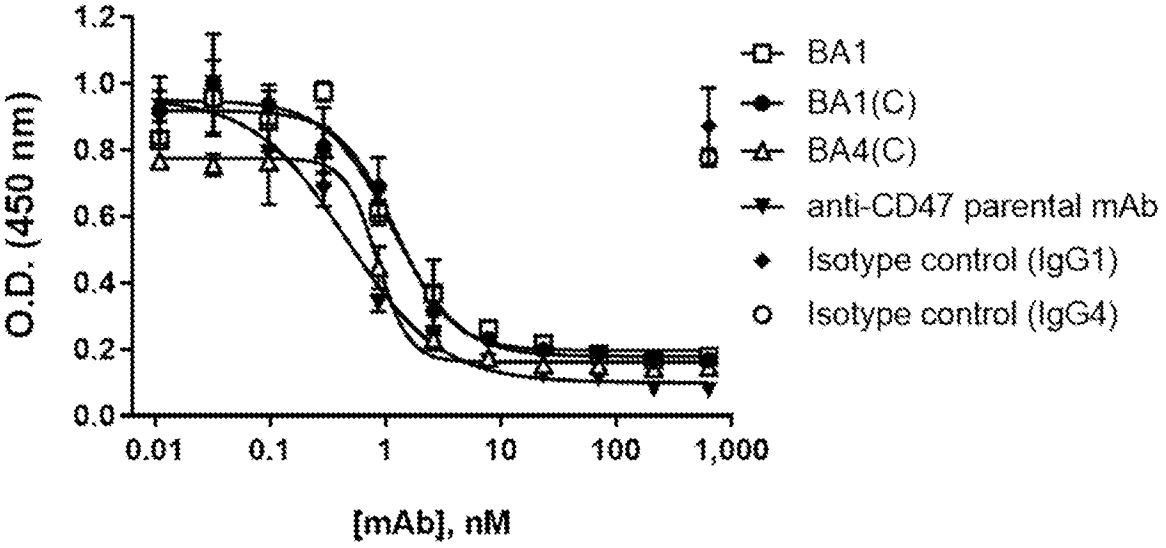

O.D. (450 nm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
100
1,000
[mAb], nM
BA1
BA1(C)
BA4(C)
anti-CD47 parental mAb
Isotype control (IgG1)
Isotype control (IgG4)

FIG. 8B

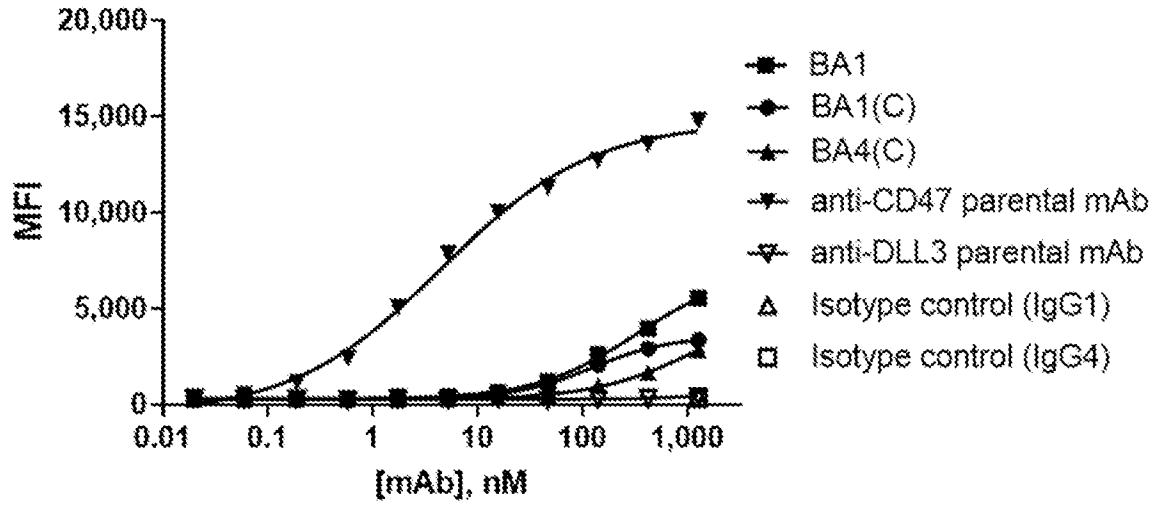

MFI
20,000
15,000
10,000
5,000
0
0.01
0.1
1
10
100
1,000
[mAb], nM
BA1
BA1(C)
BA4(C)
anti-CD47 parental mAb
anti-DLL3 parental mAb
Isotype control (IgG1)
Isotype control (IgG4)

FIG. 9

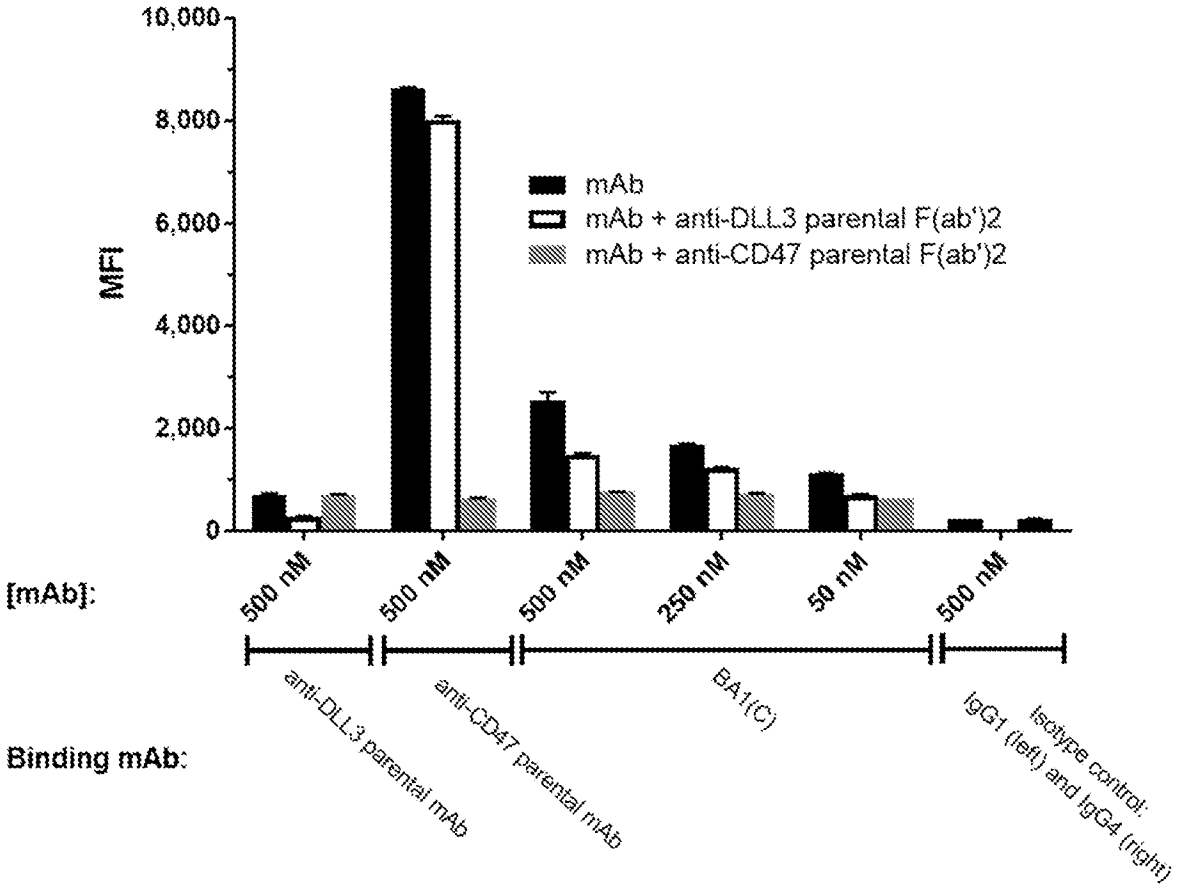
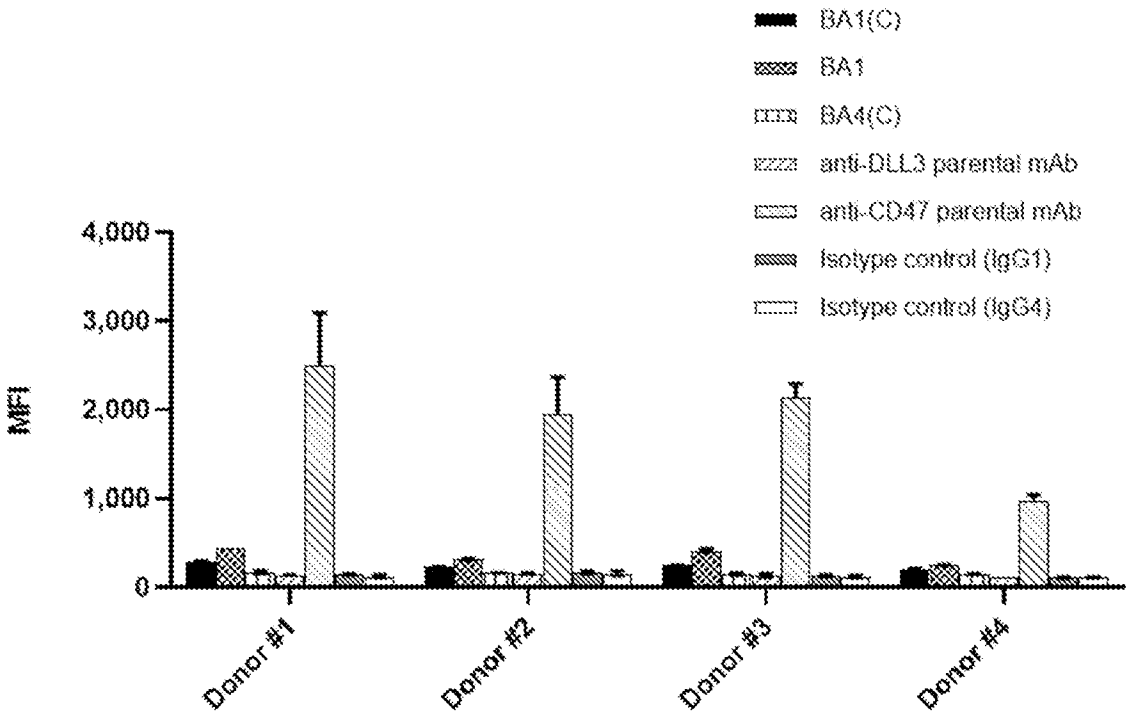
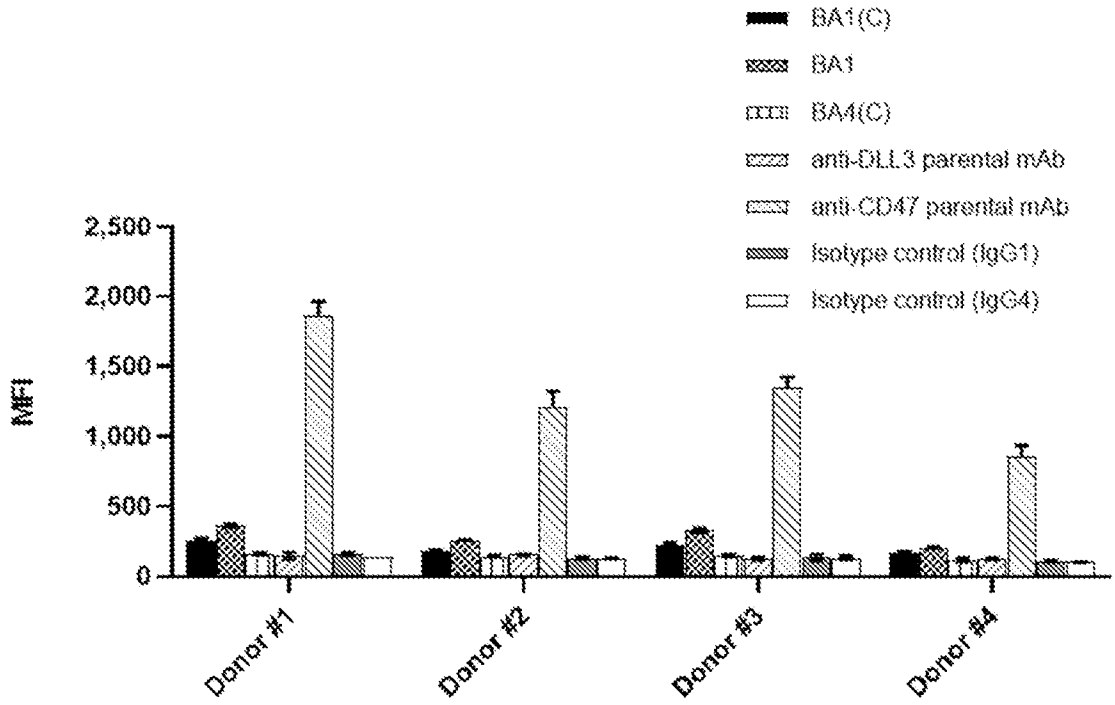

10,000
8,000
6,000
4,000
2,000
0
MFI
mAb
mAb + anti-DLL3 parental F(ab')2
mAb + anti-CD47 parental F(ab')2
[mAb]:
500 nM
500 nM
500 nM
250 nM
50 nM
500 nM
Binding mAb:
anti-DLL3 parental mAb
anti-CD47 parental mAb
BA1(C)
Isotype control:
IgG1 (left) and IgG4 (right)

FIG. 10



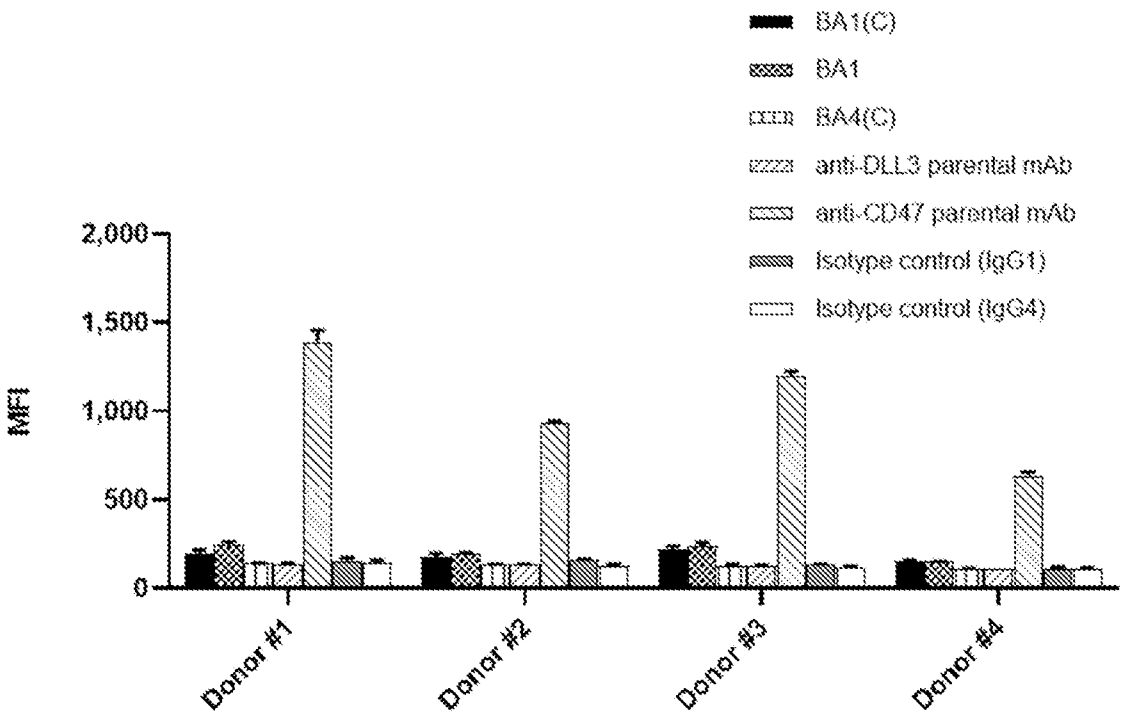
[mAb] = 1600 nM

FIG. 11A



[mAb] = 533 nM

FIG. 11B



[mAb] = 178 nM

FIG. 11C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/29888

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/395, A61P 35/00, A61P 37/02 (2019.01)
CPC - A61P 35/00, A61P 37/02, C07K 16/2809, C07K 16/2827

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2017/147368 A1 (ONCOMED PHARM INC.) 31 August 2017 (31.08.2017); para [10], [12], [52]-[56]	1, 3
A	US 2012/0195831 A1 (ZHANG et al.) 2 August 2012 (02.08.2012); SEQ ID NO: 9	1, 3
A	US 2012/0042416 A1 (SCHLEKER et al.) 16 February 2012 (16.02.2012); SEQ ID NO: 62	1, 3
A	UniProt Accession No E3RP86_PYRTT, Uncharacterized protein, submitted 11 January 2011 [online]. [Retrieved on 22 August 2019], Retrieved from the internet <URL: https://www.uniprot.org/uniprot/E3RP86>	1, 3
A	US 2016/0130356 A1 (SIAMAB THERAPEUTICS, INC) 12 May 2016 (12.05.2016) SEQ ID NO: 103	1, 3
A	US 2006/0121049 A1 (LETOURNEUR) 8 June 2006 (08.06.2006); SEQ ID NO: 18	1, 3
A	US 2011/0312505 A1 (REDDY et al.) 22 December 2011 (22.12.2011); SEQ ID NO: 131	1, 3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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

Date of the actual completion of the international search	Date of mailing of the international search report
22 August 2019	17 SEP 2019

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/29888

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

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

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

3. ☒ Claims Nos.: 4-8, 15-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

*****Continued in Supplemental Box*****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 3(in part) limited to SEQ ID NOs: 1, 2, 25, 26, 27, 61, 62 and 63

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 19/29888

Continuation of Box No. III (Observations where unity of invention is lacking):

Group I+, claims 1-3, directed to an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof. The anti-DLL3 antibody will be searched to the extent that the sequence encompasses the following sequences: a heavy chain complementarity determining region 1 (HCDR1), HCDR2, HCDR3, a light chain complementarity determining region 1 (LCDR1), LCDR2, and LCDR3, having the polypeptide sequences of SEQ ID NOs: 25, 26, 27, 61, 62 and 63, respectively, and a heavy chain variable region (VH) having the polypeptide sequence of SEQ ID NO: 1, and a light chain variable region (VL) having the polypeptide sequence of SEQ ID NO: 2. It is believed that claims 1 and 3(in part) encompass this first named invention, and thus these claims will be searched without fee to the extent that the anti-DLL3 antibody encompasses sequences SEQ ID NOs: 1, 2, 25, 26, 27, 61, 62 and 63. Additional anti-DLL3 antibody sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected anti-DLL3 antibody sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be an anti-DLL3 antibody comprising HCDR1-3 and LCDR1-3 SEQ ID Nos: 28, 29, 30, 64, 65 and 66, respectively, VH and VL SEQ ID Nos: 3 and 4, respectively, (claims 1 and 3(in part)).

Group II+, claims 9-14, directed to an isolated anti-CD47 monoclonal antibody or a humanized anti-CD47/DLL3 bispecific antibody or antigen-binding fragment. Group II+ will be searched upon payment of additional fees. The method may be searched, for example, to encompass anti-CD47/DLL3 antibody HV and LV sequences SEQ ID NOs: 175 and 171, respectively, first and second domain HCDR1-3 SEQ ID NOs: 178, 179, 180, 181, 182 and 183, respectively, and LCDR1-3 SEQ ID NOs: 184, 185 and 186, respectively, for an additional fee and election as such. It is believed that claims 9-12 read on this exemplary invention. Additional anti-CD47/DLL3 sequence(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected anti-CD47/DLL3 sequence(s). Failure to clearly identify how any paid additional invention fees are to be applied to the"+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be anti-CD47/DLL3 HV and LV sequences SEQ ID NOs: 175 and 172, and CDR sequences SEQ ID Nos: 178-183, 193, 194 and 195 (claims 9-11).

The inventions listed as Groups I+ and II+ do not relate to a single special technical feature under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special technical features

Group I+ has the special technical feature of an isolated anti-DLL3 monoclonal antibody that specifically binds DLL3, preferably human DLL3, that is not required by Group II+.

Group II+ has the special technical feature of an anti-CD47/DLL3 bispecific antibody comprising a first antigen-binding domain that specifically binds CD47, and a second antigen-binding domain that specifically binds DLL3, that is not required by Group I+.

The inventions of Group I+ and II+ each include the special technical feature of a unique amino acid sequence. Each amino acid sequence encodes a unique antibody, and is considered a distinct technical feature.

Common technical features

The inventions of Group I+ and Group II+ share the common technical feature of an isolated monoclonal antibody or antigen-binding fragment thereof comprising a HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the antibody or antigen-binding fragment thereof specifically binds DLL3.

No technical features are shared between the anti-DLL3 antibody amino acid sequences of Group I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Group I+ were considered to further share the technical features of including: an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof, these shared technical features are previously taught by WO 2017/147368 A1 to Oncomed Pharm Inc., (hereinafter Oncomed).

Additionally, even if Group II+ were considered to share the technical features of including: an isolated anti-CD47 monoclonal antibody comprising a humanized heavy chain variable region of an anti-CD47 monoclonal antibody and a humanized light chain variable region of an anti-DLL3 monoclonal antibody; or an isolated humanized anti-CD47/DLL3 bispecific antibody comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3, these shared technical features are previously taught by Oncomed.

Oncomed teaches an isolated anti-DLL3 monoclonal antibody or antigen-binding fragment thereof comprising a HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein the antibody or antigen-binding fragment thereof specifically binds DLL3 (para [10] "the TAA-binding agent is an antibody that specifically binds a TAA that includes...DLL3"; [12] "the TAA-binding agent is a monoclonal antibody"; [52]-[56] "The humanized antibody may comprise variable domains containing all or substantially all of the CDRs that correspond to the non-human immunoglobulin").

*****Continued in Supplemental Box******

Previous Page:

Oncomed teaches an isolated anti-CD47 monoclonal antibody (para [10] "the TAA-binding agent is an antibody that specifically binds a TAA...In some embodiments, a TAA includes...CD47"; [12] "the TAA-binding agent is a monoclonal antibody") comprising a humanized heavy chain variable region of an anti-CD47 monoclonal antibody and a humanized light chain variable region of an anti-DLL3 monoclonal antibody; or an isolated humanized anti-CD47/DLL3 bispecific antibody (para [10] "the TAA-binding agent is an antibody that specifically binds a TAA that includes...DLL3...In some embodiments, a TAA includes...CD47"; [60] "an antibody may be bispecific and comprise at least two antigen-binding sites with differing specificities"; [107]-[108] "in certain embodiments the antibodies to a particular TAA are multispecific...each of the one or more antigen-binding sites that an antibody contains is capable of binding (or binds) a homologous epitope on a particular TAA"; [56] "The humanized antibody may comprise variable domains containing all or substantially all of the CDRs that correspond to the non-human immunoglobulin"), comprising a first antigen-binding domain that specifically binds CD47, preferably human CD47, and a second antigen-binding domain that specifically binds DLL3, preferably human DLL3 (para [10] "the TAA-binding agent is an antibody that specifically binds a TAA that includes...DLL3...In some embodiments, a TAA includes...CD47"; [12] "the TAA-binding agent is a monoclonal antibody"; [52]-[56] "The humanized antibody may comprise variable domains containing all or substantially all of the CDRs that correspond to the non-human immunoglobulin").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Therefore, Group I+ and II+ inventions lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

NOTE, continuation of item 4 above: claims 4-8, 15-36 are held unsearchable because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).